Original Article

Effect of Uric Acid on Coronary Microvascular Endothelial Function in Women: Association with eGFR and ADMA

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***Aim*: The aim of this study was to investigate the role of uric acid (UA) in coronary endothelial function via its effects on renal function, other coronary risk factors and asymmetric dimethylarginine (ADMA) in men and women.**

***Methods*: The study population consisted of 194 consecutive patients (119 men and 75 women) without coronary artery disease. The relationships between UA and coronary endothelial function, estimated glomerular filtration rate (eGFR), ADMA or other biochemical or anthropometric parameters were investigated.**

***Results*: Monovariate analysis of female participants demonstrated that % change in coronary blood flow (CBF) induced by acetylcholine (ACh) was inversely correlated with UA, ADMA and age ($r=-0.32$, $p<0.01$; $r=-0.31$, $p<0.05$; $r=-0.23$, $p<0.05$, respectively), and positively correlated with eGFR ($r=0.27$, $p<0.05$). Stepwise regression analysis showed that UA was the only independent predictor of % change in CBF induced by ACh (F value 4.969, $p<0.05$). Similar analysis of male participants failed to show significant correlations of these variables except for age in monovariate analysis ($r=-0.19$, $p<0.05$). Meanwhile, UA was inversely correlated with eGFR in both men and in women ($r=-0.25$, $p<0.01$; $r=-0.59$, $p<0.0001$, respectively), and ADMA was positively correlated with UA and inversely correlated with eGFR ($r=0.36$, $p<0.05$; $r=-0.42$, $p<0.01$, respectively) in women but not in men.**

***Conclusion*: High concentrations of UA correlate with coronary endothelial microvascular dysfunction in women. Further, serum UA concentration is related to eGFR and ADMA only in women, which may result in impaired endothelial function in resistance coronary arteries in women but not in men.**

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Key words; Uric acid, Endothelial function, Estimated glomerular filtration rate (eGFR), Asymmetric dimethylarginine (ADMA)

Introduction

While epidemiologic studies suggest that hyperuricemia is strongly correlated with cardiovascular disease[1], it is unclear whether uric acid (UA) levels are an independent risk factor for cardiovascular disease. Multivariate analysis of the Framingham Heart study[2] cohorts failed to demonstrate a relationship between UA and cardiovascular disease. However, analysis of the First National Health and Nutrition Examination Survey (NHANES-1) study cohort suggested that increased serum UA levels are independently and significantly associated with an increased risk of car-

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diovascular mortality[3]. Furthermore, the correlation between UA levels and cardiovascular risk was stronger in women than in men in the NHANES-1 study. While the reported findings have been contradictory in male populations, it has been largely reported that cardiovascular disease is related to serum UA concentration levels in female populations[4, 5].

Recent evidence suggests that endothelial dysfunction is a fundamental mechanism whereby UA may affect cardiovascular function. For example, Zoccali and colleagues demonstrated an inverse and significant relationship between UA and acetylcholine-stimulated vasodilation in patients with untreated essential hypertension, even after adjusting for differences in traditional cardiovascular risk factors[6]. However, gender-based differences between elevations in UA and endothelial dysfunction have not been definitively investigated.

Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide (NO) synthase inhibitor. Vallance and colleagues reported that the accumulation of endogenous ADMA can impair NO synthesis and thereby contribute to hypertension and immune dysfunction associated with chronic renal failure[7]. Further, another study suggested that ADMA levels increase in response to oxidative stress and that elevated ADMA levels lead to endothelial dysfunction and atherosclerosis. Thus, ADMA may be a marker of atherosclerosis[8]. Plasma ADMA concentration was markedly higher in patients with renal disease than in control subjects[9]. UA level was also increased in patients with renal dysfunction[10]. Although these prior studies suggest that UA and ADMA levels may correlate with cardiovascular risk or renal disease, the relationship between UA and ADMA has not yet been characterized.

UA levels can vary within humans secondary to inducing factors (e.g., high purine or protein diets, alcohol consumption) or changes in excretion. Hyperuricemia can lead to the development of renal disease[11] and is associated with factors that contribute to metabolic syndrome[12]. Further, UA levels increase with menopause[13], although other mechanisms related to gender-based differences in UA levels remain unclear.

Based on these observations, the goal of this study was to investigate the gender-specific relationship between UA and coronary endothelial function.

Subjects and Methods

Study Population

From December 1999 to December 2005, 194 consecutive patients (119 men, 75 women), who had been referred to Kagoshima University Hospital for cardiac catheterization to exclude coronary artery disease, were considered for enrollment in this study.

