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**Chronic Kidney Disease Is Associated with Vascular Smooth Muscle Dysfunction
But Not with Endothelial Dysfunction**

Brief title: Vascular Function in Chronic Kidney Disease

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Disclosures

None.

Key words: chronic kidney disease, flow-mediated vasodilation, endothelial function, nitroglycerine-induced vasodilation, vascular smooth muscle dysfunction

Abstract

Backgrounds: Nitroglycerine-induced vasodilation (NID) is usually assessed as a control test for flow-mediated vasodilation (FMD). However, NID per se is impaired in patients with high cardiovascular risk. The purpose of this study was to investigate the associations of chronic kidney disease (CKD) with NID and FMD.

Methods: We measured NID and FMD in a total of 1567 adult subjects without end-stage renal disease (ESRD), 28% of whom had CKD as judged by measurements of estimated glomerular filtration rate (995 men and 572 women; mean age, 59.0 ± 16.9 years; age range, 18 to 92 years).

Results: NID was significantly smaller in patients with CKD than in those without CKD ($10.8 \pm 6.0\%$ vs. $12.7 \pm 5.7\%$, $P < 0.001$). The prevalence of vascular smooth muscle dysfunction, defined as NID of less than the division point for the lowest quartile, was significantly higher in patients with CKD than in those without CKD (37.5% vs. 21.5% , $P < 0.001$). Multivariate analysis revealed that CKD was independently associated with vascular smooth muscle dysfunction (OR: 1.36, 95% CI: 1.02 to 1.81, $P = 0.04$). FMD was significantly smaller in patients with CKD than in those without CKD ($3.1 \pm 2.8\%$ vs. $4.0 \pm 3.0\%$, $P < 0.001$). The prevalence of endothelial dysfunction, defined as FMD of less than the division point for the lowest quartile, was significantly higher in patients with

CKD than in those without CKD (31.7% vs. 23.1%, $P=0.002$). However, CKD was not independently associated with endothelial dysfunction in an age- and sex-adjusted model (OR: 0.95, 95% CI: 0.71 to 1.26, $P=0.72$).

Conclusions: Non-ESRD CKD is independently associated with vascular smooth muscle dysfunction but not with endothelial dysfunction.

Introduction

Endothelial dysfunction is an initial step in the process of atherosclerosis and plays a critical role in the development of this condition, leading to cardiovascular complications.[1] In addition, endothelial function has been shown to be an independent predictor of cardiovascular events.[2, 3] Therefore, there is considerable research interest in the assessment of endothelial function for risk stratification in subjects with cardiovascular risk factors. Recently, flow-mediated vasodilation (FMD), a vascular dilatory response of the brachial artery to reactive hyperemia, has been widely used for the assessment of endothelial function in humans.[4-7] Nitroglycerine-induced vasodilation (NID), a vascular dilatory response of the brachial artery to administered nitroglycerine, has been used as a control test for FMD measurement to confirm that the vascular dilatory response to reactive hyperemia is not affected by underlying vascular smooth muscle dysfunction or vascular structural alterations but is truly a consequence of endothelium-dependent vasodilation.[8, 9] However, recent studies have shown that NID per se is impaired in patients with multiple cardiovascular risk factors or a history of cardiovascular disease (CVD).[10-12] Moreover, we recently reported that impaired NID is associated with a higher incidence of cardiovascular events.[13] These findings suggest that NID in the brachial artery can be used not only as a diagnostic marker but

also as a prognostic marker of atherosclerosis.

Chronic kidney disease (CKD) is an independent predictor of cardiovascular events. It has been reported that patients with end-stage renal disease (ESRD) have a much higher incidence of cardiovascular death than that in the general population and that cardiac disease is the leading cause of death in patients on chronic dialysis.[14-16] Recent studies have also shown that non-ESRD CKD is independently associated with an increased risk of cardiovascular mortality and cardiovascular events.[17, 18] It has been shown that endothelial function assessed by FMD in the brachial artery is significantly impaired in patients with CKD, especially in patients with ESRD, compared with that in subjects without CKD, although it is controversial whether CKD is independently associated with FMD.[19-23] As for the relationship between CKD and NID, it has been shown that NID is significantly impaired in patients with ESRD compared with that in subjects without ESRD. However, the relationship between non-ESRD CKD and NID has not been fully investigated. Although measurement of NID in patients with non-ESRD CKD was performed as a control test in several studies, previous studies were limited to small numbers of or highly selected subjects.[19-24] In addition, it has not been determined whether non-ESRD CKD is independently associated with NID. We therefore investigated the relationship between non-ESRD

CKD and NID in a large number of well-characterized subjects with or without CKD.

Methods

Subjects

Between April 2007 and April 2017, a total of 2743 subjects were recruited for measurement of vascular function from subjects who underwent health-screening examinations or who visited the outpatient clinic at Hiroshima University Hospital. Of the 2743 subjects, 1862 subjects underwent measurement of both FMD and NID of the brachial artery. Subjects who had received nitrate treatment (n=112) and chronic hemodialysis (n=23), subjects with estimated glomerular filtration rate (eGFR) <15 ml/min/1.73 m² (n=10), and subjects with missing information on eGFR (n=150) were excluded. Finally, 1567 subjects (995 men and 572 women; mean age, 59.0±16.9 years; age range, 18 to 92 years) were enrolled in this study. Hypertension was defined as treatment with oral antihypertensive agents or systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, in a sitting position, on at least 3 different occasions.[25] Diabetes was defined according to the American Diabetes Association recommendation.[26] Dyslipidemia was defined according to the third report of the National Cholesterol Education Program.[27] We defined smokers as those who had

