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ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Downregulation of *klotho* gene expression in streptozotocin-induced diabetic rats

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Objective: The expression of *klotho*, which may function as an anti-aging hormone, is most abundant in the kidney. We have investigated the regulation of *klotho* expression in the kidneys of diabetic rats.

Methods: Diabetes was induced by a single i.v. injection of streptozotocin (STZ) at a dose of 60 mg/kg. Renal *klotho* expression was investigated 8 weeks post-STZ injection. Some rats were given losartan or deferoxamine from 5 weeks post-STZ injection until sacrifice. Klotho expression was examined by Western blot analysis and quantitative reverse transcription-polymerase chain reaction.

Results: Expression of klotho protein was reduced by approximately a third in the kidneys of diabetic rats compared to that of the untreated control rats. This downregulation was suppressed by either losartan or deferoxamine; these drugs also decreased the albuminuria. Histological study showed that, in the kidneys of the STZ-induced diabetic rats, mRNA expression of *klotho*, albeit less intense than in the untreated control, was observed in the tubular epithelial cells, and was co-localized with heme oxygenase-1, an oxidative stress-sensitive gene.

Conclusion: Expression of *klotho* was downregulated in the kidneys of diabetic rats. An activation of the renin-angiotensin system, altered iron homeostasis, and presumably enhanced oxidative stress, may play a role in this phenomenon.

Keywords: aging, AT_1 receptor, diabetes, klotho, oxidative stress.

Introduction

It was discovered approximately a decade ago that the deletion of the *klotho* gene results in phenotypes resembling those of human aging-associated disorders.[1,2] Although transcripts of this gene are expressed predominantly in the kidney, choroids plexus and parathyroid gland, the klotho protein affects many other organs by acting as a circulating hormone and modulating the signals of insulin and insulin-like growth factor (IGF)1.[3,4] It has been shown that, in human and animal models, the expression of the *klotho* gene is regulated in response to various metabolic and hemodynamic alterations.[5–7] We have reported that renal *klotho* expression is markedly downregulated in the kidneys of rats that have been made hypertensive by the continuous administration of angiotensin II.[8,9] Interestingly, the introduction of exogenous *klotho* into this hypertension animal model ameliorated renal injury. Therefore, it has

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been suggested that the downregulation of renal *klotho* expression in various disease conditions may, in turn, enhance renal damage, an hypothesis which is supported by the recent findings from other laboratories.[10] In the current study, we have investigated whether the expression of the *klotho* gene is downregulated in a rat model of diabetes, and, if so, whether the blockade of the AT_1 receptor would suppress this phenomenon.

Materials and methods

Animals

The experiments were performed in accordance with the guidelines for animal experimentation approved by the Animal Center for Biomedical Research, University of Tokyo Graduate School of Medicine. Eight-week-old male Sprague–Dawley rats (bodyweight, 230–280 g) were injected i.v. with streptozotocin (STZ) at a dose of 60 mg/kg in citrate buffer (pH 4.5) to induce diabetes after an overnight fast. At 8 weeks after the STZ injection, rats were sacrificed and renal *klotho* expression was investigated unless stated otherwise. From 3 weeks before sacrifice, some rats were given losartan via their drinking water at a dose of 20 mg/kg/day, and some rats were given s.c. injection of deferoxamine (a kind gift of Novartis, Basel, Switzerland) at a dose of 200 mg/kg/day. Rats were kept fasted overnight before sacrifice in the metabolic cages. Blood pressure was measured by the tail-cuff method.

RNA isolation and quantitative reverse transcription-polymerase chain reaction

Total RNA was isolated by the acid guanidinium thiocyanate-phenol chloroform method. After first-strand cDNA was synthesized with 2 μg of total RNA as a template using a SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA), quantitative polymerase chain reaction (PCR) with gene-specific hybriprobes was performed by LightCycler (Roche Diagnostics, Basel, Switzerland). The sense and antisense primer used for rat *klotho* were 5′-TGA GTC AGG ACA AGG AGT T-3′ and 5′-TAA ACT GAG AGA GAG TGG G-3′, respectively. The *klotho* mRNA expression was normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

Histological and immunohistochemical analyses

Prussian blue staining was used for iron staining. Immunohistochemistry was performed as described previously.[11] Antibodies against rat heme oxygenase-1 (HO-1; SPA895, StressGen, Victoria, Canada) and monocytes/macrophages (ED-1, Chemicon, Temecula, CA, USA) were used at dilution of 1:200 and 1:100, respectively.

In situ *hybridization*

Rat cDNA fragment was obtained by reverse transcription (RT)-PCR method: sense primer, 5′-GCG ACT ACC CAA AGA GTA T-3′; antisense primer, 5′-CTT GGC TAC AAC CCC GTC TA-3′. cDNA was subcloned into pGEM-T vector (Promega, Madison, WI, USA), and the orientation was confirmed by DNA sequencing. After the linearization of the plasmid, antisense and sense cRNA riboprobes were transcribed *in vitro* by using a digoxigenin (DIG) RNA labeling kit SP6/T7 (Roche Diagnostics, Mannheim, Germany). Hybridization was performed by using In Situ Hybridization Reagents (Nippongene, Tokyo, Japan) as described previously.[12]

Protein purification and Western blot analysis

Protein was isolated by homogenizing samples in the lysis buffer (50 mmol/L 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid [HEPES], 5 mmol/L ethylene diamine tetra acetate [EDTA], and 50 mmol/L NaCl; pH 7.5) containing protease inhibitors. Equal amounts of protein were loaded onto 15% sodium dodecyl sulfate (SDS) polyacrylamide gels and subsequently blotted onto Immobilon-P polyvinylidine difluoride membranes (Millipore, New Bedford, MA, USA). Antibodies against rat ferritin (Panapharm, Kumamoto, Japan), HO-1 and Klotho (a kind gift from Kyowa Hakko) were used at a dilution of 1:200. The ECL Western blotting system (Amersham Life Sciences, Arlington Heights, IL, USA) was used for detection. Bands were visualized using a lumino-analyzer (LAS-1000; Fuji Photo Film, Tokyo, Japan). Band intensity was calculated using the image analysis software, NIH Image (NIH, Research Service Branch), and was expressed as a percentage of the control value.

Statistical analysis

Data were expressed as the mean ± SEM. Results were presented as a percentage of the control value. A value of $P < 0.05$ was considered to be statistically significant.

Results

Physiological parameters and laboratory data

Compared to the age-matched untreated rats, rats that had been made diabetic by a single STZ injection weighed less and had higher systolic blood pressure, serum glucose and hemoglobin A_{1c} (Table 1). In

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Table 1 Physiological data

Variables	Untreated ($n = 6$)	STZ ($n = 12$)	STZ + LOS ($n = 12$)	STZ + DFO ($n = 9$)
Bodyweight (g)	401 ± 4*	238 ± 14	251 ± 16	255 ± 7
Systolic blood pressure (mmHg)	111 ± 5*	121 ± 7	121 ± 5	115 ± 2
Kidney weight (g)	1.38 ± 0.02*	1.60 ± 0.1	1.58 ± 0.06	1.59 ± 0.06
Serum fasting glucose (mg/dL)	154 ± 7*	529 ± 90	481 ± 98	501 ± 51
Hemoglobin A1c (%)	2.7 ± 0.1*	8.0 ± 0.6	8.1 ± 0.8	7.8 ± 0.4

*$P < 0.05$ vs streptozotocin (STZ) treatment alone group. DFO, deferoxamine; LOS, losartan.

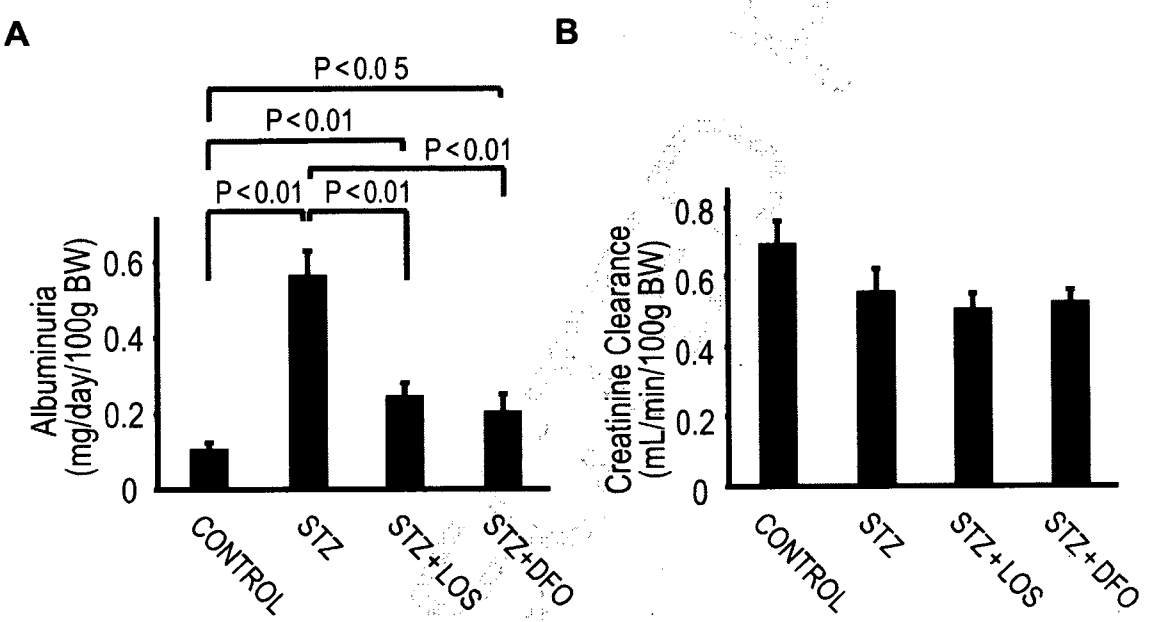

Figure 1 Effects of losartan (LOS) and deferoxamine (DFO) on the urinary excretion of [1] ~~otein and~~ albumin, and on creatinine clearance. (A) Urinary excretion of albumin. (B) Creatinine clearance. Bar graphs summarize data from 6–8 animals in each group.

diabetic rats, neither the AT_1 receptor blocker nor the iron chelator significantly affected these parameters. However, albuminuria was suppressed, although not completely, by either losartan or deferoxamine (Fig. 1a), although neither drug affected the creatinine clearance in diabetic rats (Fig. 1b).