Angiographic inclusion criteria were: 1) angiographically smooth arteries; 2) mild irregularities, less than 30% lumen diameter stenosis by visual assessment in any major conduit vessel; and 3) proximal coronary arteries greater than 2.0 mm in diameter. Patients with a history of variant angina, previous myocardial infarction, previous coronary revascularization, valvular heart disease, cardiomyopathy, or myocarditis were excluded[14].

Long-acting nitrates and calcium channel-blocking agents were withheld for 48 hours before the study to allow for the assessment of baseline coronary physiology.

Written informed consent was obtained from all patients before catheterization in accordance with guidelines established by the Committee for the Protection of Human Subjects at our institution.

Study Protocol

Diagnostic coronary angiography was performed using a 6F Judkins catheter with a standard femoral percutaneous approach. Five thousand units of heparin were administered at the beginning of the procedure. Non-ionic contrast material was used in all patients. No nitroglycerin was given prior to the diagnostic procedure. Coronary blood flow (CBF) response to papaverine, acetylcholine (ACh), and nitroglycerin was studied according to previous reports[15, 16]. After control coronary angiograms, interventions were performed as follows: 1) a 0.014-inch Doppler guidewire (Cardiometrics, Santa Anna, CA) was introduced into the left anterior descending coronary artery; 2) after obtaining a stable Doppler signal, a bolus of papaverine (an endothelium-independent vasodilator in resistance coronary arteries) (12.5 mg/5 mL) was injected through a catheter; 3) infusion of ACh (an endothelium-dependent vasodilator in resistance and conduit coronary arteries) (0.5 mL/min) at a dose of 3 μg/min for 2 min was performed via the catheter; and 4) a bolus of nitroglycerin (an endothelium-independent vasodilator in conduit coronary arteries) (200 μg/5 mL) was administered[17, 18]. There was a minimum 5-min interval between drug infusions. Coronary arteriography was performed before and 2 min after each dose of ACh and after administration of nitroglycerin. Phasic coronary blood flow velocities, arterial blood pressure, and heart rate were monitored continuously and recorded. Measurements obtained during steady state conditions were used as control values for later analysis.

Assessment of Coronary Blood Flow

Doppler flow velocity spectra were analyzed on-line to determine time-averaged peak velocity. Volumetric CBF was determined from the formula: CBF = cross-sectional area × average peak velocity × 0.5[19]. Coronary flow reserve to papaverine was calculated as the ratio of maximal CBF induced by papaverine to basal CBF, which was equivalent to the endothelium-independent function of the resistance coronary artery. Endothelium-dependent function was calculated as the percent increase in CBF in response to ACh[15, 20, 21].

Quantitative Coronary Angiographic Images

Technically suitable single-plane angiograms were selected for computer analysis. Quantitative coronary angiographic images (DBAC-1000; MID Corporation, Fukuoka, Japan) were recorded using validated densitometric analysis, as previously reported[22]. An end-diastolic still frame at each infusion (baseline, ACh, nitroglycerin) was selected from the angiographic sequence. Endothelium-dependent and -independent vasodilation of the conduit coronary artery was estimated by measuring the luminal diameter at the tip of the Doppler guidewire positioning at the proximal site of left anterior descending coronary artery. These measurements were performed by experienced observers who were unaware of the coronary vascular reactivity tests.

Baseline Measurements and Biochemical Analysis

Diagnosis of hypertension was based on systolic pressure ≥140 mmHg and/or diastolic pressure ≥90 mmHg, or current treatment with antihypertensive drugs. Diabetes was defined on the basis of a fasting plasma glucose level ≥126 mg/dL, or HbA1c level ≥6.5%, or the use of oral hypoglycemic agents or insulin. Hyperlipidemia was defined on the basis of a fasting plasma low density lipoprotein (LDL) -cholesterol ≥140 mg/dL, or triglycerides ≥150 mg/dL, or active use of lipid-lowering medication. Cigarette smoking and menstruation status were determined by self-report. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Blood samples were obtained from subjects in the fasting state. White blood cell counts were measured using commercially available kits. Serum UA was measured in local laboratories using an automated technique based on the uricase/pod method, and serum creatinine, triglycerides, high density lipoprotein (HDL) -cholesterol and LDL-cholesterol values were measured by enzymatic methods (Roche Diagnostics Co., Ltd., Basel, Switzerland), using an autoanalyzer (Modular Analytics, Roche Diagnostics Co., Ltd.). High sensitivity C-reactive protein (CRP) was measured by latex-enhanced nephelometry (Denka Seiken Co., Ltd., Tokyo, Japan). Immunoreactive insulin was determined by a specific enzyme immunoassay with various reagents (TOSOH Co., Ltd., Yamaguchi, Japan). Insulin resistance was evaluated by the homeostasis model assessment ratio (HOMA-R) index calculated as follows: fasting plasma glucose (mg/dL) × fasting plasma insulin (μU/mL)/405[23]. Glomerular filtration rate (GFR) was estimated from the Modification of Diet in Renal Disease (MDRD) equation for Japanese patients, recently proposed by a working group of the Japanese Chronic Kidney Disease Initiative: $eGFR = 0.741 \times 175 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742, \text{if female})$ mL/min/1.73 m^2 [24].

