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Title: Putative role of asymmetric dimethylarginine in microvascular disease of kidney and heart in hypertensive patients

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Abstract

Background: Despite the frequent presentation of combined cardiac and renal dysfunction, the relationship between these pathophysiological processes remains unclear. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase, which has been linked to endothelial dysfunction and atherosclerosis. This study elucidates the relationship between ADMA and intrarenal and coronary microvascular diseases.

Methods: We included 66 consecutive hypertensive patients with normal renal function or mild renal insufficiency (Cr ≤ 1.2 mg/dL) in this study. Based on their estimated glomerular filtration rate (eGFR), the patients were divided into two groups (normal group, eGFR ≥ 90 mL/min; renal insufficiency group, eGFR < 90 mL/min). Coronary flow velocity reserve (CFVR) was measured using adenosine-triphosphate stress transthoracic Doppler echocardiography. In addition, a plasma ADMA assay, echocardiography, carotid ultrasound and brachial-ankle pulse wave velocity (baPWV) measurement were performed.

Results: The plasma ADMA level was the highest in patients with both renal insufficiency and reduced CFVR. ADMA was significantly associated with eGFR ($r = -0.342$, $p = 0.006$) and CFVR ($r = -0.459$, $p < 0.001$), and eGFR and CFVR were significantly associated with each other ($r = 0.337$, $p = 0.006$). Multiple regression

analysis revealed that ADMA was an independent clinical parameter associated with both eGFR and CFVR.

Conclusions: Plasma ADMA is suggested to be an incipient biochemical marker of microvascular disease in both the kidney and heart in hypertensive patients. ADMA might play an important role in the pathogenesis of organ damage in the kidney and heart in essential hypertension.

Key words: asymmetric dimethylarginine, glomerular filtration rate, coronary flow velocity reserve, microvascular disease

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Introduction

Patients with chronic kidney disease (CKD) have a higher risk of developing cardiovascular disease (CVD) than the general population. It has been reported that not only end-stage renal disease (ESRD) but also mild renal insufficiency is associated with increased risk of CVD.[1, 2] Although CKD may be considered as an independent risk factor for CVD, the link between kidney and cardiovascular system remains unclear.

An impairment of coronary flow velocity reserve (CFVR) depending on microvascular abnormalities has been reported in various models of preclinical disease in the absence of coronary atherosclerosis.[3-5] Previously, we reported that CFVR decreases in hypertensive patients with chest pain but without coronary artery disease.[6] CFVR is thought to decrease because of impaired coronary microcirculation. Similarly, renal function depends on the integrity of the microvascular beds. Therefore, glomerular filtration rate (GFR) may be associated with renal microcirculation. It has been reported that mild renal insufficiency is associated with reduced coronary flow in patients without coronary artery disease.[7] This relationship may indicate parallel alternations in coronary and renal microcirculation.

Many studies have demonstrated that nitric oxide (NO) plays an important role in the progression of atherosclerosis.[8] NO is synthesized by endothelial, neuronal, and macrophage isoforms of the enzyme NO synthase (NOS). Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NOS, and serum ADMA levels

have been suggested to be a surrogate marker of endothelial dysfunction and/or angiosclerosis. Recently, we revealed the relationship between ADMA and coronary and peripheral endothelial dysfunction.[9] Several studies have demonstrated that serum ADMA levels increase in CKD patients and are strongly related to the severity of the atherosclerotic diseases.[10-12] Thus, ADMA is thought to be a novel biochemical marker of endothelial dysfunction and/or vascular lesions.

The present study was initiated to examine the relationship between ADMA, GFR, and CFVR in patients with normal or mild renal insufficiency and with normal or mild coronary artery disease. We hypothesized that ADMA may be involved in the underlying mechanism connecting the two pathologic conditions, and it may be an incipient biochemical marker of microvascular disease in the kidney and heart in hypertensive patients.

Methods

Study population

The study population consisted of 66 consecutive patients with essential hypertension, without significant coronary artery stenosis. All the patients had been hospitalized in our institution between 2003 and 2004. Eleven of the patients had participated in our previous study.[9] All the hypertensive patients had a well-established history of elevated casual blood pressure (BP) > 140/90 mmHg, with at least three sets of readings taken at 1-month intervals. The exclusion criteria for the present study included the presence of macrohematuria, proteinuria, renal artery stenosis, coronary artery disease, valvular heart disease, cardiomyopathy, peripheral artery disease, old cerebral infarction, diabetes mellitus, secondary hypertension, and insufficient echo imaging of the coronary arteries. Moreover, patients with arrhythmias, including atrioventricular blocks or atrial fibrillation, and bronchial asthma were excluded since the administration of adenosine triphosphate (ATP) could have worsened their symptoms. Coronary artery disease was ruled out by negative findings on exercise stress electrocardiography, scintigraphy, or coronary angiography. All the patients with positive findings on these examinations or CFVR < 2.0 underwent coronary angiography. Peripheral artery disease based on subjective and objective complaints and findings arising from leg ischemia and/or ankle-brachial index < 0.9. Renal artery stenosis was screened by Duplex ultrasonography. Diabetes mellitus was defined as a

patient's use of oral hypoglycemic agents or insulin and/or having a fasting glucose level > 126 mg/dL or a random non-fasting blood glucose level > 200 mg/dL.