Accumulation of iron

Prussian blue staining showed that iron deposition was not obvious in the kidneys of untreated rats (Fig. 2a), whereas the prominent deposition of iron could be observed in the tubular epithelial cells of STZ-induced diabetic rats (Fig. 2b). Furthermore, in these diabetic rats, iron accumulation was also found in the interstitial cells of the renal medulla, which were identified as ED-1-positive monocytes/macrophages (Fig. 2c,d). Expression of HO-1, a marker gene of oxidative stress, was also increased in the tubular epithelial and glomerular cells of the diabetic rats (Fig. 2e,f).

Effects of AT_1 receptor blockade and iron chelation

Both losartan and deferoxamine inhibited the accumulation of iron in the kidneys of diabetic rats (Fig. 3a–c). Protein expression of ferritin was markedly enhanced in the kidneys of diabetic rats, and was also found to be suppressed by losartan and deferoxamine (Fig. 3d,e).

Western blot analysis showed that the expression of ~~kl~~[2]otho protein was reduced, whereas that of HO-1

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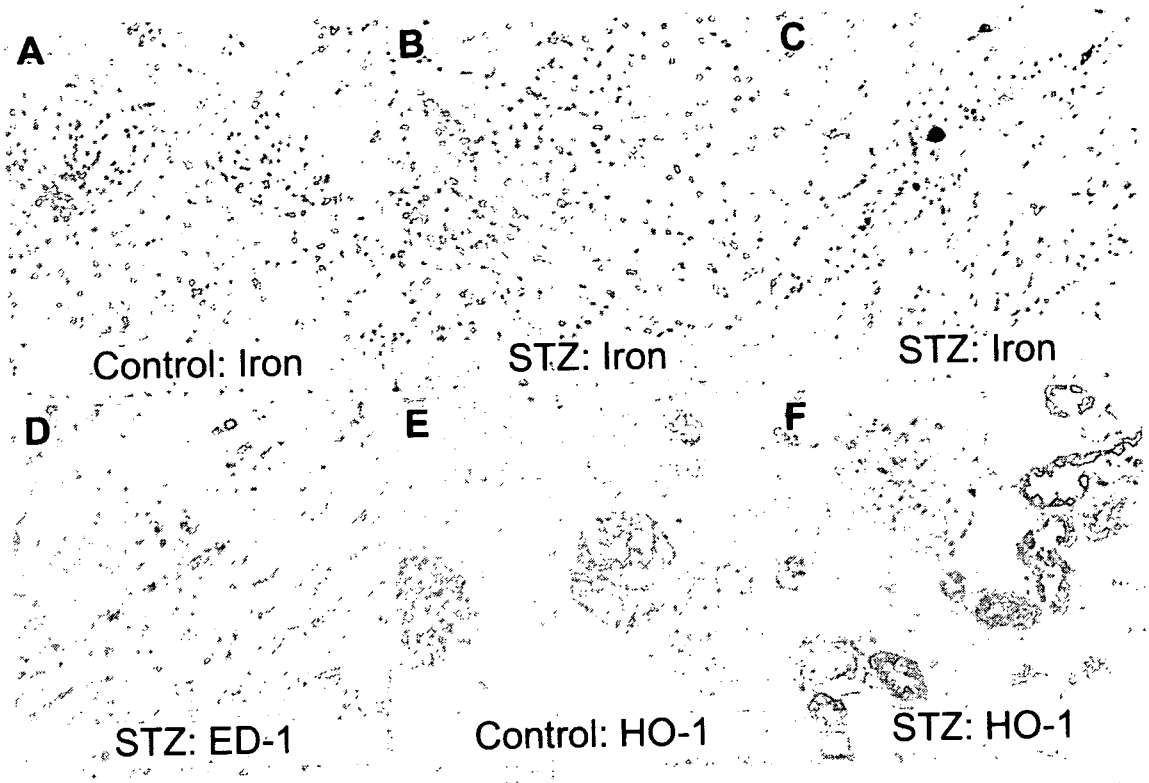

Figure 2 Accumulation of iron and induction of heme oxygenase-1 (HO-1) in diabetic kidneys. (A,E) Sections from control rats. (B–D,F) Sections from streptozotocin (STZ)-infused rats. (A–C) Iron staining. (D) ED-1 staining. (E,F) HO-1 staining. (C,D) Serial sections. In the kidneys of diabetic rats, accumulation of iron could be observed in (B) the tubular epithelial cells and (C) medullar interstitial cells, which were found to be (D) ED-1 positive. (Original magnification, ×200.)

was increased, at 4 and 8 weeks post-STZ injection (Fig. 4a). Downregulation of klotho and upregulation of HO-1 at 8 weeks post-STZ injection could be inhibited by losartan or deferoxamine (Fig. 4b–d). Quantitative RT-PCR showed that *klotho* mRNA expression was significantly reduced in STZ-infused rats, and that this was suppressed partially by losartan or deferoxamine (Fig. 4e).

Localization of klotho *mRNA*

In situ hybridization showed that *klotho* mRNA was expressed mainly in the cortical tubular epithelial and glomerular cells of the kidneys of untreated rats (Fig. 4a,b). At 8 weeks post-STZ injection, *klotho* mRNA expression became much weaker, although no apparent alteration in the localization was noted (Fig. 4d). The tubular cells that expressed *klotho* mRNA in the diabetic kidneys were found to have intense HO-1 expression (Fig. 5d,e).

Discussion

In the current study, we have demonstrated that the expression of *klotho* is reduced in the kidneys of STZ-induced diabetic rats. AT_1 receptor blockade and iron chelation suppressed this phenomenon without affecting the circulating glucose levels. It has been reported that AT_1 receptor blockade suppresses some of the unfavorable phenotypes seen in the kidneys of diabetic animals, such as increased proteinuria, reduced production of renal nitric oxide, and apoptosis of renal cells,[13–15] and, conversely, that these undesirable phenotypes can also be observed in the kidneys of rats which have received an angiotensin II infusion.[11,16,17] Considering that angiotensin II levels may be increased in kidneys of diabetic animals,[14] collectively, these findings indicate a pivotal role of angiotensin II, or alternatively the renin-angiotensin system, in the development of renal injury in diabetes. In a previous study, we have shown that the long-term administration of angiotensin II causes a

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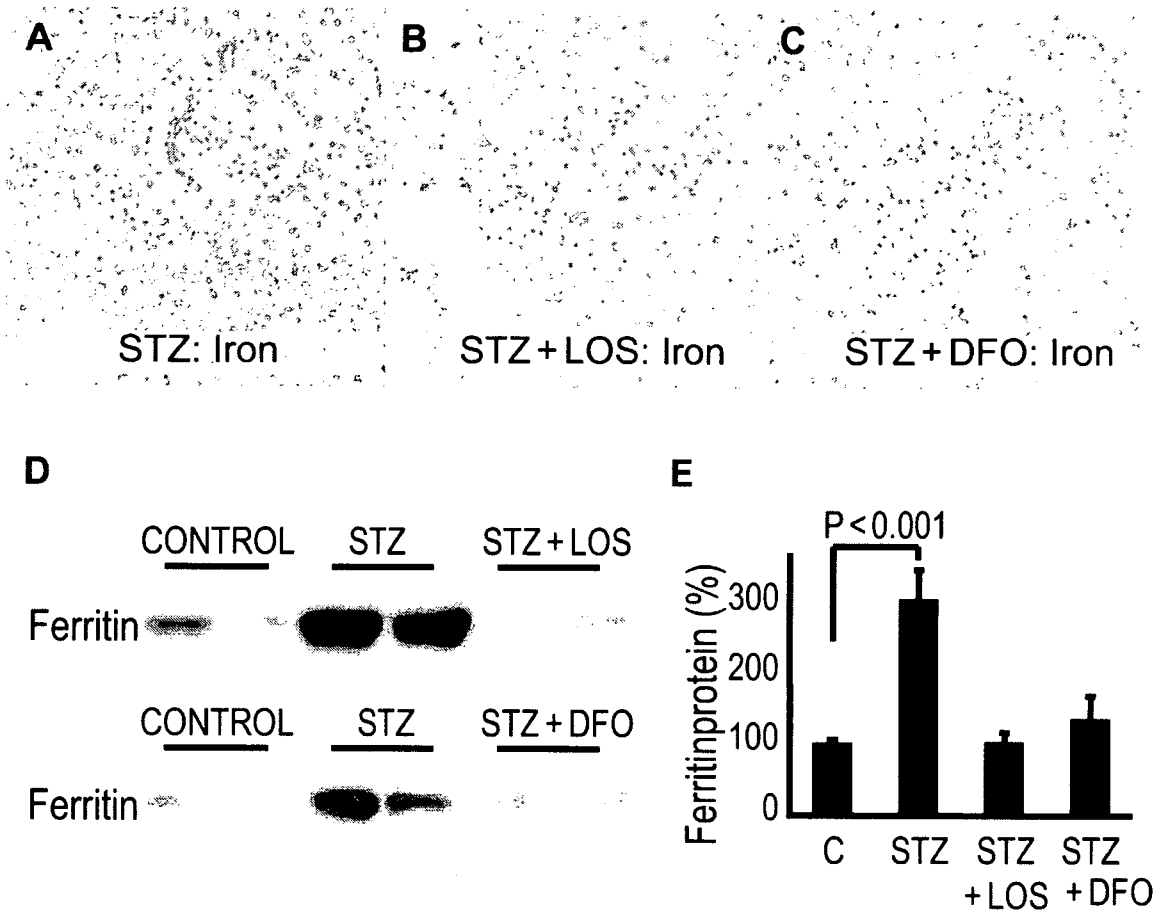