ADMA Measurements

After April 2002, 113 (64 men and 49 women) of 194 consecutive patients enrolled in the coronary flow study had their serum ADMA concentrations measured. The relationship between UA and eGFR or ADMA was also assessed.

Fasting blood samples for ADMA measurement were obtained at the time of coronary flow study and stored at −80℃ until analysis. Serum ADMA concentrations were measured by high-performance liquid chromatography (HPLC) with precolumn derivatization with o-phthalaldehyde, as described previously[25].

Statistical Analysis

Statistical analysis was performed using Stat View Version 5.0 software. Values are expressed as the mean ± SD. The two groups were compared using Student's regression analysis unpaired *t* test. Risk factors and drugs were compared between groups using Pearson's chi-square test. Predictive factors for percent change in CBF induced by ACh were determined by stepwise regression analysis. Differences between the number of risk factors and the three grades of percent change in CBF induced by ACh and serum UA level were analyzed using Spearman's rank correlation coefficient. Statistical analysis of parallelism in linear regression was employed to compare the slopes of the simple regression[26]. Statistical significance was accepted when the p value was < 0.05.

Results

Patient Characteristics

One hundered ninety-four patients were evaluated. Patient characteristics are summarized in **Table 1**. Mean age was not significantly different when com-

Table 1. Patient background 1

	Men ($n=119$)	Women ($n=75$)	p
Age (years)	63 ± 12	63 ± 13	NS
Post-menopausal		62 (83%)	
Risk Factor			
Current Smoking	44 (37%)	2 (3%)	< 0.001
Obesity (BMI ≥ 25)	36 (30%)	23 (31%)	NS
Hypertension	72 (61%)	42 (56%)	NS
Hyperlipidemia	34 (29%)	32 (43%)	NS
Diabetes mellitus	15 (13%)	13 (17%)	NS
Drugs			
ACE inhibitor	25 (21%)	14 (19%)	NS
ARB	45 (38%)	28 (37%)	NS
Calcium channel blocker	54 (45%)	33 (44%)	NS
Statin	7 (6%)	19 (25%)	< 0.001
Aspirin	45 (38%)	27 (36%)	NS
Furosemide	39 (33%)	26 (35%)	NS
Spironolactone	20 (17%)	14 (19%)	NS
Allopurinol	7 (6%)	7 (9%)	NS

Values are the means ± SD or numbers of patients (percentages).
ACE, Angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; NS: not significant

paring the two groups. Sixty-two post-menopausal women were included in the 75 women in the study population. Coronary risk factors were also similar when comparing the two groups, except for active smoking status, which was observed more frequently in men than in women ($p<0.001$). There was no significant difference between groups in the frequency of using cardiac medications, with the exception of statin use, which was more frequent in women than in men ($p<0.001$). Serum UA levels were significantly higher in men than in women (6.7 ± 1.5 mg/dL vs. 5.6 ± 2.0 mg/dL, $p<0.0001$). Serum creatinine levels were also higher in men than in women (0.88 ± 0.23 mg/dL vs. 0.70 ± 0.28 mg/dL, $p<0.0001$), while HDL-cholesterol level was significantly higher in women than in men (53 ± 15 mg/dL versus 59 ± 14 mg/dL, $p<0.01$) (**Table 2**).

Coronary hemodynamic characteristics are summarized in **Table 3**. Baseline coronary artery diameter (CAD) and CBF were similar when comparing the two groups. There was no significant difference in the percent change in CBF induced by papaverine, coronary vascular resistance, percent change in CBF and CAD induced by ACh, or percent change in CAD induced by nitroglycerin when comparing men and women (**Table 3**).