ever smoked. Coronary artery disease included angina pectoris, myocardial infarction, and unstable angina. Cerebrovascular disease included ischemic stroke, hemorrhagic stroke, and transient ischemic attack. Peripheral artery disease was defined as current intermittent claudication with ankle-brachial index <0.9 or a history of intervention, including angioplasty and bypass graft. The eGFR was calculated using the Japanese eGFR equation.[28] CKD was defined as eGFR <60 ml/min/1.73 m² and GFR stage was graded according to the Kidney Disease Improving Global Outcomes recommendations; eGFR of at least 90 ml/min/1.73 m² (G1), 60-89 to ml/min/1.73 m² (G2), 45-59 ml/min/1.73 m² (G3a), 30-44 ml/min/1.73 m² (G3b), and 15-29 ml/min/1.73 m² (G4).[29] The ethical committees of our institutions approved the study protocol. This study was performed in accordance with the Declaration of Helsinki. Written informed consent for participation in the study was obtained from all subjects.

Study protocol

We measured vascular responses to reactive hyperemia and sublingually administered nitroglycerine in the brachial artery. The subjects fasted the previous night for at least 12 hours. The subjects were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22°C to 25°C) throughout the study. A 23-gauge polyethylene

catheter was inserted into the left deep antecubital vein to obtain blood samples. FMD was measured thirty min after maintaining the supine position. After completion, we next measured NID with confirmation that the brachial artery diameter had recovered to the baseline value. The observers were blind to the form of examination.

Measurement of FMD and NID

Vascular response to reactive hyperemia in the brachial artery was used for assessment of endothelium-dependent FMD. A high-resolution linear artery transducer was coupled to computer-assisted analysis software (UNEXEF18G, UNEX Co, Nagoya, Japan) that used an automated edge detection system for measurement of brachial artery diameter. A blood pressure cuff was placed around the forearm. The brachial artery was scanned longitudinally 5 to 10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained, the transducer was held at the same point throughout the scan by a special probe holder (UNEX Co) to ensure consistency of the image. Depth and gain setting were set to optimize the images of the arterial lumen wall interface. When the tracking gate was placed on the intima, the artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using

the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. The baseline longitudinal image of the artery was acquired for 10 s, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 min. The longitudinal image of the artery was recorded continuously until 3 min after cuff deflation. Changes in brachial artery diameter were immediately expressed as percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from the baseline value $[(\text{Peak diameter} - \text{Baseline diameter}) / \text{Baseline diameter}]$.

The response to nitroglycerine was used for assessment of endothelium-independent vasodilation. After acquiring baseline rest images for 30 s, a sublingual tablet (75 μg nitroglycerine) was given, and images of the artery were recorded continuously until the dilation reached a plateau after administration of nitroglycerine. We carefully checked in the mouth to confirm that tablet had been dissolved and absorbed a few minutes after administration of nitroglycerine. Subjects in whom the sublingually administered nitroglycerine tablet was not dissolved during the measurement were excluded from this study. NID was automatically calculated as a

percent change in peak vessel diameter from the baseline value. Percentage of NID $[(\text{Peak diameter} - \text{Baseline diameter})/\text{Baseline diameter}]$ was used for analysis.

Statistical analysis

Results are presented as means \pm SD. All reported probability values were 2-sided, and a probability value of <0.05 was considered statistically significant. Categorical variables were compared by means of chi-square test. Continuous variables were compared by using unpaired Student's t test. Univariate linear regression analyses were performed to assess the relationships between NID, FMD, and eGFR. Multiple logistic regression analyses were performed to identify independent variables associated with vascular smooth muscle dysfunction and endothelial dysfunction. The data were processed using JMP version 11 (SAS institute, Cary, NC).

Results

Baseline clinical characteristics

The baseline clinical characteristics are summarized in Table 1. Of the 1567 subjects, 995 (63.4%) were men, 1203 (76.8%) had hypertension, 1072 (68.6%) had dyslipidemia, 463 (29.6%) had diabetes mellitus, 847 (54.4%) were smokers, 182 (11.7%) had

coronary artery disease, 113 (7.3%) had cerebrovascular disease, 131 (8.6%) had peripheral artery disease, and 341 (21.8%) had CKD. The mean eGFR was 73.4 ± 19.3 mL/min/1.73 m². The mean value of FMD was $3.8 \pm 3.0\%$ and that of NID was $12.3 \pm 5.8\%$. The clinical characteristics of subjects classified according to the GFR stage are summarized in Table 2.

Relationship between CKD and NID

Univariate regression analysis revealed that eGFR was significantly correlated with NID ($r=0.23$, $P<0.001$) (Figure 1A). NID was significantly smaller in patients with CKD than in those without CKD ($10.8 \pm 6.0\%$ vs. $12.7 \pm 5.7\%$, $P<0.001$) (Figure 2A). NID decreased significantly in relation to increase in the GFR stage ($14.6 \pm 5.0\%$ in stage G1, $12.1 \pm 5.5\%$ in stage G2, $11.1 \pm 6.0\%$ in stage G3a, $10.6 \pm 6.5\%$ in stage G3b, and $8.5 \pm 4.5\%$ in stage G4, $P<0.001$) (Table 2). The division point for the lowest quartile of NID was 8.3%. Therefore, vascular smooth muscle dysfunction was defined as NID of $<8.3\%$. The prevalence of vascular smooth muscle dysfunction was significantly higher in patients with CKD than in those without CKD (37.5% vs. 21.5% , $P<0.001$). The prevalence of vascular smooth muscle dysfunction increased significantly in relation to increase in the GFR stage (13.7% , 23.7% , 33.5% , 42.9% , and 63.0% , $P<0.001$) (Table