Figure 3 Effect of losartan (LOS) and deferoxamine (DFO) on the accumulation of iron and the induction of ferritin in the diabetic rat. The kidney sections were from rats given (A) STZ alone, (B) STZ plus LOS, and (C) STZ plus DFO. (D) Representative Western blot analysis of ferritin protein expression. (E) Summary of the data of ferritin protein from 4–6 experiments for each group.

decrease in the *klotho* expression in the rat kidney. In that study, either the administration of losartan, which reversed the downregulation of *klotho*, or transfer of exogenous *klotho* gene decreased proteinuria induced by the angiotensin II infusion, suggesting that preservation of *klotho* expression may have a physiological role in the kidneys of these animals.

Here we showed iron deposition in the tubular epithelial cells in the diabetic rats. Accumulation of iron, presumably originated from tubular fluid, in the kidney of STZ-induced diabetic rats was first described by Nankivell *et al.*[18] That iron was reabsorbed from the tubular fluid may explain the observed association between iron accumulation and proteinuria in the current and angiotensin II-infused animal models. It has also been reported that expression of iron transport proteins are regulated in the kidney of STZ-induced diabetic animals,[19] which might also play a role in accumulating iron in the kidney of diabetic animals. We found that administration of angiotensin II regulated several iron transporting proteins in mRNA levels;[20] therefore, whether or not AT_1 receptor blockade have any effect on expression of iron transporting proteins in the kidney of STZ-induced diabetic animals should be investigated in future studies.

Although the expression of *klotho* is known to be regulated in the kidney by various stimuli, not much is known about the underlying mechanism. The finding in the current study that losartan reversed the downregulation of *klotho* in diabetic rats may suggest that activation of the AT_1 receptor is involved in the downregulation of *klotho* expression in the kidneys of diabetic

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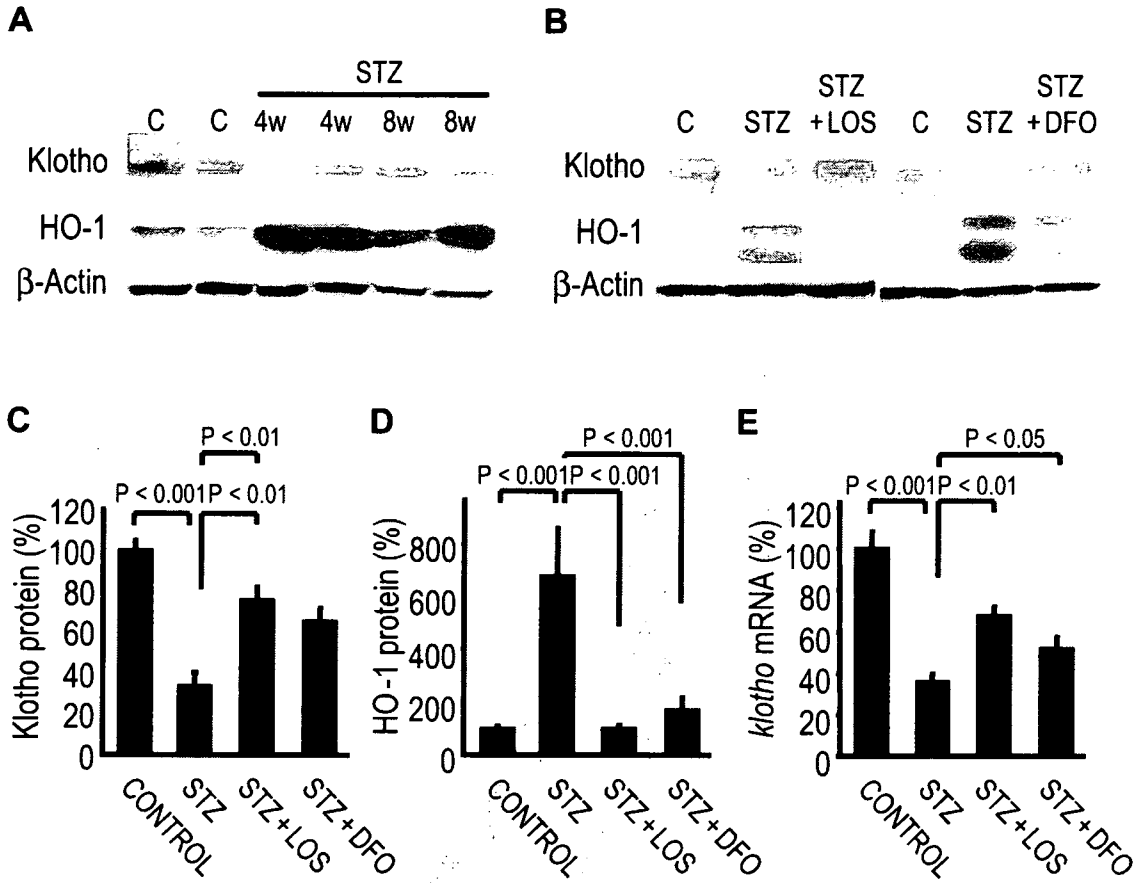

Figure 4 Effect of losartan (LOS) and deferoxamine (DFO) on the expression of *klotho* and heme oxygenase-1 (HO-1). (A) Representative Western blot analysis showing the time course of protein expression of klotho and HO-1. (B) Representative Western blot analysis showing the effects of LOS and DFO on the diabetes-induced regulation of HO-1 and klotho protein at 8 weeks post-STZ infusion. (C,D) Summary of the data from 4–6 experiments for each group. (E) Summary of the expression of *klotho* mRNA from 4–6 experiments for each group.

animals. Support for this scenario comes from the finding that angiotensin II-induced downregulation of *klotho* expression has also been observed in renal cells *in vitro*, where it is possibly dependent on the activation of RhoA.[21]

Enhanced oxidative stress may represent another possible mechanism that is involved in mediating *klotho* regulation in diabetes. Expression of *klotho* has been found to be downregulated by hydrogen peroxide *in vitro*.[22] It is of note that AT_1 blockade decreases the extent of *in vivo* oxidative stress in the angiotensin II-infused animals[23] as well as in the kidneys of STZ-induced diabetic rats.[24] It has been shown that expression of HO-1, an oxidative stress-sensitive gene, is upregulated in the kidney of STZ-induced diabetic animals.[25] Administration of an antioxidative agent suppressed this upregulation,[26] suggesting that, in these diabetic rats, HO-1 was induced in response to the enhanced oxidative stress. Furthermore, in the current study, deferoxamine, which would prevent the generation of reactive oxygen species,[27,28] also suppressed the downregulation of *klotho*. These findings are consistent with the notion that enhanced oxidative stress underlies the downregulation of *klotho* in diabetes.

The relationship between oxidative stress and aging is one of the main topical issues of today; however, little is known about the downstream signaling events that mediate the oxidative stress-induced downregulation of *klotho* expression. Recently, studies have shown that oxidative stress may activate Rho-kinase,[29] and that the regulation of expression plasminogen activator

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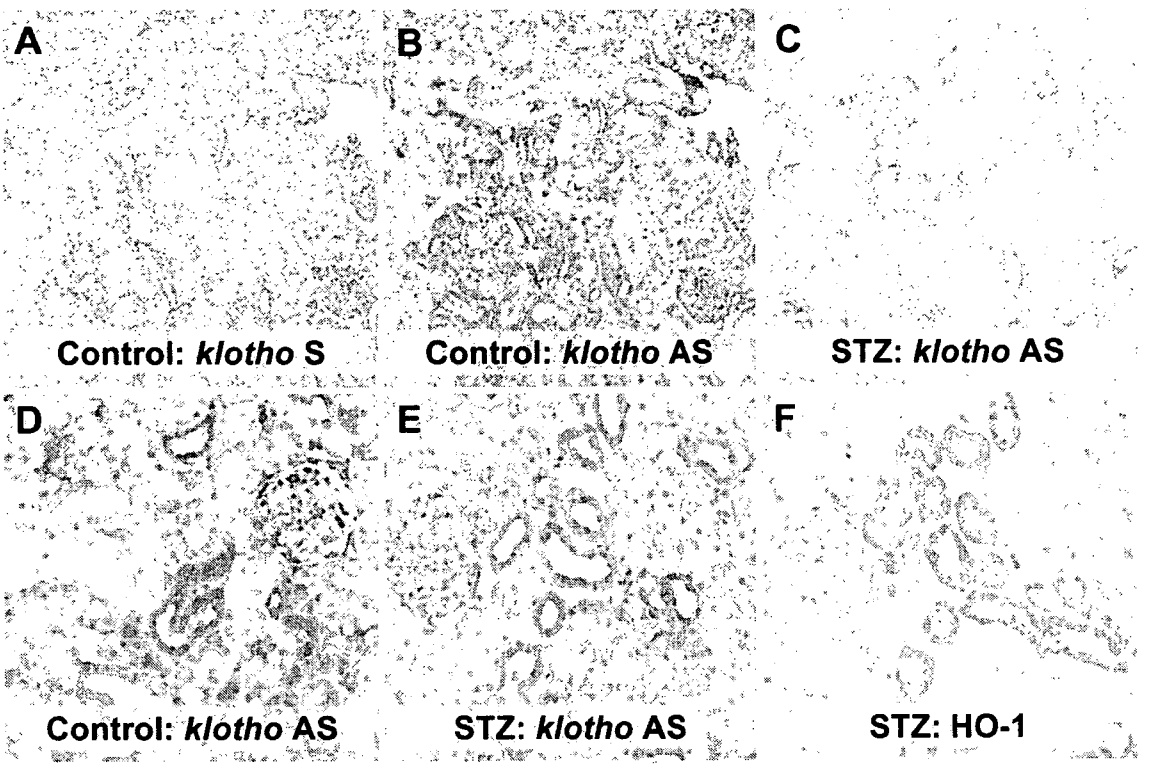