Table 2. Patient background 2

	Men ($n=119$)	Women ($n=75$)	p
Mean BP (mmHg)	92 ± 16	90 ± 14	NS
Uric Acid (mg/dL)	6.7 ± 1.5	5.6 ± 2.0	< 0.0001
WBC (/μL)	5,505 ± 1,349	5,346 ± 1,963	NS
hsCRP (mg/dL)	0.25 ± 0.44	0.17 ± 0.31	NS
Body mass index (kg/m^2)	23.3 ± 3.6	23.4 ± 3.8	NS
LDL-Cholesterol (mg/dL)	111 ± 30	119 ± 33	NS
HDL-Cholesterol (mg/dL)	53 ± 15	59 ± 14	< 0.01
Triglycerides (mg/dL)	122 ± 85	127 ± 85	NS
HOMA-R	2.0 ± 2.5	1.8 ± 1.6	NS
Creatinine (mg/dL)	0.88 ± 0.23	0.70 ± 0.28	< 0.0001
eGFR (mL/min/1.73 m^2)	70.2 ± 19.3	72.3 ± 25.2	NS

Values are the means ± SD.
BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL-Cholesterol, high density lipoprotein cholesterol; HOMA-R, homeostasis assessment of insulin resistance index; hsCRP, highly sensitive C-reactive protein; LDL-Cholesterol, low density lipoprotein cholesterol; WBC, white blood cell; NS, not significant

Table 3. Coronary hemodynamic characteristics

	Men ($n=119$)	Women ($n=75$)	p
CAD at baseline (mm)	2.9 ± 0.7	2.8 ± 0.6	NS
CBF at baseline (mL/min)	73.5 ± 46.1	72.8 ± 45.3	NS
% change in CBF induced by papaverine (%)	210.4 ± 97.4	183.7 ± 96.0	NS
Coronary vascular resistance (mmHg min/mL)	1.8 ± 1.2	1.9 ± 1.3	NS
% change in CBF induced by acetylcholine (%)	38.9 ± 58.8	32.0 ± 54.6	NS
% change in CAD induced by acetylcholine (%)	3.2 ± 17.4	1.2 ± 10.0	NS
% change in CAD induced by nitroglycerin (%)	15.4 ± 21.2	14.1 ± 17.3	NS

Values are the mean ± SD.
CAD, coronary artery diameter; CBF, coronary blood flow; NS: not significant

Gender Difference in Coronary Endothelial Function

In univariate analysis of the female study population, serum UA, ADMA level and age inversely correlated with the percent change in CBF induced by ACh ($r=-0.32$, $p<0.01$; $r=-0.31$, $p<0.05$; $r=-0.23$, $p<0.05$, respectively) (**Fig. 1**, **2**). Further, eGFR positively correlated with the percent change in CBF induced by ACh ($r=0.27$, $p<0.05$) (**Fig. 3**). Stepwise regression analysis showed that serum UA level was the only independent predictor of percent change in CBF induced by ACh in women (**Table 4**). In con-

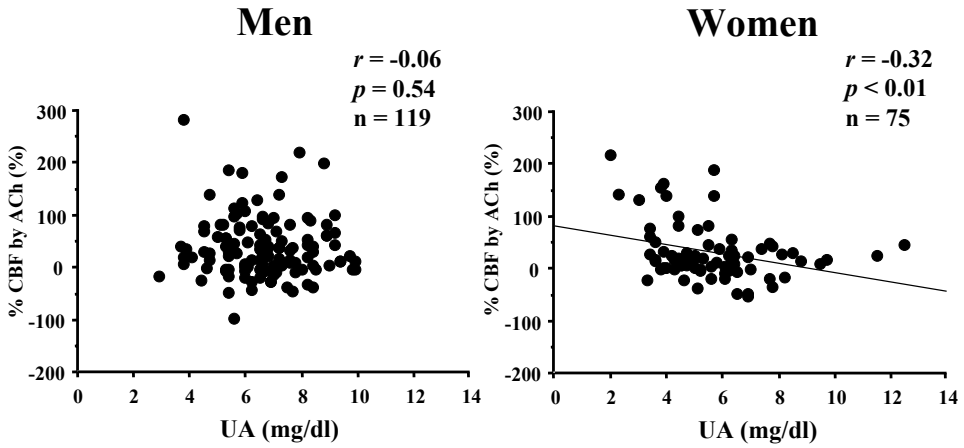

Fig. 1. Relationship between serum UA level and percent change in CBF induced by ACh.
ACh, acetylcholine; CBF, coronary blood flow; UA, uric acid