For the patients taking antihypertensive drugs, medication was withdrawn approximately 1 week before the examination to exclude direct effects on vasodilation. Individuals with a smoking habit abstained from smoking for 1 week before the examination. All the patients abstained from caffeine the day before the examination. In brief, BP was measured by trained personnel using a calibrated mercury sphygmomanometer with appropriate cuffs (2 sizes), keeping the subject in a supine position, and ensuring standardized conditions. Three readings were taken and the average of the last two readings was used for the analyses. The experimental protocols were approved by the appropriate institutional review committee and an informed consent was obtained from all the subjects. This study was performed prospectively and all the examinations were conducted by several investigators who were blinded to the patients' characteristics.

We divided the patients into two groups based on their estimated glomerular filtration rate (eGFR). Patients with eGFR ≥ 90 mL/min were classified into group A (n = 33; normal or CKD stage 1), while those with eGFR ≤ 90 mL/min were placed in group B (n = 33; CKD stage 2 or 3).

GFR determination

GFR was estimated using the Cockroft and Gault equation, which is defined as eGFR = (140 − age) × body weight/(72 × serum creatinine). A correction factor of 0.85 was used for the females.

Measurement of CFVR

CFVR was measured using transthoracic Doppler echocardiography (TTDE), which has been described previously.[9] Briefly, TTDE examinations were conducted with a Siemens Sequoia digital ultrasound system at a frequency of 7.0 MHz (Siemens USA, Mountain View, CA, USA). The ultrasound beam was transmitted toward the heart to visualize coronary blood flow in the distal portion of the left anterior descending (LAD) coronary artery by color Doppler flow mapping. First, the left ventricle was imaged in cross section along the longitudinal axis, and then the ultrasound beam was inclined laterally. Next, coronary blood flow in the distal LAD was examined under the guidance of color Doppler flow mapping. After positioning a sample volume on the color signal in the distal LAD, Doppler spectral tracings of flow velocity were recorded by fast Fourier transformation analysis. All the results were recorded on 0.5-inch S-VHS videotapes for off-line analysis.

We first recorded baseline spectral Doppler signals in the distal LAD. ATP was administered (140 μg kg^{-1} min^{-1} IV) for 3 min to record spectral Doppler signals during

hyperemic conditions. All the patients underwent continuous heart rate and blood pressure monitoring throughout the study period.

Analysis of coronary flow velocity was conducted off-line by tracing the contour of the spectral Doppler signal using an ultrasound system computer. Mean diastolic velocity (MDV) was measured at baseline and peak hyperemic conditions. Measurements were averaged over three cardiac cycles. CFVR was defined as the ratio of hyperemic to basal MDV. We adopted a CFVR of less than 2.0 as the cut-off value for the presence of significant coronary microvascular disease, as used in the previous studies.[13]

Echocardiographic study

Two-dimensional guided M-mode echocardiography was conducted to measure left ventricular wall mass. Left ventricular diastolic and systolic diameters (LVDd/LVDs), in addition to the diastolic thickness of the left ventricular posterior wall (LVPWT) and interventricular septum (IVST), were assessed in M-mode images in the parasternal longitudinal-axis view. The M-mode analysis was conducted according to the guidelines of the American Society of Echocardiography. The left ventricular mass index (LVMI) (g/m^2) was calculated using the following formula: [14]

$$\text{LVMI } (g/m^2) = [1.04 \times \{(\text{IVST} + \text{LVPWT} + \text{LVDd})^3 - \text{LVDd}^3\} - 13.6]/\text{body surface area}$$

Carotid ultrasonographic study

The subjects were investigated in the supine position with the head slightly turned from the sonographer. The carotid arteries were carefully examined for wall changes from different longitudinal (anterior oblique, lateral, and posterior oblique) and transverse views. The common carotid arteries were examined in all the subjects. A region about 15 mm proximal to the carotid bifurcation was identified, and the intima-media thickness (IMT) of the far wall was evaluated as the distance between the luminal-intimal interface and the medial-adventitial interface. One transversal and two longitudinal measurements of IMT were obtained from 10 contiguous sites at 1-mm intervals, and the average of the 10 measurements was used for the analysis. The IMT was measured at a site free of any discrete plaques.