Figure 5 Localization of *klotho* mRNA in untreated and STZ-treated rat kidneys. (A,B,D) Sections from control rats. (C,E,F) Sections from STZ-infused rats. (A–B,E–F) Serial specimens. (B–E) *In situ* hybridization using *klotho* sense (background) and antisense. (F) HO-1 staining. Note that tubular epithelial cells weakly expressing *klotho* mRNA were positive for HO-1. (Original magnifications [A,B] ×100, [C–F] ×200.)

inhibitor-1 under hyperglycemic conditions may be mediated by Rho-kinase in an oxidative stress-dependent manner.[30] In addition, the inhibition of Rho-kinase resulted in an upregulation of the expression of ~~klotho~~ mRNA in cultured renal cells.[21] Taken together, ~~these results suggest~~ that the activation of the RhoA/Rho-kinase system might be one of the underlying mechanisms linking oxidative stress and the regulation of *klotho* expression. This should be investigated further in future studies.

Recent studies have shown that ~~kl~~tho protein may affect insulin and IGF1 signaling. In addition, it has been shown that circulating glucose levels are reduced in *klotho*-deficient mice,[1] and insulin levels are lower in ~~klotho~~-deficient animals whereas higher in ~~klotho~~-overexpressing animals,[4] suggesting that *klotho* expression is somehow related to insulin sensitivity. Future studies should investigate whether the expression of *klotho* is regulated according to circulating insulin levels, and whether, from the viewpoint of insulin action, the downregulation of *klotho* would act in a beneficial manner in STZ-induced diabetic animals, in which insulin secretion is decreased.

In conclusion, we have demonstrated that the expression of k~~l~~tho is downregulated in the kidneys of STZ-induced diabetic rats. This downregulation was suppressed by losartan or deferoxamine. Whether the downregulation of *klotho* expression is involved in the acceleration of aging-associated phenotypes in diabetes, such as arteriosclerosis and osteoporosis, needs to be elucidated in future studies.

Acknowledgements

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Original Article

Association between Chronic Kidney Disease and Carotid Intima-Media Thickening in Individuals with Hypertension and Impaired Glucose Metabolism

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We investigated whether chronic kidney disease (CKD) was associated with carotid intima-media thickening in 1,351 male individuals undergoing general health screening. Glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease equations using 0.881 as a coefficient for Japanese, and low estimated GFR (eGFR) was defined as an eGFR value of <60 mL/min/1.73 m^2. Albuminuria was defined as a urine albumin–to–urine creatinine ratio of ≥30 mg/g, and CKD was defined when low eGFR and/or albuminuria was present. After adjusting for age, CKD was associated with carotid intima-media thickening with an odds ratio of 1.47 (95% confidence interval [CI] 1.05–2.06, p=0.0024). After adjusting for age, fasting plasma glucose, and smoking status, both albuminuria and low eGFR were significantly associated with intima-media thickening in individuals with hypertension with an odds ratio of 1.85 (95% CI 1.13–3.03, p=0.015) and 1.79 (95% CI 1.09–2.94, p=0.022), respectively. On the other hand, neither of them was associated with carotid intima-media thickening in individuals without hypertension. Similarly, after adjusting for age, systolic blood pressure, and smoking status, both albuminuria and low eGFR were significantly associated with intima-media thickening in individuals with high fasting glucose (defined as fasting plasma glucose levels of ≥110 mg/dL or current use of anti-diabetic medication), but not in those without. Our data indicate that CKD or its components (low eGFR and albuminuria) may be associated with early carotid atherosclerosis in low-risk individuals, such as those undergoing general health screening, who have hypertension and/or impaired glucose metabolism. (*Hypertens Res* 2007; 30: 1035–1041)

***Key Words*: chronic kidney disease, carotid intima-media thickening, hypertension, risk factors, cross-sectional study**

Introduction

An increasing prevalence of end-stage renal disease that may require hemodialysis is a worldwide public health problem owing to poor outcomes and high costs. A mild decline in renal function may already be associated with a substantially higher prevalence of renal failure, coronary artery disease, arteriosclerosis, and premature death (*1*, *2*), and thus mild renal dysfunction has gathered more attention recently (*3*, *4*).

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Table 1. Clinical Characteristics and Laboratory Data

	No CKD (n=973)	Albuminuria (n=166)	Low eGFR (n=251)
Age (years)	56.0±10.3	61.8±10.5	62.5±9.3
Body mass index (kg/m^2)	24.1±2.9	24.8±3.1	24.1±2.6
Systolic blood pressure (mmHg)	130±18	139±20	130±19
Diastolic blood pressure (mmHg)	80±11	87±13	82±11
Laboratory data			
Serum urea nitrogen (mg/dL)	14.6±3.4	15.5±4.5	17.2±4.0
Serum creatinine (mg/dL)	0.8±0.1	0.9±0.2	1.1±0.1
Median (interquarilte range) of serum creatinine (mg/dL)	0.8 (0.8–0.9)	0.8 (0.8–1.0)	1.0 (1.0–1.1)
eGFR (mL/min/1.73 m^2)	72±8	68±13	55±5
Median (interquarilte range) of eGFR (mL/min/1.73 m^2)	71 (65–76)	68 (60–75)	56 (53–58)
Uric acid (mg/dL)	6.1±1.1	6.3±1.2	6.6±1.2
γ-GTP (IU/L)	57±52	73±84	54±47
Total cholesterol (mg/dL)	209±31	216±35	209±31
HDL-cholesterol (mg/dL)	55±13	54±14	54±12
Triglycerides (mg/dL)	138±99	151±122	133±95
Fasting glucose (mg/dL)	102±19	111±29	100±16
Haemoglobin A1c (%)	5.4±0.7	5.8±1.1	5.4±0.6
HOMA-IR	1.8±1.4	2.7±7.0	1.9±1.3
Smoking status			
Never/former/current (%)	30/43/27	25/52/23	37/47/16
Drinking status			
Never/former/current (%)	10/6/84	7/13/81	15/11/74

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; GTP, glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance.

According to the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (K/DOQI) criteria, chronic kidney disease (CKD) is defined as the presence of either of the following two conditions for three months or more: a glomerular filtration rate (GFR) of <60 mL/min/1.73 m^2; or kidney damage, as ascertained by the presence of proteinuria (*5*). The purpose of the current study was to investigate whether CKD or its components (decreased GFR and albuminuria) are associated with carotid atherosclerosis in Japanese men.

Methods

Study Subjects

The study was approved by the Ethical Committee of Mitsui Memorial Hospital. Between April 2005 and May 2006 at Mitsui Memorial Hospital, 6,351 men underwent a general health screen and fully responded a questionnaire concerning alcohol drinking and cigarette smoking. Of these 6,351 subjects, 1,351 underwent carotid ultrasonography as a part of the health screening, and were enrolled in the present study.

In Japan, regular health check-ups for employees are legally mandated, and all or most of the costs of the screening are usually paid either by the company to which a subject belongs or by the subject themselves. At our institute, several types of health screening programs are available, the choice of which is dependent on the decision of individuals and/or the companies to which they belong. Some courses of general health screening include carotid ultrasonography, while others do not. Therefore, it should be noted that the subjects enrolled may not be a random selection of all health screening participants. Indeed, among individuals who underwent general health screening during the study period, individuals who underwent carotid ultrasonography (n=1,351; *i.e.*, study subjects) were significantly older than those who did not (n=5,000) (58±10 and 53±10 years old, respectively, p<0.0001). Therefore, it could be said that there might have been some selection bias for participants planning carotid ultrasound. However, this was never the decision or the recommendation of any attending physician.

Laboratory Analysis

Blood samples were taken from the subjects after an overnight fast. Serum levels of total cholesterol (TC), high-density lipoprotein (HDL)-cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method, haemoglobin A1c was determined using the latex agglutination immunoas-

say, and creatinine was determined by the enzymatic method. Plasma glucose was measured by the hexokinase method and serum insulin was measured by enzyme immunoassay. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated in these individuals according to the following formula: HOMA-IR = [fasting immunoreactive insulin (μU/mL) × fasting plasma glucose (FPG; mg/dL)]/405. An increased insulin resistance was defined as a HOMA-IR of ≥2.5. Metabolic syndrome was defined as described previously (*6*).