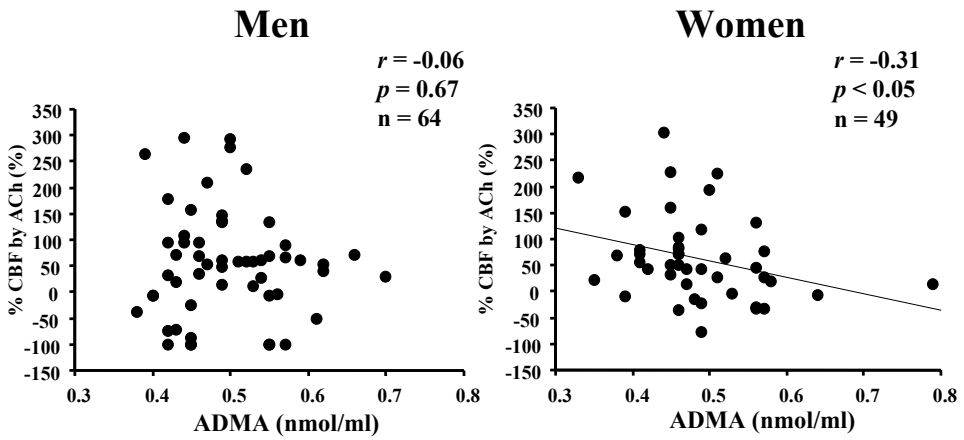

Fig. 2. Relationship between serum ADMA level and percent change in CBF induced by ACh.
ACh, acetylcholine; ADMA, asymmetric dimethylarginine; CBF, coronary blood flow

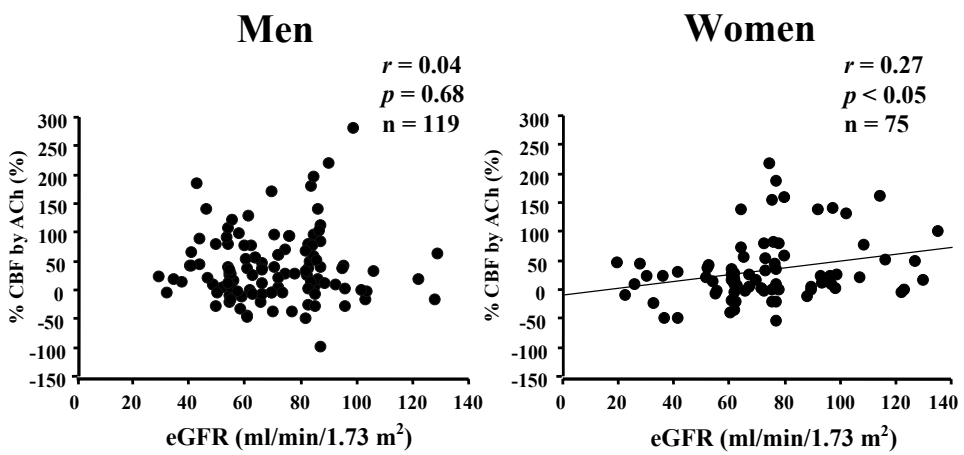

Fig. 3. Relationship between eGFR and percent change in CBF induced by ACh.
ACh, acetylcholine; CBF, coronary blood flow; eGFR, estimated glomerular filtration rate

Table 4. Percent change in CBF induced by acetylcholine: Potential predictive factors

Parameter	Men				Women			
	Univariate Analysis		Stepwise Multiple Regression analysis		Univariate Analysis		Stepwise Multiple Regression Analysis	
	r	*p*	*F*	*p*	*r*	*p*	*F*	*p*
Age	−0.19	<0.05	0.569	NS	−0.23	<0.05	0.139	NS
Mean BP	0.04	NS	0.070		0.08	NS	0.001	
Body mass index	0.05	NS	0.130		−0.05	NS	0.368	
Uric acid	−0.06	NS	0.246		−0.32	<0.01	4.969	<0.05
WBC	0.06	NS	0.026		0.04	NS	0.138	
hsCRP	−0.02	NS	0.024		−0.21	NS	0.791	
LDL-cholesterol	−0.07	NS	0.960		−0.06	NS	0.119	
HDL-cholesterol	0.10	NS	0.402		0.08	NS	0.108	
Triglycerides	−0.08	NS	0.620		−0.17	NS	1.001	
HbA1c	−0.14	NS	3.987		−0.07	NS	3.355	
HOMA-R	−0.15	NS	1.153		−0.11	NS	0.464	
Creatinine	−0.01	NS	0.433		−0.21	NS	0.032	
eGFR	0.04	NS	1.079		0.27	<0.05	0.356	NS