2). There was a significant association between CKD and vascular smooth muscle dysfunction in unadjusted analysis (OR: 2.20, 95% CI: 1.70 to 2.85, $P < 0.001$). Multiple logistic regression analysis revealed that CKD was independently associated with vascular smooth muscle dysfunction even after adjustment for age, sex, heart rate, hypertension, dyslipidemia, diabetes mellitus, smoking, and cardiovascular disease (OR: 1.36, 95% CI: 1.02 to 1.81, $P = 0.04$) (Table 3). When systolic blood pressure and antihypertensive drug treatment were simultaneously entered into the model as covariates instead of hypertension, CKD was independently associated with vascular smooth muscle dysfunction (OR: 1.35, 95% CI: 1.01 to 1.79, $P = 0.04$) and there were significant associations of vascular smooth muscle dysfunction with systolic blood pressure ($P = 0.003$) and antihypertensive drug treatment ($P = 0.001$) (Table 3). Of 1567 subjects, we have data on white blood cell (WBC) count in 1364 subjects (87.0%). There was no significant correlation between NID and WBC count ($r = 0.03$, $P = 0.28$). When WBC count was additionally entered into the multivariate model, CKD remained independently associated with vascular smooth muscle dysfunction (OR: 1.42, 95% CI: 1.05 to 1.94, $P = 0.03$) with an insignificant association between WBC count and vascular smooth muscle dysfunction ($P = 0.20$).

We divided subjects into two groups according to the presence ($n = 463$) or

absence (n=1104) of diabetes mellitus. eGFR correlated significantly with NID in patients with diabetes mellitus ($r=0.18$, $P<0.001$) and those without diabetes mellitus ($r=0.22$, $P<0.001$). NID was significantly smaller in patients with CKD than in those without CKD regardless of the presence of diabetes mellitus ($10.1\pm 6.0\%$ vs. $11.4\pm 5.4\%$, $P=0.03$) or absence of diabetes mellitus ($11.2\pm 6.0\%$ vs. $13.1\pm 5.8\%$, $P<0.001$). NID decreased significantly in relation to increase in the GFR stage in patients with diabetes mellitus ($12.7\pm 5.3\%$, $11.1\pm 5.3\%$, $10.4\pm 5.9\%$, $10.6\pm 7.4\%$, and $7.6\pm 2.8\%$, $P=0.01$) and those without diabetes mellitus ($15.1\pm 6.0\%$, $12.5\pm 5.6\%$, $11.4\pm 6.0\%$, $10.5\pm 5.6\%$, and $10.1\pm 6.3\%$, $P<0.001$). The prevalence of vascular smooth muscle dysfunction was significantly higher in patients with CKD than in those without CKD regardless of the presence of diabetes mellitus (43.0% vs. 28.7% , $P=0.003$) or absence of diabetes mellitus (34.0% vs. 18.8% , $P<0.001$). The prevalence of vascular smooth muscle dysfunction increased significantly in relation to increase in the GFR stage in subjects with diabetes mellitus (25.9% , 29.2% , 37.5% , 43.3% , and 70.6% , $P=0.004$) and those without diabetes mellitus (10.7% , 21.5% , 31.3% , 42.4% , and 50.0% , $P<0.001$).

Among the subjects receiving antihypertensive drug treatment, 139 subjects were receiving angiotensin II receptor blockers (ARBs)/angiotensin-converting enzyme inhibitors (ACEIs) monotherapy (ARBs/ACEIs group) and 307 were receiving calcium

channel blockers (CCBs) monotherapy (CCBs group). There was no significant difference in the prevalence of vascular smooth muscle dysfunction between ARBs/ACEIs group and CCBs group (23.7% vs. 26.7%, $P=0.50$). We next categorized subjects into 3 groups according to the number of prescribed antihypertensive drugs: 0 ($n=509$), 1 ($n=479$), and ≥ 2 ($n=560$). The prevalence of vascular smooth muscle dysfunction increased significantly in relation to increase in the number of prescribed antihypertensive drugs (15.5%, 24.8%, and 33.6%, $P<0.001$), indicating that the number of antihypertensive drugs is associated with vascular smooth muscle dysfunction. Therefore, we investigated the association between CKD and vascular smooth muscle dysfunction in each group stratified according to the number of prescribed antihypertensive drugs. The prevalence of vascular smooth muscle dysfunction was significantly higher in subjects with CKD than in those without CKD in all groups (28.0% vs. 14.2%, $P=0.02$, in subjects without antihypertensive drug treatment; 36.5% vs. 21.6%, $P=0.003$, in subjects treated with 1 antihypertensive drug; and 40.7% vs. 30.2%, $P=0.01$, in subjects treated with ≥ 2 antihypertensive drugs).