Serum creatinine was calibrated using the following formula: serum creatinine (Jaffe method) = 0.2 + serum creatinine (enzyme method). GFR was estimated by equations from the simplified version of the Modification of Diet in Renal Disease (MDRD) (*7*), in which 0.881 is a coefficient for eGFR specific to the Japanese population (*8*): eGFR = 186.3 × (serum creatinine)$^{-1.154}$ × (age)$^{-0.203}$ × 0.881. Individuals were classified as having low eGFR when their eGFR values were <60 mL/min/1.73 m^2 (*5*). For the diagnosis of albuminuria, spot urine samples were collected and analyzed; albuminuria was expressed as the ratio of urinary albumin to urinary creatinine, designated as the albumin excretion index (AEI). Normoalbuminuria, microalbuminuria, and macroalbuminuria were defined as AEI <30 mg/g, 30–300 mg/g, and >300 mg/g, respectively. An eGFR of <60 mL/min/1.73 m^2 was designated as low eGFR, and an AEI ≥30 mg/g was designated as albuminuria. Individuals were said to have CKD when they had either or both of a low eGFR and albuminuria (*5*).

Carotid Ultrasonography

Carotid artery status was studied and analyzed as described previously (*9*). In brief, carotid artery status was assessed by high resolution B-mode ultrasonography, using a machine (Sonolayer SSA270A; Toshiba, Tokyo, Japan) equipped with a 7.5 MHz transducer (PLF-703ST; Toshiba). The carotid arteries were examined bilaterally at the levels of the common carotid, the bifurcation, and the internal carotid arteries from transverse and longitudinal orientations by trained sonographers. The intima-media thickness was measured using a computer-assisted method by experienced sonographers who were unaware of the subjects' clinical and laboratory findings. Carotid intima-media wall thickening was said to have occurred when the intima-media thickness measured at the far wall of the distal 10 mm of the common carotid artery was ≥1.0 mm. Carotid plaque was considered to be present when there was a portion of the artery for which the thickness of the intima-media complex was ≥1.1 mm (*10*) with a focal protrusion or point(s) of inflexion. The difference in the prevalence of carotid plaque in health screening participants between the current and previous studies (*9*) was likely due to the difference in diagnostic criteria for carotid plaque.

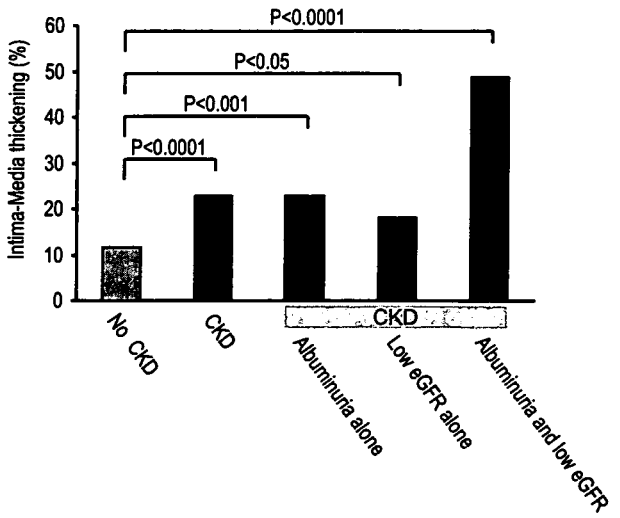

Fig. 1. *Prevalence of intima-media thickening in individuals with or without CKD or its components.*

Statistical Analysis

The data in this study were analyzed by the χ^2 test, ANOVA, and univariate and multivariate logistic regression analysis using computer software StatView ver. 5.0 (SAS Institute, Cary, USA). A value of $p<0.05$ was taken to be statistically significant. Results are expressed as the means±SD unless stated otherwise.

Results

Baseline Characteristics

The mean age±SD of the individuals enrolled was 57.7±10.5 years (Table 1). Of the 1,351 individuals examined, 166 (12%) had albuminuria: 142 (11%) had microalbuminuria and the remaining 24 (2%) had macroalbuminuria. Low eGFR was found in 251 individuals (19%), and 39 (3%) had both albuminuria and low eGFR. Therefore, 378 subjects (28%) were said to have CKD in our study population. If the coefficient for eGFR specific to the Japanese population (0.881) was not used for the calculation of eGFR, only 4.9% (66/1,351) of subjects were judged to have an eGFR of <60 mL/min/1.73 m^2. Individuals with albuminuria had a greater HOMA-IR value than those without CKD (Table 1). After adjusting for age and smoking status, logistic regression analysis showed that albuminuria was significantly associated with increased insulin resistance (*i.e.*, HOMA-IR of ≥2.5): the odds ratio was 2.18 (95% CI 1.52–3.13, $p<0.0001$) for all enrolled subjects, and 1.91 (95% CI 1.16–3.14, $p=0.011$) for individuals who had an FPG level of <126 mg/dL and were not taking antidiabetic medication ($n=1,096$). A positive association between low eGFR and increased insulin resistance was also observed; however, it did not reach statistical

Table 2. Logistic Regression Analysis for CKD or Its Components as Independent Variables and Carotid Intima-Media Thickening as a Dependent Variable According to Hypertension Status

	Odds ratio (95% CI) of CKD	*p* value	Odds ratio (95% CI) of albuminruia	*p* value	Odds ratio (95% CI) of low eGFR	*p* value
Whole (n=1,351)						
Unadjsted	2.26 (1.66–3.09)	<0.0001	2.81 (1.93–4.09)	<0.0001	2.00 (1.42–2.82)	<0.0001
Adjusted for age	1.47 (1.05–2.06)	0.024	2.07 (1.38–3.11)	0.0005	1.30 (0.90–1.88)	0.17
Adjusted for age, SBP, and smoking status	1.39 (0.99–1.95)	0.058	1.74 (1.14–2.65)	0.0098	1.34 (0.92–1.96)	0.13
Adjusted for age, SBP, FPG, and smoking status	1.38 (0.98–1.94)	0.065	1.64 (1.07–2.52)	0.023	1.40 (0.96–2.04)	0.085
Subjects with hypertension (n=563)						
Unadjsted	2.24 (1.47–3.42)	0.0002	2.56 (1.61–4.04)	<0.0001	2.13 (1.34–3.39)	0.0014
Adjusted for age	1.75 (1.13–2.72)	0.013	2.06 (1.27–3.34)	0.0034	1.67 (1.03–2.72)	0.038
Adjusted for age, FPG, and smoking status	1.71 (1.09–2.66)	0.019	1.85 (1.13–3.03)	0.015	1.79 (1.09–2.94)	0.022
Subjects without hypertension (n=788)						
Unadjsted	1.84 (1.13–2.99)	0.014	1.93 (0.90–4.14)	0.089	1.70 (1.00–2.90)	0.050
Adjusted for age	0.97 (0.57–1.67)	0.92	1.40 (0.61–3.23)	0.43	0.90 (0.50–1.62)	0.72
Adjusted for age, FPG, and smoking status	0.99 (0.57–1.70)	0.96	1.31 (0.56–3.05)	0.53	1.01 (0.49–2.11)	0.97

CKD, chronic kidney disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; FPG, fasting plasma glucose.

significance (odds ratio 1.45 [95% CI 0.97–2.25, p=0.068] for individuals who had an FPG level of <126 mg/dL and were not taking antidiabetic medication). After adjusting for age and smoking habits, albuminuria and low eGFR were each associated with metabolic syndrome with an odds ratio of 2.63 (95% CI 1.81–3.83, p<0.0001) and 1.54 (95% CI 1.07–2.20, p=0.020), respectively.

Association between CKD and Carotid Atherosclerosis

Intima-media thickening was more frequently found in the individuals with CKD than in those without (Fig. 1). The prevalence of intima-media thickening was more than two times greater in individuals with CKD than in those without. Age-adjusted logistic regression analysis showed that the odds ratios of no-CKD (n=973), low eGFR alone (n=212), albuminuria alone (n=127), and both low eGFR and albuminuria (n=39) were 1 (reference), 1.07 (95% CI 0.69–1.64, p=0.77), 1.60 (0.98–2.61, p=0.060), and 4.38 (2.13–8.99, p<0.0001), respectively. After adjusting for age, CKD was found to be associated with intima-media thickening. After adjustment for age, systolic blood pressure (SBP), FPG, and smoking status, albuminuria, but not low eGFR, was positively associated with intima-media thickening (Table 2). Neither CKD, albuminuria nor low eGFR was significantly associated with carotid plaque after adjusting for age (data not shown).