Pulse wave velocity (PWV) measurement

Brachial-ankle PWV (baPWV) was measured using a volume-plethysmographic apparatus (Form/ABI; Colin Co., Ltd., Komaki, Aichi, Japan). The subjects were examined while resting in the supine position. Electrocardiographic electrodes were placed on both wrists, and cuffs were wrapped on the bilateral brachia and ankles. Pulse volume waveforms at the brachium and ankle were recorded using a semiconductor pressure sensor after a rest period of at least 5 min.

Measurement of plasma ADMA levels and other laboratory determinations

On the same day as CFVR measurement, venous blood was collected from the patients after a 20-min period of supine rest in the morning, following overnight fasting. Blood was drawn into chilled citrate tubes on ice. Plasma was separated by centrifugation at 2,500 g for 10 min at 4ºC and stored at −20ºC until analysis. Plasma ADMA levels were determined at Fujimoto Biomedical Laboratories (Matsubara, Osaka, Japan) with a novel high-performance liquid chromatography (HPLC) method. This method used the Hitachi L-7480 system (Hitachi, Tokyo, Japan) equipped with a fluorescence detector for excitation at 348 nm and emission at 450 nm with an ODS column using ortho phthaldialdehyde for fluorescence determination. Other laboratory tests were conducted by standardized clinical laboratory methods.

Statistical analysis

We used the computer software application StatView 5.0 (SAS Institute, Cary, NC, USA) for all statistical analyses. Values are presented as mean ± SD. The significance of differences between the two groups was analyzed by the Student's *t*-test for continuous variables and by the χ^2 test for categorical variables. The differences among three groups were examined by ANOVA. Relationships between variables were assessed using univariate linear regression analysis. To determine the independent

biochemical markers for GFR and CFVR, we performed a multiple regression analysis.

A *p* value of less than 0.05 was considered statistically significant.

Results

Patients' characteristics

The main characteristics of the subjects are shown in Table 1. The patients in group B (CKD stage 2 or 3) were older than those in group A (normal or CKD stage 1). As for the other clinical parameters measured, in group B, eGFR was significantly lower, and IMT, baPWV and A/E ratio were significantly higher as compared with group A. Prior to withdrawal of antihypertensive drugs, there were no significant differences in blood pressure between the two groups (group A, systolic/diastolic blood pressure: 127 ± 16/76 ± 13 mmHg; group B, systolic/diastolic blood pressure: 131 ± 28/79 ± 28 mmHg).

Plasma ADMA concentrations and microvascular disease

The plasma ADMA level was significantly higher in group B than in group A. To further evaluate the relationship between plasma ADMA levels and coronary microvascular disease, we divided the study patients into two groups based on their CFVR value (normal CFVR ≥ 2.0; n = 52, abnormal CFVR < 2.0; n = 14). Mean plasma ADMA levels were significantly higher in the patients abnormal CFVR (group with CFVR ≥ 2.0; 0.49 ± 0.07 pg/mL, group with CFVR < 2.0; 0.56 ± 0.06 pg/mL: Figure 1A). In addition, the patients in group B had significantly lower CFVR than those in group A (group A; 2.70 ± 0.63, Group B; 2.30 ± 0.50: Figure 1B). Furthermore, the

patients were divided into the following three groups based on the presence of microvascular disease: group 1, CFVR ≥ 2.0 and eGFR ≥ 90 mL/min; group 2, CFVR < 2.0 or eGFR < 90 mL/min; group 3, CFVR < 2.0 and eGFR < 90 mL/min. Plasma ADMA level tended to be higher in group 2 than in group 1, and it was significantly higher in group 3 than in groups 1 and 2 (Figure 2).

Correlation between ADMA, eGFR and CFVR

Plasma ADMA levels were significantly associated with age (r= 0.481, $p < 0.001$), high sensitivity C-reactive protein (hsCRP) (r = 0.293, $p = 0.030$), IMT (r = 0.464, $p < 0.001$), and A/E ratio (r = −0.304, $p = 0.020$). Furthermore, ADMA had a significant correlation with both eGFR (r = −0.342, $p = 0.006$; Figure 3A) and CFVR (r = −0.459, $p < 0.001$; Figure 3B). We also found CFVR to be significantly correlated with eGFR (r = 0.337, $p = 0.006$; Figure 3C).