Relationship between CKD and FMD

Univariate regression analysis revealed that eGFR was significantly correlated with

FMD ($r=0.24$, $P<0.001$) (Figure 1B). FMD was significantly smaller in patients with CKD than in those without CKD ($3.1\pm 2.8\%$ vs. $4.0\pm 3.0\%$, $P<0.001$) (Figure 2B). The division point for the lowest quartile of FMD was 1.7%. Therefore, endothelial dysfunction was defined as FMD of $<1.7\%$. The prevalence of endothelial dysfunction was significantly higher in patients with CKD than in those without CKD (31.7% vs. 23.1% , $P=0.002$). Although there was a significant association between CKD and endothelial dysfunction in unadjusted analysis (OR: 1.54, 95% CI: 1.18 to 2.01, $P=0.002$), multiple logistic regression analysis revealed that the association between CKD and endothelial dysfunction was no longer significant after adjustment for age and sex (OR: 0.95, 95% CI: 0.71 to 1.26, $P=0.72$) and other risk factors, including heart rate, hypertension, dyslipidemia, diabetes mellitus, smoking, and cardiovascular disease (OR: 0.98, 95% CI: 0.73 to 1.32, $P=0.90$) (Table 3). When systolic blood pressure and antihypertensive drug treatment were simultaneously entered into the model as covariates instead of hypertension, CKD was not independently associated with endothelial dysfunction (OR: 0.94, 95% CI: 0.70 to 1.27, $P=0.70$) and there were significant associations of endothelial dysfunction with systolic blood pressure ($P=0.04$) and antihypertensive drug treatment ($P<0.001$) (Table 3). There was a significant correlation between WBC count and FMD ($r=0.07$, $P=0.01$). However, when WBC count was

additionally entered into the multivariate model, neither CKD (OR: 1.06, 95% CI: 0.77 to 1.46, $P=0.71$) nor WBC count ($P=0.65$) was independently associated with FMD.

When subjects were divided into two groups according to the presence or absence of diabetes mellitus, eGFR correlated significantly with FMD in patients with diabetes mellitus ($r=0.11$, $P=0.02$) and those without diabetes mellitus ($r=0.26$, $P<0.001$). In patients without diabetes mellitus, FMD ($3.4\pm 2.9\%$ vs. $4.3\pm 3.1\%$, $P<0.001$) was significantly smaller and the prevalence of endothelial dysfunction (28.6% vs. 20.0% , $P=0.009$) was significantly higher in patients with CKD than in those without CKD. However, in patients with diabetes mellitus, there was no significant difference in FMD ($2.7\pm 2.6\%$ vs. $3.1\pm 2.6\%$, $P=0.10$) and the prevalence of endothelial dysfunction (36.3% vs. 31.4% , $P=0.31$) between patients with and without CKD.

There was no significant difference in the prevalence of endothelial dysfunction between ARBs/ACEIs group and CCBs group (30.9% vs. 26.7% , $P=0.36$). When subjects were categorized into 3 groups according to the number of prescribed antihypertensive drugs (0, 1, and ≥ 2), the prevalence of endothelial dysfunction increased significantly in relation to increase in the number of prescribed antihypertensive drugs (15.5% , 24.8% , and 33.6% , $P<0.001$). The prevalence of endothelial dysfunction was significantly higher in subjects with CKD than in those

without CKD in subjects without antihypertensive drug treatment (20.0% vs. 9.6%, $P=0.04$), whereas there was no significant difference in the prevalence of endothelial dysfunction between subjects with and without CKD in subjects treated with 1 antihypertensive drug (27.9% vs. 25.9%, $P=0.68$) and those treated with ≥ 2 antihypertensive drugs (36.8% vs. 37.3%, $P=0.91$).

Discussion

In the present study, we demonstrated that eGFR was positively correlated with NID and FMD and that the prevalence of vascular smooth muscle dysfunction and endothelial dysfunction was significantly higher in patients with CKD than in those without CKD. CKD was independently associated with vascular smooth muscle dysfunction even after multivariable adjustment, whereas the association between CKD and endothelial dysfunction was no longer significant after adjustment for age and sex. To our knowledge, this is the first report showing that non-ESRD CKD is associated with vascular smooth muscle dysfunction.

It is well known that patients with ESRD have high rates of cardiovascular morbidity and mortality. The rate of cardiovascular mortality has been reported to be 10 to 20-times higher in patients treated by dialysis than in the general population even

after stratification for age, sex, race, and diabetes mellitus.[30] Recent studies have shown that risks of cardiovascular events are higher not only in patients with ESRD but also in patients with CKD not undergoing maintenance dialysis and that CKD is an independent risk factor for CVD.[16, 17, 31] The association between CKD and endothelial function assessed by FMD has been investigated.[19-23] However, there is little information on the relationship between CKD and NID.

NID, a vasodilatory response of the brachial artery to sublingual nitroglycerine administration, is usually assessed as a control test for FMD in the brachial artery to confirm that the vasodilatory response of the brachial artery to reactive hyperemia is not affected by vascular smooth muscle dysfunction or structural alterations but is truly a consequence of endothelium-dependent vasodilation. However, several investigators, including us, have recently demonstrated that NID per se is impaired in patients with multiple cardiovascular risk factors or established CVDs, such as coronary artery disease, peripheral artery disease, and heart failure with preserved ejection fraction.[10-12, 32-35] In addition, impaired NID in the brachial artery has been shown to be associated with a significantly higher incidence of cardiovascular events.[13] Therefore, not only FMD but also NID in the brachial artery could be a useful marker for risk stratification in subjects with cardiovascular risk factors.

Measurement of NID was performed in several studies in which the relationship between CKD and vascular function was investigated.[19-24] NID has been shown to be significantly impaired in patients with ESRD compared to that in patients without CKD.[22, 24] However, the relationship between non-ESRD CKD and NID has not been fully investigated. It is unclear whether non-ESRD CKD is independently associated with NID since the relationship between non-ESRD CKD and NID was not evaluated because of the small number of subjects or highly selected subjects in previous studies. In the present study, we demonstrated that NID was significantly smaller in patients with non-ESRD CKD than in those without non-ESRD CKD and that non-ESRD CKD was independently associated with vascular smooth muscle dysfunction in a large number of well-characterized subjects with or without CKD.