Association between CKD and Carotid Intima-Media Thickening According to Hypertension Status

Next, we assessed the association between CKD and carotid intima-media thickening after subdividing individuals according to their hypertension status. For this analysis, hypertension was defined as SBP ≥140 mmHg or diastolic blood pressure ≥90 mmHg or current use of an antihypertensive medication. Of the 1,351 enrolled subjects, 563 were considered to have hypertension. After adjusting for age, logistic regression analysis showed that CKD was associated with intima-media thickening in individuals with hypertension, but not in those without (Table 2). Similar results were obtained after further adjustment for FPG and smoking status; both albuminuria and low eGFR were significantly associated with intima-media thickening in individuals with hypertension, but, again, not in those without after adjusting for age, FPG, and smoking status,

Association between CKD and Intima-Media Thickening According to Glucose Metabolism

We then assessed the association between CKD and carotid intima-media thickening after subdividing individuals according to their fasting glucose levels. For this analysis, high fasting glucose was defined as FPG ≥110 mg/dL or current use of an antidiabetic medication. Of the 1,351 enrolled subjects, 251 had FPG ≥110 mg/dL, 50 were taking an antidiabetic medication, and 46 fell into both categories; therefore, 255 were considered to have high fasting glucose. Logistic regression analysis after adjusting for age, SBP, and smoking

Table 3. Logistic Regression Analysis for CKD or Its Components as Independent Variables and Carotid Intima-Media Thickening as a Dependent Variable According to Fasting Glucose Status

	Odds ratio (95% CI) of CKD	*p* value	Odds ratio (95% CI) of albuminruia	*p* value	Odds ratio (95% CI) of low eGFR	*p* value
Subjects with high fasting glucose (n=255)						
Unadjsted	2.55 (1.41–4.61)	0.0021	2.65 (1.40–5.02)	0.0029	2.82 (1.39–5.71)	0.0040
Adjusted for age	2.12 (1.15–3.92)	0.017	2.30 (1.19–4.46)	0.013	2.15 (1.03–4.49)	0.042
Adjusted for age, SBP, and smoking status	1.99 (1.06–3.72)	0.031	2.00 (1.01–3.95)	0.046	2.39 (1.11–5.14)	0.026
Subjects without high fasting glucose (n=1,096)						
Unadjsted	2.08 (1.44–3.01)	0.0001	2.45 (1.51–3.97)	0.0003	1.89 (1.26–2.83)	0.019
Adjusted for age	1.22 (0.82–1.84)	0.33	1.66 (0.97–2.83)	0.063	1.16 (0.75–1.81)	0.50
Adjusted for age, SBP, and smoking status	1.16 (0.77–1.75)	0.48	1.41 (0.81–2.43)	0.23	1.17 (0.75–1.83)	0.48

CKD, chronic kidney disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.

status showed that CKD, albuminuria, and low eGFR were each significantly associated with intima-media thickening in individuals with high fasting glucose, but not in those without (Table 3).

Association between CKD and Intima-Media Thickening According to Smoking Status

Logistic regression analysis after adjusting for age, SBP, and FPG showed that CKD was significantly associated with intima-media thickening in current smokers (n=337) with an odds ratio of 2.67 (95% CI 1.26–5.68, p=0.011) but not in former smokers (n=596, odds ratio 1.04 [95% CI 0.64–1.69, p=0.87]) or in never smokers (n=418, odds ratio 1.27 [95% CI 0.67–2.42, p=0.47]).

Discussion

We found that 28% of the male individuals undergoing general health screening in the present series had CKD (albuminuria, 12%: low eGFR, 19%: both, 3%). Several previous studies have reported on the prevalence of CKD in the general population in Japan. Ninomiya *et al.* reported that 11% (324/2,736) of subjects had an eGFR of less than 60 mL/min/1.73 m^2 at the beginning of their study, although the number of each gender was not specified (*11*). Nakamura *et al.* reported that the prevalence of CKD was 4.7% (146/3,047) in a randomly selected Japanese men (*12*). In these two studies, CKD was defined solely by the eGFR criterion. The greater prevalence of CKD in the current study is in part due to the fact that we included the albuminuria criterion for the diagnosis of CKD. Nevertheless, the prevalence of low eGFR itself seems to have been greater in the current study than in the previous reports. This is, at least in part, because we used 0.881 as a coefficient for Japanese to calculate an eGFR value specific to the Japanese population (*8*). Tanaka *et al.* have diagnosed CKD considering both albuminuria and low eGFR and reported that the prevalence of CKD in individuals included in a hospital-based registry was 13.7% (*13*); however, the coefficient for Japanese may not be used to estimate eGFR in their study. In the present study, when we estimated GFR without using the coefficient for Japanese, the prevalence of CKD was calculated to be 16.0%. Micro- and macroalbuminuria were found in 10.5% and 1.8%, respectively, of individuals in the current study. These values are comparable to those found in the general population in Japan by Konta *et al.* (13.7% and 1.7%, respectively) (*14*).

After adjusting for age, SBP, FPG, and smoking status, albuminuria was found to be associated with carotid intima-media thickening in the current study. An association between albuminuria and atherosclerotic diseases has been reported in a number of previous papers; albuminuria is known to be a predictor of cardiovascular mortality, although its capabilities may differ according to gender, race, and ethnicities (*15*, *16*), and albuminuria is an independent predictor for carotid intima-media thickness in diabetic (*17*) as well as non-diabetic (*18*) subjects. There have also been several studies that have assessed the possible association of low eGFR with carotid atherosclerosis in individuals who had only a mild decline in renal function, and in the general population. Preston *et al.* reported that common carotid artery intima-media thickness was greater in CKD patients. In their study, the mean GFR was 29.6±18.4 mL/min/1.73 m^2, which is much lower than that in the current study (*19*). Taniwaki *et al.* reported that decreasing GFR was significantly correlated with carotid intima-media thickness in diabetic patients (*20*), and this correlation may be independent of albuminuria status. In their study, the decrease in GFR was much milder than that in the study of Preston *et al.* (*19*): the mean GFR values were 127±26 mL/min/1.48 m^2 and 119±27 mL/min/1.48 m^2, respectively, in patients with and without microalbuminuria. In addition, by analyzing the data from a population-based survey, Rodondi *et al.* showed that carotid intima-media thickness increased with decreasing eGFR, although this association did not retain its statistical significance after adjusting for age (*21*).

Interestingly, albuminuria was associated with carotid intima-media thickening in individuals with hypertension, but not in those without. Similarly, the association between albuminuria and carotid intima-media thickening was statistically significant in individuals with high fasting glucose, but not in those without. In addition, in individuals with hypertension or high fasting glucose, not only albuminuria, but also low eGFR was significantly associated with intima-media thickening after multivariate adjustment. Previous studies showed that reduced GFR was an independent risk factor for worse cardiovascular outcomes in patients with hypertension (*22*, *23*) and diabetes (*24*). Our data suggest that a mild decline in GFR may also be a risk factor for early atherosclerosis in Japanese men with relatively low risk profiles, especially when individuals have hypertension and diabetes, which is agreement with the previous findings (*25*). As CKD was associated with carotid intima-media thickening in current smokers but not in former smokers or nonsmokers in the current study, it is possible that the presence of CKD might increase the prevalence of early carotid atherosclerosis when some atherogenic risk factors are present.

We also showed that CKD was associated with metabolic syndrome, although we cannot determine a causal or resultant relationship. Ninomiya *et al.* have reported in their Hisayama Study that metabolic syndrome was an independent risk factor for CKD based on the 5-year cumulative data for the disease (*11*), suggesting that the clustering of hemodynamic/metabolic syndrome may be a reason for the increase in CKD in the general population. Whether the clustering of the hemodynamic/metabolic risk factors can explain the observed association between CKD and carotid intima-media thickening should be analyzed in future studies.

In conclusion, albuminuria, low eGFR, and CKD were found in 12%, 19%, and 28%, respectively, of male individuals undergoing general health screening in the present study. CKD was found to be a risk factor for carotid intima-media thickening after the adjustment for age, SBP, FPG, and smoking status. Both albuminuria and low eGFR were significantly associated with carotid intima-media thickening in individuals with hypertension and in those with high fasting glucose, but not in those without either condition. To what extent maintaining blood pressure and/or plasma glucose levels within a preferable range suppresses the future development of carotid atherosclerosis in CKD patients would be a meaningful question to be addressed in future longitudinal studies.

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Original Article

Association between Obesity and Chronic Kidney Disease in Japanese: Differences in Gender and Hypertensive Status?

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Obesity is a known risk factor for hypertension and diabetes, both of which ultimately promote renal dysfunction. In the current study, we investigated the association between body mass index (BMI) and chronic kidney disease (CKD) in 8,168 Japanese individuals (2,924 women, 5,244 men) who underwent general health screening. CKD was diagnosed if the estimated glomerular filtration rate (eGFR) was less than 60 mL/min/1.73 m^2 (designated as low eGFR) and/or if the urinary albumin/creatinine value was equal to or greater than 30 mg/g (designated as albuminuria). Logistic regression analysis adjusted for age, systolic blood pressure, fasting glucose, and smoking habits showed that, in men, both overweight (BMI 25–29 kg/m^2) and obesity (BMI ≥30 kg/m^2) were associated with increased prevalence of low eGFR and albuminuria, whereas, in women, obesity was associated with albuminuria, but neither overweight nor obesity was associated with low eGFR. After multivariate adjustment, logistic regression analysis showed that BMI had a graded association with both low eGFR and albuminuria in men. On the other hand, in women, the second and third BMI quartiles were associated with a lower prevalence of albuminuria in comparison with the first BMI quartile. Essentially the same results were obtained when the subjects were subdivided according to the presence and absence of hypertension. Our data showed that overweight and obesity were associated with increased risk for CKD in Japanese individuals undergoing a general health screening, irrespective of the presence or absence of hypertension, although there was a gender difference in these associations. (*Hypertens Res* 2007; 30: 1059–1064)

***Key Words*: obesity, chronic kidney disease, gender difference, hypertension**

Introduction

As in Western countries (*1*, *2*), the prevalence of obesity among Japanese is increasing, especially in men and women older than 40 years of age (*3*). Several cross-sectional and longitudinal epidemiological studies have shown that obesity may increase the prevalence and incidence of chronic kidney disease (CKD) (*4–8*). Care must be taken in interpreting these data, since several of the studies have diagnosed CKD based on an estimated glomerular filtration rate (eGFR) lower than a certain cutoff value, while others have defined CKD based on either low eGFR or the presence of albuminuria/proteinuria. Nevertheless, it is of note that one study found a gender-related difference in the association between obesity and CKD (*9*), whereas other reports did not (*4*, *5*). Obesity

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increases renal sodium reabsorption, which results in an elevation of blood pressure (*6*, *10*, *11*) and may explain the observed link between obesity and CKD, as hypertension is one of the most important factors associated with the progression of both diabetic and nondiabetic CKD (*7*). On the other hand, Kramer *et al.* noted a significant association between obesity and the risk for CKD, defined as ≥1+ proteinuria and/or eGFR <60 mL/min/1.73 m^2, in a cohort of hypertensive adults, which was statistically significant after adjustment for blood pressure and diabetes (*8*). This finding suggested that obesity and overweight increase the incidence of CKD independent of the degree of hypertension.