ADMA, asymmetric dimethylarginine; BP, blood pressure; CBF, coronary blood flow; eGFR, estimated glomerular filtration rate; HDL-cholesterol, high density lipoprotein cholesterol; HOMA-R, homeostasis assessment of insulin resistance index; hsCRP, highly sensitive C-reactive protein; LDL-cholesterol, low density lipoprotein cholesterol; WBC, white blood cell; NS, not significant

trast, age inversely correlated with the percent change in CBF by ACh ($r=-0.19$, $p<0.05$) in univariate analysis in the male study population. However, serum UA and ADMA levels did not correlate with the percent change in CBF by ACh in univariate analysis in this population ($r=-0.06$, $p=0.54$; $r=-0.06$, $p=0.67$, respectively). Stepwise regression analysis showed no independent predictor of percent change in CBF in response to ACh (**Table 4**).

As previously described, coronary endothelial function, as reflected by ACh-induced percent change in CBF, was classified into three different grades: poor (<0%), fair (0%–50%), and good (>50%)[14]; therefore, the mean UA concentration was also analyzed after stratifying according to CBF grade. Mean UA level increased as CBF grade worsened in women (good: UA 4.2±1.3 mg/dL, fair: UA 5.6±2.0 mg/dL, poor: UA 5.8±1.4 mg/dL, $p<0.001$) but not in men (good: UA 6.6±1.5 mg/dL, fair: UA 6.7±1.6 mg/dL, poor: UA 6.6±1.5 mg/dL, $p=0.83$).

Relationship between UA Concentration and eGFR or ADMA

The correlation between UA and eGFR is shown in **Fig. 4**. eGFR significantly decreased with increasing UA in both groups (eGFR decreased 3.1 and 7.7 mL/min/1.73 m^2 for every 1 mg/day increase in UA, respectively), suggesting that renal function decreased in response to increasing UA concentrations ($r=-0.25$, $p<0.01$; $r=-0.59$, $p<0.0001$, respectively). The slope for women was significantly ($p<0.01$) steeper than for men, suggesting that renal dysfunction progressed more rapidly in response to elevated UA concentration in women than in men. Serum UA levels positively correlated with ADMA only in women ($r=0.36$, $p<0.05$) (**Fig. 5**). Moreover, serum ADMA levels inversely correlated with eGFR in women ($r=-0.42$, $p<0.01$) but not in men (**Fig. 6**).

Relationship between BMI and UA Concentrations

Serum UA levels were measured in obese (BMI ≥25) and non-obese (BMI <25) subjects. The mean UA concentration was significantly higher in obese men than in non-obese men (7.2±1.4 mg/dL vs. 6.5±1.6 mg/dL, $p<0.05$), but there was no difference in UA concentrations when comparing obese women and non-obese women (5.4±1.5 mg/dL vs. 5.7±2.1 mg/dL). Moreover, serum UA levels positively correlated with BMI in men ($r=0.24$, $p<0.05$) but not in women.

Relationship between Number of Coronary Risk Factor and UA Concentrations

Coronary risk factors include hypertension, diabetes mellitus, hyperlipidemia and obesity. Smoking status was excluded from analysis because there were

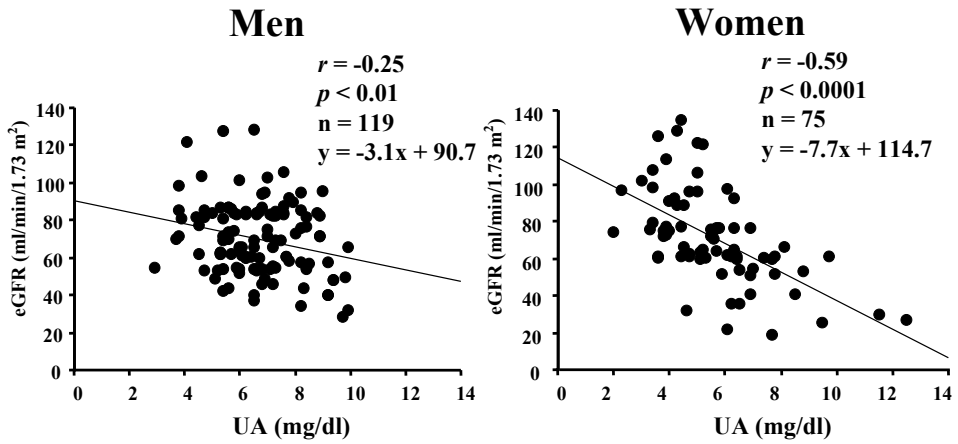

Fig. 4. Relationship between serum UA level and eGFR.
eGFR, estimated glomerular filtration rate; UA, uric acid