Traditional cardiovascular risk factors, such as older age, hypertension, diabetes mellitus and dyslipidemia, tend to be highly prevalent as underlying diseases in patients with CKD. Indeed, in the present study, patients with CKD were older than patients without CKD and they had a higher prevalence of hypertension, dyslipidemia, and diabetes mellitus. These traditional cardiovascular risk factors have been shown not only to cause renal function impairment but also to be associated with impaired NID in the brachial artery.[10, 12] Therefore, impairment of NID in patients with CKD might

be in part attributed to these coexisting traditional cardiovascular risk factors. However, in the present study, the association between CKD and vascular smooth muscle dysfunction remained significant even after adjustment for these traditional risk factors. In addition, the prevalence of vascular smooth muscle dysfunction was significantly higher irrespective of the status of diabetes mellitus or antihypertensive drug treatment. These findings suggest that CKD is not merely a marker for traditional CVD risk factors but a risk factor independently associated with vascular smooth muscle dysfunction. Non-traditional risk factors associated with reduced renal function may contribute to the vascular smooth muscle dysfunction in patients with CKD, although WBC count, a measure of inflammation, was not associated with NID in the present study.

The Framingham Heart Study showed that there was no significant difference in FMD between subjects with non-ESRD CKD and those without non-ESRD CKD in either an age- and sex-adjusted model or a multivariable-adjusted model in a large community-based population, though NID was not assessed in that study.[36] Consistent with those previous observations, our results also showed that the association between endothelial dysfunction and non-ESRD CKD was no longer significant in either an age- and sex-adjusted model or a multivariable-adjusted model, though FMD was positively correlated with eGFR and was significantly smaller in patients with CKD

than in those without CKD in unadjusted analysis. Non-traditional risk factors associated with reduced renal function, such as enhanced oxidative stress, may theoretically contribute not only to vascular smooth muscle dysfunction but also to endothelial dysfunction in patients with non-ESRD CKD.[37] However, in the present study, CKD was independently associated with vascular smooth muscle dysfunction but not with endothelial dysfunction. Although we do not know the precise reason for the difference in the association of CKD with vascular smooth muscle dysfunction and endothelial dysfunction, it might be due to the difference in the stage of atherosclerosis at which vascular smooth muscle dysfunction and endothelial dysfunction are initiated. Endothelial dysfunction is the initial step in the process of atherosclerosis, and endothelial function assessed by FMD in the brachial artery is already impaired at an early stage of atherosclerosis.[1, 38] In addition, impairment of FMD has been shown to be an early event that precedes the impairment of NID.[12] Therefore, it is postulated that FMD is already reduced under the condition in which traditional cardiovascular risk factors are present as underlying diseases of CKD and that addition of non-traditional risk factors associated with reduced renal function does not cause an additional impairment of FMD, resulting in the non-independent association between CKD and FMD. Indeed, in patients with diabetes mellitus or patients receiving antihypertensive

drug treatment, there was no significant difference in FMD or the prevalence of endothelial dysfunction between patients with and those without CKD.

There are some limitations in the present study. First, we cannot deny the possibility that some medications, such as antihypertensive agents, affect brachial reactivity. However, Gokce et al. reported that administration of non-nitrate vasoactive agents had no significant effect on FMD and NID in healthy subjects and in patients with coronary artery disease.[39] As for the relationship between antihypertensive medication and NID, there was no significant difference in the prevalence of vascular smooth muscle dysfunction between subjects treated with ARBs/ACEIs monotherapy and those treated with CCBs monotherapy, and the prevalence of vascular smooth muscle dysfunction was significantly higher in subjects with CKD than in those without CKD irrespective of the number of antihypertensive drugs, indicating that the independent association of CKD with vascular smooth muscle dysfunction was not influenced by the type or the number of antihypertensive medications. In addition, we excluded individuals receiving nitrate treatment in the present study. Second, the enrollment period of the present study spanned over ten years, during which operators changed. However, all of the operators in the present study received training for a standard protocol of FMD and NID measurements and training for scanning and analysis of the record from experienced

operators, and supervised measurements were performed before independent scanning and reading. Third, the number of patients with severe CKD was small. Further study with a large number of patients who have severe CKD would enable more specific conclusions to be drawn regarding the relationship between vascular function and severity of CKD.

In conclusions, non-ESRD CKD is independently associated with vascular smooth muscle dysfunction but not with endothelial dysfunction. CKD is not merely a marker for traditional cardiovascular risk factors but is an independent risk factor associated with vascular smooth muscle dysfunction.

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Table 1. Clinical Characteristics of the Subjects