In the current study, we sought to investigate whether body mass index (BMI) was associated with CKD and its components, which are low eGFR and albuminuria, in Japanese individuals who underwent general health screening, and whether the mode of this association, if present, differed according to gender and hypertensive status.

Methods

Study Population

The study was approved by the Ethical Committee of the Mitsui Memorial Hospital. Between April 2004 and August 2006, 12,535 individuals (4,481 women and 8,054 men) undergoing a general health screen at this institute, including an estimation of urinary excretion of albumin, and who completed a questionnaire concerning the amount and duration of their alcohol consumption, were enrolled in the present study. In Japan, regular health check-ups for employees are a legal requirement; all or most of the costs of the screening are paid for either by the place of employment or by the subjects themselves. Ideal BMI, overweight, and obesity were defined as BMI <25 kg/m^2, BMI 25–29 kg/m^2, and BMI ≥30 kg/m^2, respectively.

Laboratory Analysis

Blood samples were taken from the subjects after an overnight fast. Serum levels of total cholesterol (TC), high-density lipoprotein (HDL)-cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically. Serum uric acid (UA) was measured by the uricase-peroxidase method, hemoglobin A1c was determined using the latex agglutination immunoassay, and creatinine was determined by the enzymatic method. Plasma glucose was measured by the hexokinase method and serum insulin was measured by enzyme immunoassay. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated in these individuals according to the following formula: HOMA-IR = [fasting immunoreactive insulin (μU/mL) × fasting plasma glucose (FPG; mg/dL)]/405. Metabolic syndrome was said to be present when three or more of the following conditions were met: TG levels ≥150 mg/dL; HDL-C levels <40 mg/dL; FPG levels ≥110 mg/dL or current use of antidiabetic medication; systolic blood pressure (SBP) ≥130 mmHg or diastolic blood pressure (DBP) ≥85 mmHg or current use of antihypertensive medication; BMI ≥25 kg/m^2.

Table 1. Baseline Characteristics

	Women (n=2,924)	Men (n=5,244)
Age (years)	52.0±10.7	53.8±10.5
Waist circumferrence (cm)	76.8±9.0	85.8±7.8
Body mass index (kg/m^2)	21.2±3.0	23.7±2.9
Systolic blood pressure (mmHg)	117±19	125±19
Diastolic blood pressure (mmHg)	73±12	79±11
Lipid data		
LDL-cholesterol (mg/dL)	127±33	129±31
HDL-cholesterol (mg/dL)	68±15	55±13
Triglycerides (mg/dL)	85±47	133±89
Glucose metabolism		
Fasting glucose (mg/dL)	90±15	100±21
Hemoglobin A1c (%)	5.2±0.5	5.4±0.8
HOMA-IR	1.2±0.9	1.7±1.3
Metabolic syndrome (n (%))	103 (3.5)	744 (14.2)
Renal data		
Serum urea nitrogen (mg/dL)	13.6±3.5	14.6±3.5
Serum creatine (mg/dL)	0.63±0.24	0.86±0.20
eGFR (mL/min/1.73 m^2)	69±10	70±10
Urinary albumin/creatinine ratio (mg/g)	18±68	26±182
Smoking status		
Non-smokers (n (%))	2,510 (86)	1,743 (33)
Former smokers (n (%))	177 (6)	1,893 (36)
Current smokers (n (%))	243 (8)	1,608 (31)

LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; eGFR, estimated glomerular filtration rate.

Estimated Glomerular Filtration Rate, Albuminuria, and Definition of CKD

Serum creatinine was calibrated using the following formula: serum creatinine (Jaffe method) = 0.2 + serum creatinine (enzyme method). Serum creatinine was measured in mg/dL and age in years; GFR was estimated using the equation from a simplified version of the Modification of Diet in Renal Disease (MDRD) (*12*) as follows: eGFR (mL/min/1.73 m^2) = $186.3 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times 0.881$ (× 0.742 if female). In this MDRD formula, 0.881 is a coefficient for eGFR specific to the Japanese population (*13*). An eGFR of <60 mL/min/1.73 m^2 was designated as low eGFR. For the diagnosis of albuminuria, spot urine samples were collected and analyzed; albuminuria was considered to be present when the urinary albumin excretion ratio (UAER) was ≥30 mg/g-creatinine. Normoalbuminuria, microalbuminuria, and macroalbuminuria were defined as an UAER of <30 mg/g, 30–

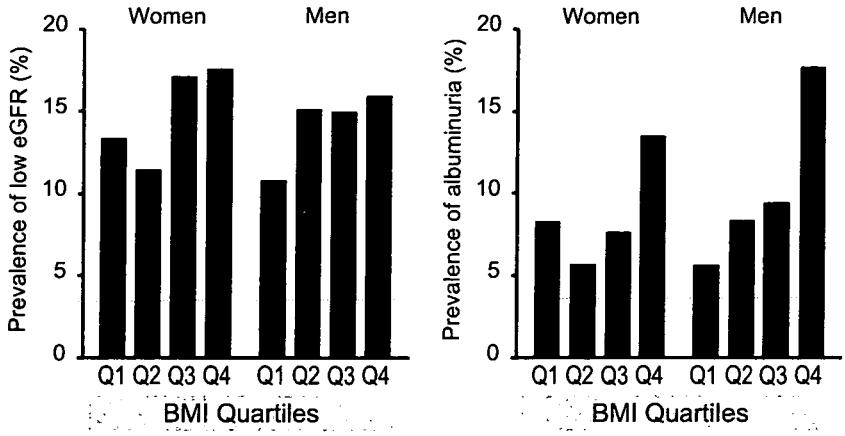

Fig. 1. *Prevalence of low eGFR and albuminuria according to BMI quartiles.*

299 mg/g, and 300 mg/g, respectively (*14*). Individuals were said to have CKD when they had either or both of low eGFR and albuminuria (*15*).

Statistical Analysis

The data in this study were analyzed by multivariate logistic regression analysis using computer software, StatView ver. 5.0 (SAS Institute, Cary, USA). A value of $p<0.05$ was taken to be statistically significant. Results are expressed as the means±SD unless stated otherwise.

Results

Baseline Characteristics

Characteristics of the enrolled subjects are shown in Table 1. The numbers of women with overweight and obesity were 254 (8.7%) and 32 (1.1%), respectively, and the numbers in men were 1,361 (26.0%) and 147 (2.8%), respectively.

Among women, 432 (14.8%) had low eGFR, 254 (8.7%) had albuminuria, and 55 (1.9%) had both low eGFR and albuminuria. Therefore, 631 (21.6%) women were considered to have CKD. In men, low eGFR was found in 739 (14.1%) and albuminuria in 535 (10.2%), and both low eGFR and albuminuria were present in 122 (2.3%). Therefore, 1,152 men (22.0%) were considered to have CKD. Micro- and macroalbuminuria were found in 236 (8.1%) and 18 (0.6%) women, respectively, and in 467 (8.9%) and 68 (1.3%) men, respectively. Pearson's correlation coefficients for the relationship between BMI and UA were 0.29 ($p<0.0001$) in women and 0.23 ($p<0.0001$) in men.

The interquartile cutoff values for BMI were 19.2, 20.7, and 22.8 kg/m^2 in women and 21.9, 22.5, and 25.3 kg/m^2 in men. The correlation coefficient between WC and BMI was 0.79 in women and 0.84 in men.

Association among BMI, Overweight, Obesity and CKD

In both genders, the prevalence of low eGFR and that of albuminuria was greatest in the highest BMI quartile (Fig. 1). Age-adjusted logistic regression analysis showed that women in the second and third BMI quartiles had a lower prevalence of CKD and albuminuria after adjustment for age, SBP, FPG, and smoking status. On the other hand, in men, the prevalence of CKD and albuminuria was increased according to BMI in the multivariate adjusted model. BMI showed a graded association with low eGFR in men (Table 2). When data on only male never smokers ($n=1,743$) were analyzed, the first, second, third, and fourth BMI quartiles were associated with low eGFR with an odds ratio of 1 (referent), 1.21 (95% confidence interval [CI] 0.83–1.77, $p=0.33$), 1.18 (95% CI 0.80–1.74, $p=0.41$), and 1.76 (95% CI 1.16–2.67, $p=0.0077$), respectively.

We then analyzed the association between overweight and obesity and CKD components in a logistic regression analysis after adjusting for age, SBP, FPG, and smoking status; the ideal BMI was used as the reference (Table 3). Both overweight and obesity were associated with low eGFR in men, but again not in women. Obesity in women and both overweight and obesity in men were associated with albuminuria.