Univariate and multivariate predictors of eGFR and CFVR

The correlation coefficients between eGFR or CFVR and clinical parameters had been evaluated. The univariate analysis revealed that eGFR was significantly correlated with age (r = −0.583, $p < 0.001$), sex (r = −0.240, $p < 0.001$), BMI (r = −0.384, $p = 0.002$), homeostasis model assessment (HOMA-R) (r = −0.368, $p = 0.011$) and ADMA (r = −0.342, $p = 0.006$), and CFVR was significantly correlated with age (r = −0.434, p

< 0.001), sex (r = −0.264, p = 0.032), IMT (r = −0.359, p = 0.003), plasma aldosterone concentration (PAC) (r = 0.262, p = 0.037), endothelin-1 (ET-1) (r = −0.270, p = 0.028) and ADMA (r = −0.459, p < 0.001). Multiple regression analysis was conducted between eGFR or CFVR and the clinical parameters listed in Table 2 and 3. Body weight or BMI, and age greatly influence the results because they are used in the Cockroft and Gault equation for eGFR. The results of multiple regression analysis including them show that age and BMI had a significant independent association with eGFR (Table 2). When age was not included in the model, only ADMA showed a statistically significant independent relationship with eGFR and CFVR (Table 3). When ADMA was added to a model with the other covariates, the absolute value of the magnitude of the regression coefficient for age was reduced by 23 %.

Discussion

Our study demonstrated that (1) plasma ADMA level is already elevated at the early stage of kidney and heart disease in hypertensive patients; (2) ADMA is significantly correlated with age, IMT, A/E ratio and hsCRP; (3) there is a significant association between ADMA, GFR, and CFVR; and (4) ADMA is a statistically significant independent clinical parameter associated with both GFR and CFVR at the early stage of kidney and heart disease.

Recently, accumulating evidence has shown that not only end-stage renal disease but also minor renal dysfunction is a significant cardiovascular risk factor.[1] An American Heart Association statement published in 2003 recommended that patients with CKD should be considered as members of the highest risk group for subsequent CVD events.[15] It is thought that even mild renal dysfunction and/or the presence of albuminuria is correlated with increased cardiovascular mortality and morbidity.[16, 17] Thus, despite growing recognition of the cardio-renal association, its detailed mechanisms are not well understood.

Impaired CFVR was thought to arise during advanced stages of disease, when left ventricular hypertrophy (LVH) becomes obvious.[5] However, recent studies have suggested that CFVR decreases even at an early disease stages in hypertensive patients without LVH.[4] Impaired blood flow in small intramural resistance vessels or in the coronary capillary system results in decreased coronary microcirculation. Opherk et al.

suggested that the reduced CFVR in hypertensive patients may be attributed to abnormalities in small intramyocardial vessels that cannot be visualized by coronary angiography.[18] These abnormalities are currently considered a critical step in the development of angiosclerosis. Our recent study also showed similar deterioration in coronary and peripheral vascular territories.[9] Thus, impaired CFVR could be an indicator of systemic early organ damage due to microcirculation abnormalities. Our present findings also showed that CFVR decreased even in patients with mild renal insufficiency. Thus, hypertensive patients may have already had microvascular disease and/or atherosclerotic lesions before the presence of overt renal and cardiac disease.

In the last few decades, many studies have revealed an important role for NO, a potent anti-atherosclerotic molecule, in the development of endothelial dysfunction.[14] Decreased local NO production leads to progressive damage due to impaired microcirculation in the kidneys, heart, and other systemic organs. The role of increased plasma ADMA levels and vascular injury has been studied in not only in in vivo studies [19, 20] but also in various clinical conditions such as diabetes, hypercholesterolemia, and CVD.[21-23] Suda et al. reported that long-term treatment with ADMA leads to coronary microvascular lesions in an experimental study.[20] In addition, Kielstein et al. demonstrated that systemic ADMA infusion decreases cardiac output and renal blood flow in a dose-related manner in a human study.[24] Thus, elevation of plasma ADMA levels is thought to be the first step in the process of microvascular disease and/or

atherosclerosis. Numerous studies have suggested that an increased concentration of plasma ADMA might account for endothelial dysfunction in patients with CKD.[11, 25, 26] Indeed, in patients with renal disease as well as in those without renal disease, elevated plasma ADMA levels have a strong relationship with the severity of the atherosclerotic disease. Furthermore, plasma ADMA levels have been associated with increased cardiovascular risk factors in CKD patients, such as C-reactive protein (CRP), IMT, LVH, and left ventricular dysfunction.[27, 28] Our data have demonstrated that ADMA was significantly correlated with hsCRP and IMT but not LVH. We consider that the reason is because our study includes only patients with normal or mildly impaired renal function and without CVD. Kielstein et al. reported that ADMA might be an indicator of incipient renal disease, even when GFR is within the normal range.[11] Our study patients had a significant negative relationship between plasma ADMA levels and eGFR despite having normal or mildly impaired renal function. Considering these results, elevated plasma ADMA levels in patients with CKD do not seem to be the result of only its accumulation due to decreased renal function. Furthermore, our data also indicated that ADMA had a significant association with both eGFR and CFVR and plasma ADMA levels were the highest in patients with both renal and coronary microvascular disease. Therefore, we speculated that microvascular disease might cause parallel deleterious effects in both kidney and heart and ADMA may play an important role in the progression of kidney and heart disease. In our study, ADMA concentration

showed a statistically significant independent association with both eGFR and CFVR among the clinical parameters. Thus, elevated plasma ADMA level is suggested to reflect the degree of microvascular disease in the kidney and heart.