Variables	Total (n=1567)	Non-CKD (n=1226)	CKD (n=341)	P value
Age, y	59.1±16.8	55.9±17.1	70.3±9.6	<0.001
Male, n (%)	995 (63.4)	797 (65.0)	198 (58.1)	0.02
Body mass index, kg/m ²	23.9±3.9	24.0±4.0	23.7±3.4	0.24
Systolic blood pressure, mmHg	131.4±18.4	131.2±17.7	132.2±20.6	0.38
Diastolic blood pressure, mmHg	78.0±12.0	78.5±12.0	76.3±11.9	0.004
Heart rate, bpm	70.0±12.1	69.9±11.8	70.3±12.9	0.51
Total cholesterol, mmol/L	4.97±0.98	4.96±0.95	5.00±1.07	0.52
Triglycerides, mmol/L	1.57±1.09	1.56±1.08	1.62±1.13	0.37
HDL cholesterol, mmol/L	1.53±0.43	1.53±0.43	1.50±0.42	0.16
LDL cholesterol, mmol/L	2.89±0.87	2.89±0.86	2.90±0.91	0.77
Glucose, mmol/L	6.26±2.14	6.17±2.03	6.59±2.49	0.002
HbA1c, %	5.6±0.9	5.6±0.8	5.8±1.1	<0.001
Creatinine, μmol/L	72.9±24.0	65.8±13.0	98.3±34.8	<0.001
eGFR, ml/min/1.73 m ²	73.4±19.3	80.3±15.3	48.8±10.1	<0.001
CKD stage				
G1/G2, n	278/948	278/928	0/0	
G3a/G3b/G4, n	251/63/27	0/0/0	251/63/27	
Smoker, n (%)	847 (54.4)	663 (54.5)	184 (54.1)	0.89
Complications				
Hypertension, n (%)	1203 (76.8)	910 (74.2)	393 (85.9)	<0.001
Dyslipidemia, n (%)	1072 (68.6)	806 (66.0)	266 (78.2)	<0.001
Diabetes mellitus, n (%)	463 (29.6)	328 (26.8)	135 (39.6)	<0.001
Cardiovascular disease, n (%)	326 (21.3)	212 (17.6)	114 (34.8)	<0.001
Coronary artery disease, n (%)	182 (11.7)	98 (8.1)	84 (24.9)	<0.001
Cerebrovascular disease, n (%)	113 (7.3)	66 (5.5)	47 (14.0)	<0.001
Peripheral artery disease, n (%)	131 (8.6)	89 (7.4)	42 (12.7)	0.003
Medication use				
Antihypertensive drug, n (%)	988 (63.3)	729 (59.7)	259 (76.2)	<0.001
Antidiabetic drug, n (%)	283 (18.1)	195 (15.9)	88 (25.8)	<0.001
Statin, n (%)	534 (34.5)	358 (29.5)	176 (52.2)	<0.001
Insulin, n (%)	37 (2.4)	17 (1.4)	20 (5.9)	<0.001
Baseline brachial artery diameter, mm	4.11±0.66	4.10±0.66	4.14±0.66	0.30
Flow-mediated vasodilation, %	3.8±3.0	4.0±3.0	3.1±2.8	<0.001
Endothelial dysfunction, n (%)	391 (25.0)	284 (23.1)	108 (31.7)	0.002

Nitroglycerine-induced vasodilation, %	12.3±5.8	12.7±5.7	10.8±6.0	<0.001
Vascular smooth muscle dysfunction, n (%)	391 (25.0)	263 (21.5)	128 (37.5)	<0.001

CKD indicates chronic kidney disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate.

Table 2. Clinical Characteristics of the Subjects According to the Severity of Chronic Kidney Disease

Variables	G1 (n=278)	G2 (n=948)	G3a (n=251)	G3b (n=63)	G4 (n=27)	P value
Age, y	42.4±17.8	59.9±14.7	70.2±9.4	71.0±8.6	69.9±13.2	<0.001
Male, n (%)	199 (71.6)	598 (63.1)	138 (55.0)	41 (65.1)	19 (70.4)	0.002
Body mass index, kg/m ²	23.2±4.3	24.2±3.9	23.7±3.4	23.7±3.3	23.7±3.4	0.005
Systolic blood pressure, mmHg	127.8±17.3	132.2±17.6	130.2±19.5	136.0±24.0	141.4±20.8	<0.001
Diastolic blood pressure, mmHg	76.1±12.4	79.2±11.8	76.0±11.6	77.1±12.4	77.5±13.1	<0.001
Heart rate, bpm	70.5±12.6	69.7±11.6	70.0±13.0	71.9±13.1	69.7±12.4	0.60
Total cholesterol, mmol/L	4.88±1.02	4.98±0.93	5.00±0.97	5.02±1.33	4.98±1.30	0.57
Triglycerides, mmol/L	1.55±1.24	1.56±1.02	1.54±0.94	1.71±1.25	2.13±2.00	0.07
HDL cholesterol, mmol/L	1.55±0.46	1.53±0.42	1.55±0.42	1.38±0.42	1.25±0.29	<0.001
LDL cholesterol, mmol/L	2.81±0.89	2.91±0.85	2.90±0.87	2.96±1.03	2.83±0.93	0.45
Glucose, mmol/L	6.06±2.51	6.20±1.87	6.30±1.73	7.16±3.88	8.04±3.73	<0.001
HbA1c, %	5.5±1.0	5.6±0.8	5.7±0.8	6.0±1.0	6.9±3.0	<0.001
Creatinine, µmol/L	57.4±11.3	68.3±12.4	84.2±13.4	114.9±21.1	190.6±39.9	<0.001
eGFR, ml/min/1.73 m ²	102.8±11.9	73.7±8.3	53.8±4.1	39.6±4.2	23.7±4.0	<0.001
Smoker, n (%)	149 (54.4)	514 (54.6)	135 (54.0)	38 (60.3)	11 (40.7)	0.57
Complications						
Hypertension, n (%)	143 (51.4)	767 (80.9)	208 (82.9)	58 (92.1)	27 (100.0)	<0.001
Dyslipidemia, n (%)	141 (50.7)	665 (70.4)	201 (80.4)	46 (73.0)	19 (70.4)	<0.001
Diabetes mellitus, n (%)	54 (19.4)	274 (29.0)	88 (35.1)	30 (47.6)	17 (63.0)	<0.001
Cardiovascular disease, n (%)	39 (14.2)	173 (18.6)	77 (32.0)	24 (40.0)	13 (48.2)	<0.001
Coronary artery disease, n (%)	11 (4.0)	87 (9.3)	63 (25.5)	16 (25.4)	5 (18.5)	<0.001
Cerebrovascular disease, n (%)	10 (3.6)	56 (6.0)	26 (10.5)	12 (19.4)	9 (8.0)	<0.001
Peripheral artery disease, n (%)	26 (9.5)	63 (6.8)	19 (7.9)	13 (21.3)	10 (37.0)	<0.001
Medication use						
Antihypertensive drug, n (%)	102 (36.8)	627 (66.4)	180 (72.0)	53 (84.1)	26 (96.3)	<0.001
Antidiabetic drug, n (%)	34 (2.2)	161 (17.0)	62 (24.7)	18 (28.6)	8 (29.6)	<0.001
Statin, n (%)	42 (15.4)	316 (33.7)	126 (50.8)	35 (56.5)	15 (55.6)	<0.001
Insulin, n (%)	5 (1.8)	12 (1.3)	10 (4.0)	5 (8.1)	5 (18.5)	<0.001
Baseline brachial artery diameter, mm	3.90±0.55	4.16±0.68	4.13±0.66	4.16±0.66	4.14±0.66	<0.001
Flow-mediated vasodilation, %	5.3±3.2	3.6±2.9	3.1±2.8	3.0±2.5	3.3±2.8	<0.001
Endothelial dysfunction, n (%)	34 (12.2)	249 (26.3)	84 (33.5)	16 (25.4)	8 (29.6)	<0.001
Nitroglycerine-induced	14.6±5.9	12.1±5.5	11.1±6.0	10.6±6.5	8.5±4.5	<0.001