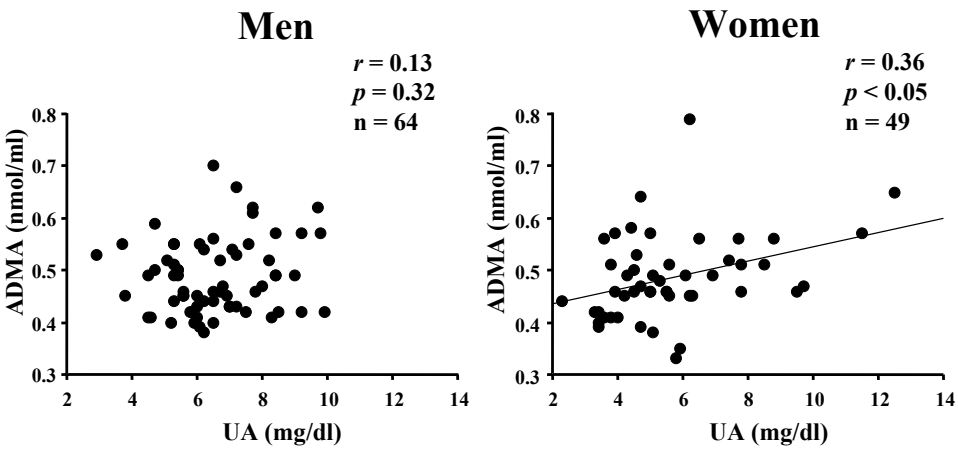

Fig. 5. Relationship between serum UA level and ADMA.
ADMA, asymmetric dimethylarginine; UA, uric acid

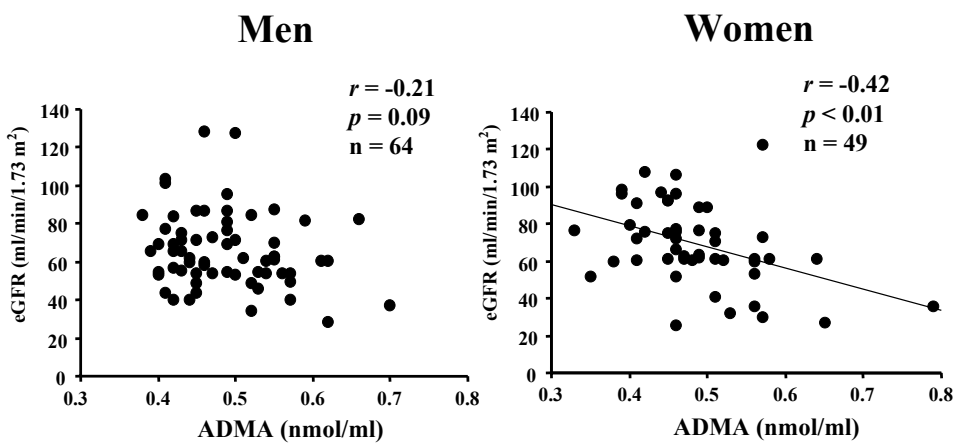

Fig. 6. Relationship between serum ADMA level and eGFR.
ADMA, asymmetric dimethylarginine; eGFR, estimated glomerular filtration rate

relatively few women smokers in the study population. In men, the mean UA concentration increased along with an increasing number of coronary risk factors. (0 risk factors, 6.3 ± 1.6 mg/dL; 1 risk factor, 6.5 ± 1.6; 2 risk factors, 6.9 ± 1.5 mg/dL; 3 or 4 risk factors, 7.4 ± 1.3 mg/dL, $p < 0.05$). By contrast, there was no correlation between the number of risk factors and the mean UA level in women (0 risk factors, 5.4 ± 2.2 mg/dL; 1 risk factor, 5.7 ± 1.7 mg/dL; 2 risk factors, 5.9 ± 2.5 mg/dL; 3 or 4 risk factors, 5.4 ± 1.4 mg/dL). Even after considering other risk factors (**Table 4**), UA remained the only independent predictor of ACh-induced percent change in CBF.