In the present study, the significant association of ADMA with eGFR disappeared on the multivariate analysis including age. As previous study mentioned [29], we also considered that the reason may be because the Cockroft and Gault equation includes age. Therefore, in the present study, we excluded the parameter on the additional multivariate analysis with eGFR and could not completely elucidate the degree of the influence of ADMA on GFR compared with age. Therefore, we chose 20 patients from group A and 20 patients matched for age from group B and compared ADMA levels between the two groups. The result showed that ADMA levels were significantly higher in group B (group A: 0.49 ± 0.07 pg/mL, group B: 0.53 ± 0.07 pg/mL: $p < 0.05$). Furthermore, the relationship between age and ADMA was preserved (Data not shown). As another reason, the influence of age on renal function may be mediated partly by ADMA or an ADMA related process. It has demonstrated that microcirculation deteriorates due to vascular structural and functional changes with age progression.[30, 31] It may be aggravated through such a mechanism.

There are several limitations in this study that require to be mentioned. First, there is the possibility that GFR might not reflect intrarenal microvascular damage, because we did not perform renal biopsy. However, the renal etiology of all the patients in the

present study was considered to be nephrosclerosis. We looked at clinical records and blood examination results, excluded macrohematuria and proteinuria using the urine strip test, and we did not detect chronic glomerulonephritis or other renal diseases in our study patients. It has been reported that intrarenal arteriolar lesions are associated with impaired GFR in patients with nephrosclerosis.[32, 33] Therefore, we thought that eGFR is related to intrarenal microvascular damage in the present study. Second, we estimated GFR not using the inulin-clearance technique but the Cockroft and Gault equation. This does not reflect exact GFR. It is very difficult to perform the GFR measurement using inulin-clearance method for all the subjects despite almost normal renal function in the clinical setting. To clarify that exactly, however, we will perform a further study using inulin-clearance method. Third, a cut-off value of CFVR ≤ 2.0 was originally used for significant coronary artery stenosis; however, it is very difficult to find the cut-off value of CFVR for coronary microvascular disease. We considered that patients with CFVR ≤ 2.0 despite having no significant coronary artery stenosis have quite severe microvascular disease. Therefore, we adopted it as a cut-off value for microvascular disease.

Conclusions

Our data indicate that ADMA may be a useful biochemical marker to detect early damage in the kidneys and heart. We speculate that renal and coronary microvascular

diseases are closely linked and ADMA may play an important role in the pathogenesis. Therefore, we consider that the analysis of ADMA might contribute to detect the progression of kidney and heart disease in hypertensive patients. Further pharmacological interventional studies are necessary to test the potential benefit.

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Table 1. Clinical characteristics of the patients