vasodilation, %								
Vascular	smooth	muscle	38 (13.7)	225 (23.7)	84 (33.5)	27 (42.9)	17 (63.0)	<0.001
dysfunction, n (%)								

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate.

Table 3. Multivariate Analysis of the Relationship between Vascular Smooth Muscle Dysfunction and Endothelial Dysfunction and Variables

Model	Covariates	Vascular smooth muscle dysfunction		Endothelial dysfunction	
		Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
1	CKD (yes/no)	2.20 (1.70 to 2.85)	<0.001	1.54 (1.18 to 2.01)	0.002
2	1+age, sex	1.39 (1.05 to 1.83)	0.02	0.95 (0.71 to 1.26)	0.72
3	2+other variables	1.36 (1.02 to 1.81)	0.04	0.98 (0.73 to 1.32)	0.90
4	2+other variables	1.35 (1.01 to 1.79)	0.04	0.94 (0.70 to 1.27)	0.70

CKD indicates chronic kidney disease; CI, confidence interval.

Model 3: Other variables included body mass index, heart rate, hypertension, dyslipidemia, diabetes mellitus, smoking, and cardiovascular disease.

Model 4: Other variables included body mass index, heart rate, systolic blood pressure, antihypertensive drug treatment, dyslipidemia, diabetes mellitus, smoking, and cardiovascular disease.

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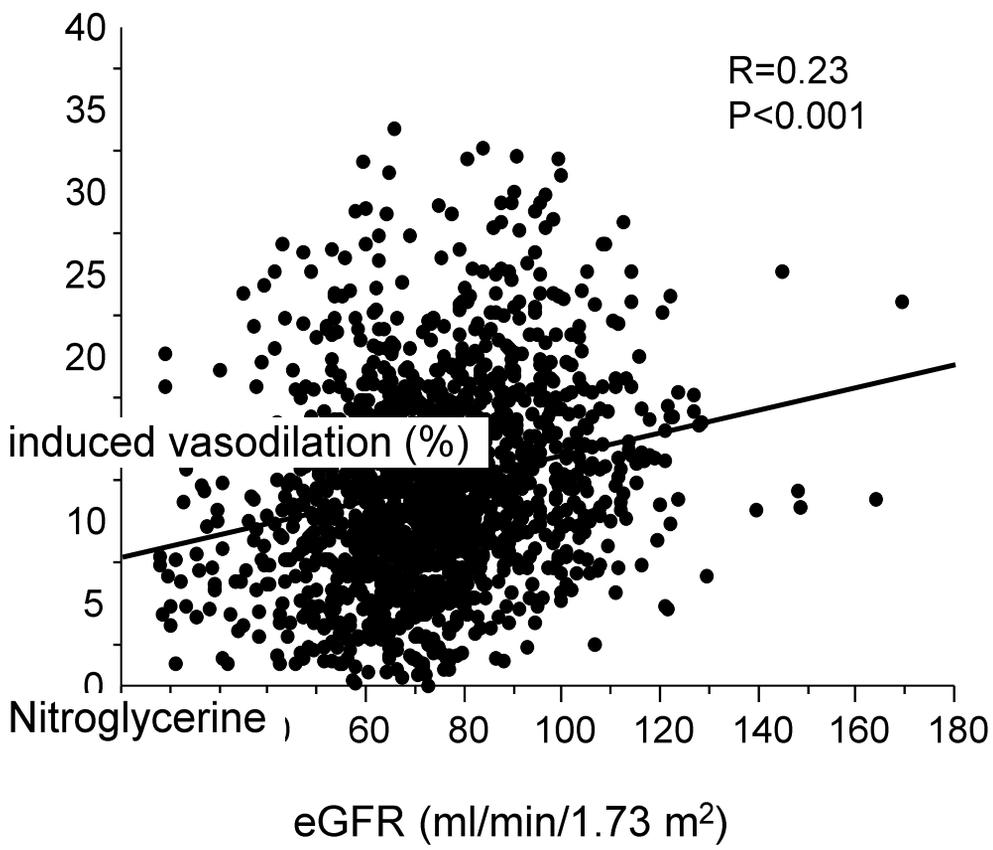
Figure legends

Figure 1. Scatter plots show the relationship between eGFR and nitroglycerine-induced vasodilation (A) and flow-mediated vasodilation (B).