Association between BMI and CKD Components in Hypertensive and Non-Hypertensive Subjects

We next evaluated whether hypertension modified the association between BMI and CKD. Hypertension was found in 502 (17.2%) of the women and 1,658 (31.6%) of the men. The mode of association between BMI and CKD (and its components) was similar between hypertensive and non-hypertensive individuals (Table 4). In women without hypertension, individuals in the second BMI quartile had significantly lower prevalence of low eGFR when compared with those in the

Table 2. Association between Body Mass Index and CKD and Its Components

	Odds raio (95% CI)		Odds raio (95% CI)		Odds raio (95% CI)	
	For CKD	*p* value	For low eGFR	*p* value	For albuminuria	*p* value
Women						
Model 1						
BMI-Q1	1	—	1	—	1	—
BMI-Q2	0.63 (0.48–0.84)	0.0017	0.69 (0.50–0.97)	0.032	0.61 (0.40–0.92)	0.020
BMI-Q3	0.86 (0.66–1.13)	0.28	0.93 (0.68–1.27)	0.64	0.76 (0.51–1.11)	0.16
BMI-Q4	0.99 (0.76–1.29)	0.96	0.86 (0.63–1.18)	0.36	1.39 (0.98–1.96)	0.065
Model 2						
BMI-Q1	1	—	1	—	1	—
BMI-Q2	0.60 (0.45–0.80)	0.0004	0.72 (0.51–1.01)	0.055	0.48 (0.32–0.74)	0.0009
BMI-Q3	0.80 (0.61–1.05)	0.10	0.99 (0.72–1.36)	0.94	0.57 (0.38–0.85)	0.0062
BMI-Q4	0.82 (0.62–1.09)	0.17	1.01 (0.72–1.41)	0.97	0.73 (0.50–1.07)	0.11
Men						
Model 1						
BMI-Q1	1	—	1	—	1	—
BMI-Q2	1.50 (1.22–1.85)	0.0001	1.40 (1.11–1.78)	0.0055	1.45 (1.06–1.98)	0.019
BMI-Q3	1.56 (1.27–1.92)	<0.0001	1.44 (1.13–1.83)	0.0028	1.71 (1.27–2.32)	0.0005
BMI-Q4	2.66 (2.18–3.25)	<0.0001	1.71 (1.35–2.17)	<0.0001	3.81 (2.88–5.03)	<0.0001
Model 2						
BMI-Q1	1	—	1	—	1	—
BMI-Q2	1.41 (1.14–1.74)	0.0016	1.43 (1.12–1.82)	0.040	1.27 (0.92–1.75)	0.16
BMI-Q3	1.38 (1.11–1.70)	0.0032	1.47 (1.15–1.88)	0.0019	1.32 (0.96–1.82)	0.088
BMI-Q4	2.05 (1.66–2.54)	<0.0001	1.82 (1.42–2.34)	<0.0001	2.26 (1.68–3.06)	<0.0001

Model 1: adjusted for age. Model 2: adjusted for age, systolic blood pressure, fasting glucose and smoking habits. BMI, body mass index; CKD, chronic kidney disease; CI, confidence interval; eGFR, estimated glomerular filtration rate.

first BMI quartile, and individuals in the second or third BMI quartiles had significantly lower prevalence of albuminuria when compared with those in the first BMI quartile.

Discussion

In this cross-sectional study, we investigated the association between BMI and CKD components in individuals who underwent general health screening. BMI had a graded association with CKD, low eGFR, and albuminuria in men, whereas women in the second and the third BMI quartiles had a significantly lower prevalence of CKD and albuminuria. Modes of association between BMI and CKD (and its components) were similar between hypertensive and non-hypertensive individuals, especially in men.

Several previous studies have examined the association between BMI and CKD. In a community-based longitudinal cohort study in the U.S., BMI was found to be related to the development of kidney disease (*16*). Kramer *et al.* reported that overweight (BMI 25–29.9 kg/m^2) and obesity (BMI ≥30 kg/m^2) were associated with CKD, defined as the presence of 1+ or greater proteinuria and/or eGFR <60 mL/min/1.73 m^2, in hypertensive subjects, and that this association remained statistically significant even after adjustment for blood pressure and diabetes (*8*). Gelber *et al.* reported that BMI was associated with low eGFR in a 14-year follow-up of apparently healthy men (*17*).

In the current study, we found a gender difference in the association between BMI and low eGFR: an increased BMI was a risk factor for low eGFR only in men, but not in women. Iseki *et al.* reported in their 17-year cohort study that elevations in BMI had a graded association with the cumulative incidence of end-stage renal disease (ESRD) compared with individuals with a baseline BMI of <21 kg/m^2 (*9*); however, this association was only found in men. In the same study, Iseki *et al.* suggested that this gender difference might be partly attributable to a difference in the prevalence of cigarette smoking (*9*). This was because cigarette smoking, prevalence of which is higher in men, is a risk factor for the ESRD and proteinuria (*18*, *19*).

It is possible that the difference in the mode of association between BMI and low eGFR (or albuminuria) between genders was attributable to the usage of sex-specific quartiles, which resulted in different cutoff values between genders. And in fact, when we used the same cutoff values, the association between obesity and albuminuria was observed in both genders with similar odds ratios (Table 4). It should be noted, however, that overweight and obesity were associated with low eGFR in men, but again, not in women (Table 4). Thus, this concept could not explain the gender difference in the

Table 3. Association between Overweight, Obesity, and CKD and Its Components

	Odds ratio (95% CI)		Odds ratio (95% CI)		Odds ratio (95% CI)	
	For CKD	*p* value	For low eGFR	*p* value	For albuminuria	*p* value
Women						
Ideal BMI	1	—	1	—	1	—
Overweight	1.00 (0.72–1.39)	>0.99	1.06 (0.72–1.55)	0.78	1.07 (0.71–1.61)	0.74
Obesity	2.20 (0.98–4.96)	0.056	0.27 (0.03–2.09)	0.21	2.74 (1.20–6.27)	0.017
Men						
Ideal BMI	1	—	1	—	1	—
Overweight	1.56 (1.33–1.82)	<0.0001	1.44 (1.20–1.73)	0.0001	1.70 (1.38–2.08)	<0.0001
Obesity	2.77 (1.91–4.02)	<0.0001	2.14 (1.32–3.47)	0.0021	2.72 (1.77–4.17)	<0.0001

Odds ratios were obtained after adjusting for age, systolic blood pressure, fasting glucose and smoking habits. CKD, chronic kidney disease; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate.

Table 4. Association between Body Mass Index and CKD and Its Components According to Hypertensive Status

	Odds raio (95% CI)		Odds raio (95% CI)		Odds raio (95% CI)	
	For CKD	*p* value	For low eGFR	*p* value	For albuminuria	*p* value
Women						
HT (+)						
BMI-Q1	1	—	1	—	1	—
BMI-Q2	0.73 (0.32–1.63)	0.44	1.17 (0.43–3.18)	0.75	0.75 (0.30–1.87)	0.53
BMI-Q3	0.82 (0.38–1.78)	0.61	1.13 (0.43–2.94)	0.81	0.79 (0.33–1.92)	0.61
BMI-Q4	1.08 (0.53–2.22)	0.83	1.34 (0.55–3.26)	0.52	1.16 (0.53–2.57)	0.71
HT (−)						
BMI-Q1	1	—	1	—	1	—
BMI-Q2	0.60 (0.44–0.81)	0.0011	0.67 (0.47–0.97)	0.033	0.45 (0.27–0.74)	0.0017
BMI-Q3	0.85 (0.63–1.14)	0.28	1.01 (0.72–1.41)	0.97	0.57 (0.36–0.91)	0.018
BMI-Q4	0.80 (0.57–1.10)	0.17	1.00 (0.69–1.46)	1.00	0.61 (0.38–1.00)	0.052
Men						
HT (+)						
BMI-Q1	1	—	1	—	1	—
BMI-Q2	1.36 (0.91–2.01)	0.13	1.45 (0.90–2.35)	0.13	1.19 (0.72–1.96)	0.49
BMI-Q3	1.56 (1.06–2.28)	0.023	1.78 (1.13–2.82)	0.014	1.28 (0.79–2.07)	0.32
BMI-Q4	1.90 (1.32–2.74)	0.0006	1.81 (1.15–2.83)	0.0097	1.86 (1.18–2.91)	0.0071
HT (−)						
BMI-Q1	1	—	1	—	1	—
BMI-Q2	1.43 (0.11–1.85)	0.0053	1.45 (0.19–1.93)	0.010	1.19 (0.77–1.83)	0.44
BMI-Q3	1.25 (0.96–1.64)	0.099	1.32 (0.98–1.79)	0.070	1.14 (0.74–1.78)	0.55
BMI-Q4	2.13 (1.61–2.81)	<0.0001	1.87 (1.35–2.61)	0.0002	2.20 (1.44–3.34)	0.0002

Odds ratios were obtained after adjusting for age, systolic blood pressure, fasting glucose and smoking habits. BMI, body mass index; CKD, chronic kidney disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; HT (+), hypertensive; HT (−), non-hypertensive.

association between obesity and low eGFR in both genders. A study in the U.S. showed a relationship between obesity and nephrosclerosis in women only (*20*), and a study in Norway showed an association between obesity (BMI ≥30 kg/m^2) and decreased eGFR (eGFR <45 mL/min/1.73 m^2) in both genders. Together with these previous findings, our data suggested that the gender difference in the mode of association between BMI and CKD might differ according to racial groups and ethnicities. On the other hand, recent studies analyzing the data of Japanese hypertensive patients showed that the mode of association between UA and left ventricular hypertrophy (LVH) (*21*), and that between insulin resistance and LVH (*22*) differed according to gender. Considering the possible association between CKD and LVH (*23*), it is possible that the gender difference in the association between BMI and CKD components that was observed in the current study