Clinical characteristics	All patients (n = 66)	Group A (n = 33)	Group B (n = 33)	*p*
Age (yo)	62 ± 10	57 ± 10	66 ± 6	<0.001
Male (%)	31 (47.0)	18 (54.5)	13 (39.4)	0.224
Body weight (kg)	62.0 ± 11.7	68.2 ± 9.8	58.0 ± 9.6	<0.001
BMI (kg/m^2)	24.7 ± 3.4	25.8 ± 3.3	23.5 ± 3.2	0.005
Smoking (%)	57 (86.4)	28 (84.8)	29 (87.9)	0.919
Hyperlipidemia (%)	23 (34.8)	14 (42.4)	9 (27.3)	0.202
SBP (mmHg)	151 ± 23	144 ± 16	160 ± 26	0.003
DBP (mmHg)	86 ± 14	85 ± 14	87 ± 13	0.534
Cr (mg/dL)	0.77 ± 0.19	0.73 ± 0.17	0.82 ± 0.19	0.043
BUN (mg/dL)	16.1 ± 2.0	15.8 ± 4.0	16.5 ± 4.1	0.487
Total cholesterol (mg/dL)	195.6 ± 29.8	196.7 ± 32.0	194.6 ± 27.9	0.778
TG (mg/dL)	116.5 ± 48.7	130.0 ± 50.8	103.0 ± 43.2	0.024
HDL-C (mg/dL)	50.3 ± 16.4	47.6 ± 14.7	52.9 ± 17.7	0.196
LDL-C (mg/dL)	122.1 ± 31.7	123.0 ± 32.3	121.1 ± 31.4	0.805
Glucose (mg/dL)	96.3 ± 13.9	93.5 ± 13.0	99.1 ± 14.4	0.103
HbA1c (%)	5.5 ± 0.8	5.4 ± 0.7	5.7 ± 1.0	0.120
PRA (ng/mL·hr)	1.2 ± 1.3	0.9 ± 0.8	1.5 ± 1.6	0.100
PAC (ng/dL)	15.2 ± 7.4	14.6 ± 8.1	15.7 ± 6.9	0.563
ET-1 (pg/mL)	3.44 ± 1.32	3.15 ± 1.17	3.73 ± 1.41	0.073
ADMA (nmol/mL)	0.51 ± 0.07	0.49 ± 0.06	0.53 ± 0.08	0.043
IRI (mU/L)	6.2 ± 4.0	5.4 ± 3.8	6.9 ± 4.1	0.126
hsCRP (mg/dL)	0.135 ± 0.161	0.122 ± 0.169	0.149 ± 0.156	0.536
HOMA-R	1.58 ± 1.15	1.33 ± 1.09	1.85 ± 1.17	0.122
eGFR (mL/min)	94.9 ± 32.1	120.2 ± 23.9	69.5 ± 14.1	<0.001
IMT (mm)	1.46 ± 0.71	1.32 ± 0.64	1.75 ± 0.92	0.031
baPWV (cm/s)	1871.9 ± 452.2	1689.1 ± 390.7	2066.6 ± 436.6	<0.001
LVMI (g/m^2)	119.5 ± 26.2	119.4 ± 23.7	119.6 ± 28.4	0.972
A/E ratio	1.21 ± 0.29	1.12 ± 0.28	1.30 ± 0.28	0.012

Group A: eGFR ≥ 90 mL/min, Group B: eGFR < 90 mL/min.

BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Cr, creatinine; BUN, blood urea nitrogen; TG, triglyceride; HDL-C, high-density

lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; PRA, plasma renin activity; PAC, plasma aldosterone concentration; ET-1, endothelin-1; ADMA, asymmetric dimethylarginine; IRI, immunoreactive insulin; hsCRP, high sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; IMT, intima-media thickness; baPWV, brachial-ankle pulse wave velocity; LVMI, left ventricular mass indexed for body surface area; HOMA-R, homeostasis model assessment.

Values are mean ± SD.

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Table 2. Analysis of the relationship between eGFR or CFVR and clinical parameters by multivariate linear regression; model 1

	eGFR		CFVR	
	β	*p*	β	*p*
Age	−0.514	0.002	−0.068	0.761
BMI	0.287	0.023	0.143	0.397
Sex	−0.175	0.200	−0.242	0.205
SBP	0.039	0.766	0.056	0.762
IMT	−0.006	0.966	0.076	0.699
A/E	0.055	0.413	0.084	0.658
HOMA-R	−0.125	0.307	−0.135	0.432
LDL-C	0.145	0.211	0.125	0.440
hsCRP	−0.086	0.486	−0.135	0.436
PAC	−0.139	0.306	0.228	0.235
ET-1	−0.116	0.336	−0.240	0.159
ADMA	−0.194	0.189	−0.407	0.048

eGFR, estimated glomerular filtration rate; CFVR, coronary flow velocity reserve; BMI, Body mass index; SBP, systolic blood pressure; IMT, intima-media thickness;

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HOMA-R, homeostasis model assessment; LDL-C, low density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; PAC, plasma aldosterone concentration; ET-1, endothelin-1; ADMA, asymmetric dimethylarginine.

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Table 3. Analysis of the relationship between eGFR or CFVR and clinical parameters by multivariate linear regression; model 2

	eGFR		CFVR	
	β	*p*	β	*p*
BMI	0.360	0.016	0.153	0.351
Sex	−0.282	0.076	−0.255	0.162
SBP	−0.028	0.858	0.048	0.792
IMT	0.110	0.499	0.091	0.630
A/E	−0.014	0.931	0.075	0.682
HOMA-R	−0.202	0.163	−0.125	0.449
LDL-C	0.087	0.519	0.117	0.454
hsCRP	−0.154	0.289	−0.143	0.393
PAC	0.002	0.988	0.246	0.172
ET-1	−0.066	0.639	−0.234	0.159
ADMA	−0.403	0.015	−0.433	0.023

eGFR, estimated glomerular filtration rate; CFVR, coronary flow velocity reserve; BMI, Body mass index; SBP, systolic blood pressure; IMT, intima-media thickness; HOMA-R, homeostasis model assessment; LDL-C, low density lipoprotein cholesterol;

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hsCRP, high sensitivity C-reactive protein; PAC, plasma aldosterone concentration;

ET-1, endothelin-1; ADMA, asymmetric dimethylarginine.