Figure 2. Nitroglycerine-induced vasodilation (A) and flow-mediated vasodilation (B) in non-CKD patients and CKD patients.

Figure 1

A



B

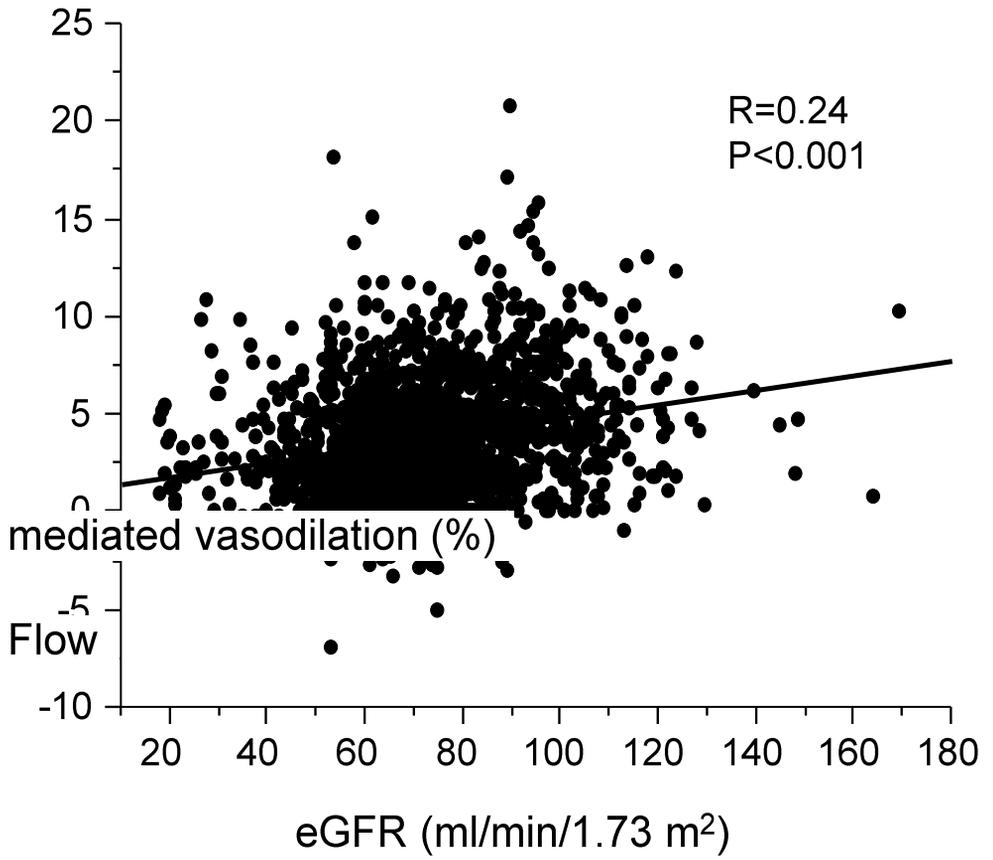
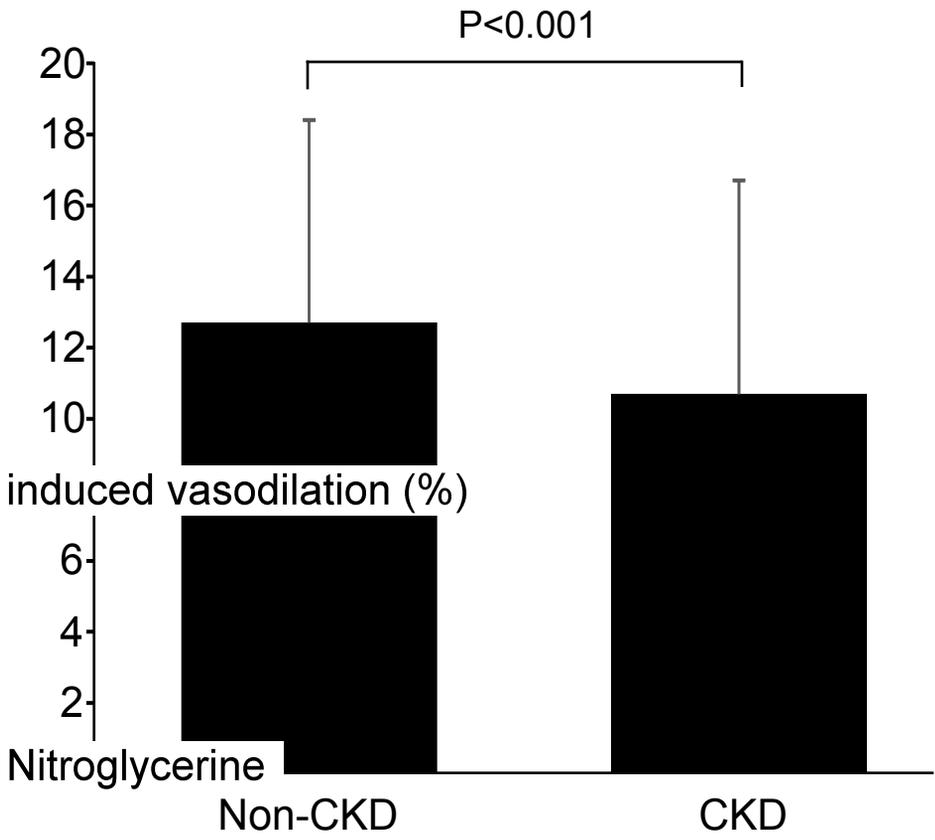


Figure 2

A



B