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Figure Legends

Figure 1.

(A) Average values of plasma asymmetric dimethylarginine (ADMA) concentration in the group with CFVR ≥ 2.0 and the group with CFVR < 2.0.

(B) Average values of coronary flow velocity reserve (CFVR) in groups A and B.

Values are shown as mean ± SD.

Figure 2. Average values of plasma asymmetric dimethylarginine (ADMA) concentration depending on the presence of microvascular disease.

Group 1, CFVR ≥ 2.0 and eGFR ≥ 90 mL/min; Group 2, CFVR < 2.0 or eGFR < 90 mL/min: Group 3, CFVR < 2.0 and eGFR < 90 mL/min.

Values are shown as mean ± SD.

Figure 3. Relationship between the three parameters: (A) Coronary flow velocity reserve (CFVR) and plasma asymmetric dimethylarginine (ADMA) concentration, (B) estimated glomerular filtration rate (GFR) and plasma ADMA concentration, and (C) CFVR and eGFR.

Figure 1

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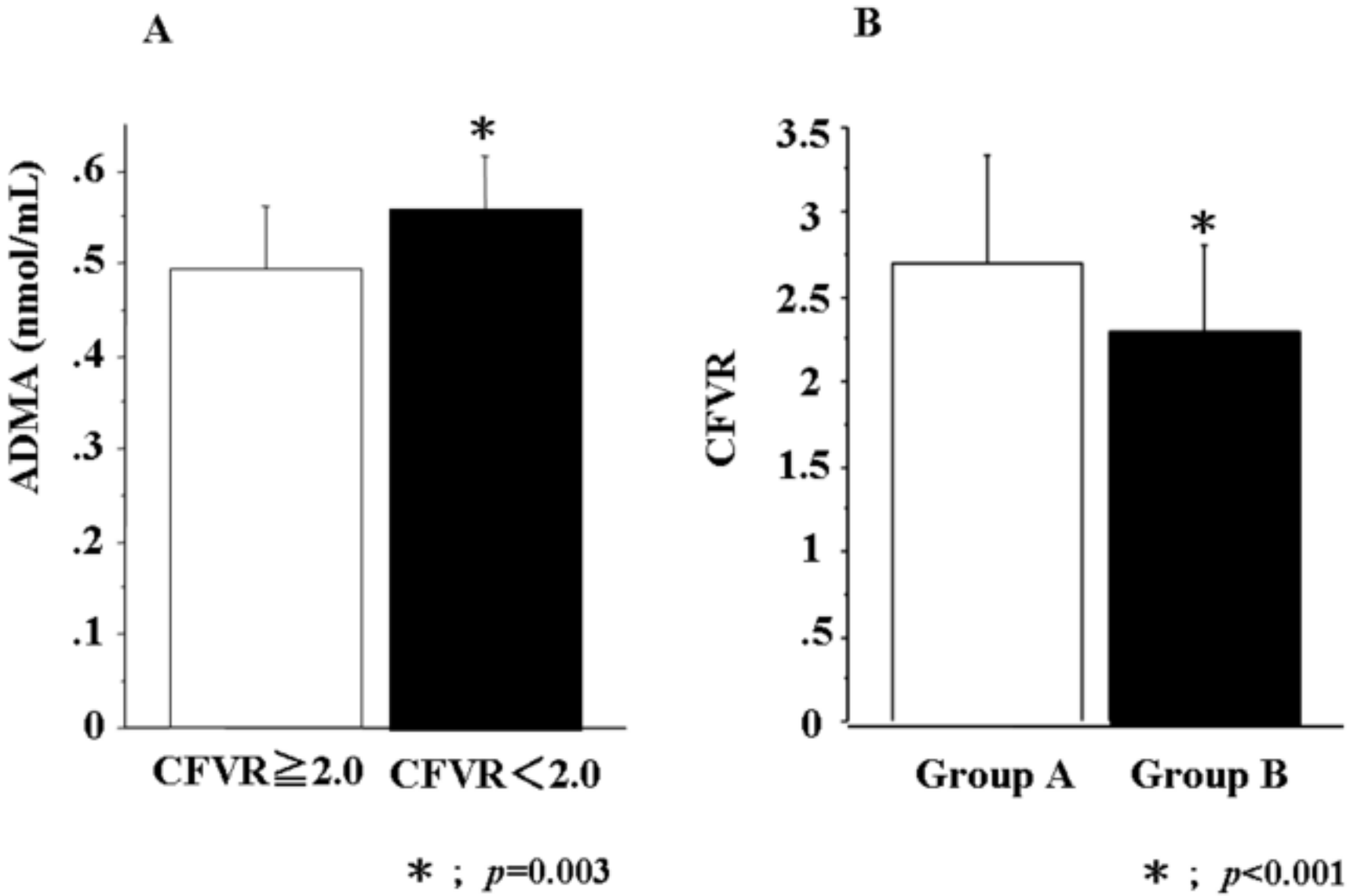

Figure 2

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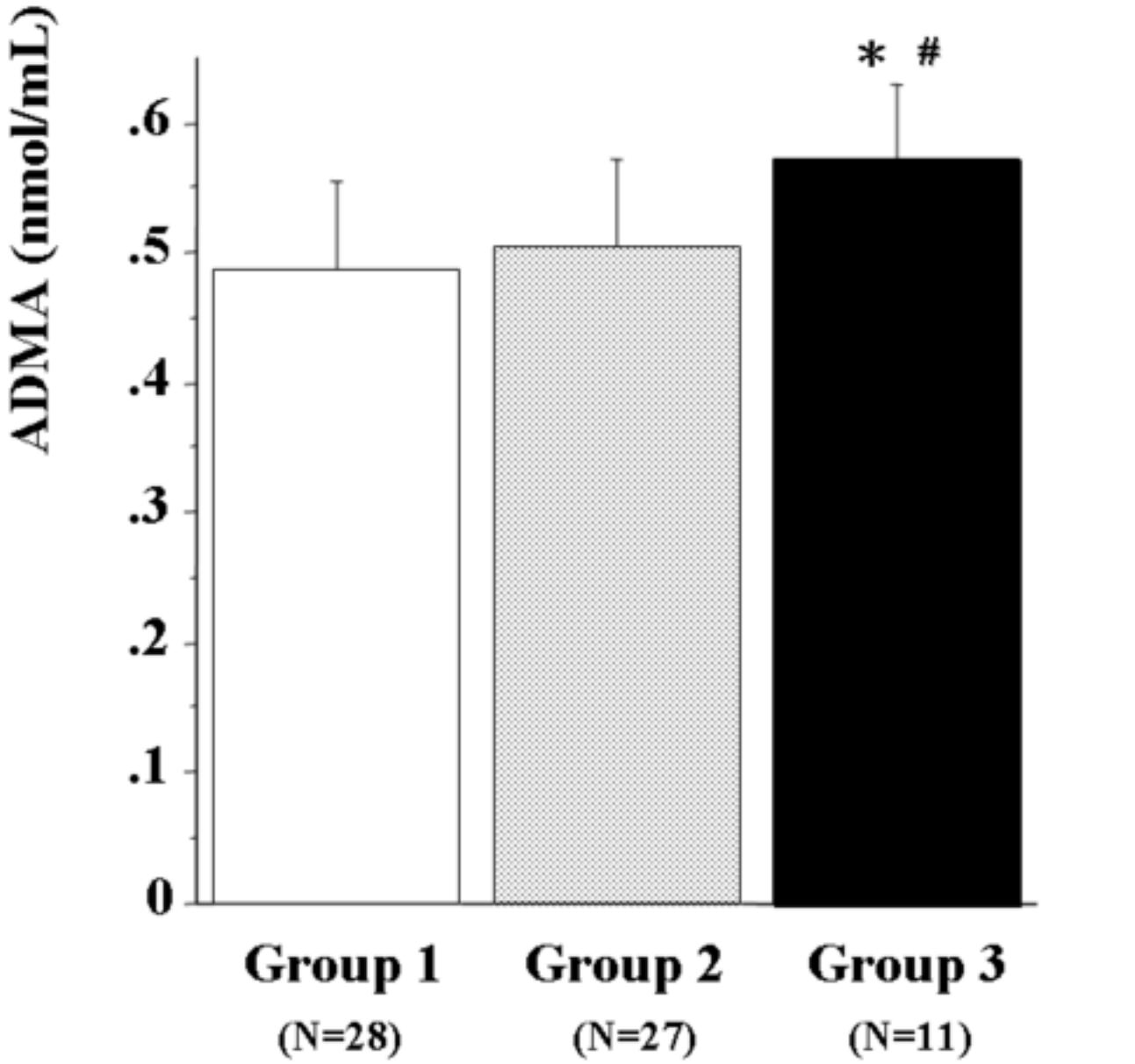

*; Group 1 v.s Group 3, $p<0.001$, #; Group 2 v.s Group 3, $p<0.01$

Figure 3

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