Discussion

Relationship between Coronary Endothelial Function and Serum UA Level in Women

The present study demonstrated that serum UA level inversely correlated with endothelium-dependent vasodilation of the resistance coronary arteries in women but not in men. In the Worksite Study, serum UA level was independently and specifically associated with cardiovascular events in hypertensive patients. Despite blood pressure control, serum UA level increased during treatment and was significantly and directly associated with cardiovascular disease. In fact, that study predicted that a serum UA level greater than 7.5 mg/dL in men and greater than 6.2 mg/dL in women was associated with an elevated risk of cardiovascular disease[27]. In another study, coronary endothelial function was classified according to ACh-induced percent change in CBF into one of three different grades: poor (<0%), fair (0%–50%), and good (>50%)[14]. Using this classification system to evaluate the present study population revealed that 10 women with normal UA (<6.0 mg/dL) had a poor CBF response to ACh (**Fig. 1**). This is consistent with the observations from the Progetto Ipertensione Umbria Monitoraggio Ambulatoriale (PIUMA) study, in which patients in the highest quartile of serum UA (>6.2 mg/dL in men; >4.6 mg/dL in women) had an increased risk of subsequent cardiovascular events and death from all causes. Based on these data, the PIUMA investigators proposed that "healthy" UA levels were 4.5–6.2 mg/dL in men and 3.2–4.6 mg/dL in women[28]. Based upon the solubility limit of urate in serum, hyperuricemia is generally defined as a serum UA level above 7 mg/dL in both genders[29]. Even in patients with gout, reduction of the serum UA level to below 6 mg/dL is recommended to prevent recurrence and assist in resolution of tophi[30]. The PIUMA investigators, as well as the results from the present study, suggest that reduction of UA to even lower levels is associated with additional increments of endothelial function in women.

Kielstein *et al.*[9] demonstrated that eGFR correlated with ADMA in patients with renal dysfunction. Comparing men without renal dysfunction (eGFR ≥60, $n = 37$) and men with renal dysfunction (eGFR <60, $n = 27$) in the present study, ADMA inversely correlated with eGFR in men with renal dysfunction ($r = -0.43$, $p = 0.026$), but not in men without renal dysfunction ($r = -0.18$, $p = 0.275$). Thus, the inclusion of men without renal dysfunction likely resulted in the absence of a significant correlation between ADMA and eGFR when considering the whole study population of men.

Several previous studies have demonstrated that UA elevation in men may be related to urate overproduction associated with obesity rather than decreased urate excretion[31-34]. These reports are consistent with the results from the present study that the UA level did not correlate with ADMA. Several studies also showed that hyperuricemia lead to cardiovascular events in men as well as women[27, 28]. However, these studies had different set values for UA relative to the elevated risk of cardiovascular disease between men and women (men: 6.2–7.5 mg/dL; women: 4.6–6.2 mg/dL). We investigated the relationship between the UA level and endothelial dysfunction for the consequences in all patients, so our study population included many men with a normal range of UA, which may explain why we did not observe a significant correlation between UA and endothelial dysfunction in men.

Relationship between UA and Renal Function Include of eGFR and ADMA Level in Women

Measurement of eGFR is the gold standard for the assessment of renal function[35]. In our study, there was a correlation between UA and eGFR in both men and women, as illustrated in **Fig. 4**. The correlation between these variables was stronger in women than in men, which suggests that renal dysfunction and UA levels were more strongly related in women than in men. Recent epidemiologic data suggest that hyperuricemia (≥6.0 mg/dL) is an independent predictor of end-stage renal disease in women[36]. Meanwhile, a decrease in eGFR may result in hyperuricemia. As estrogen stimulates urinary urate excretion, premenopausal women have lower serum UA levels than men or postmenopausal women[13]. Generally, hyperuricemia results from factors that increase urate generation or decrease urate excretion[1], and decreased urate excretion may be a more predominant cause of hyperuricemia in women. Indeed, the present study demon-

strated that renal dysfunction and the UA level were more closely related in women than men. Further, most of the women enrolled in this study were postmenopausal and hence, can be assumed to have decreased urate excretion or hypoestrogenemia.

Endothelial dysfunction is characterized by reduced endogenous NO activity, which may be attributed to elevated ADMA[37]. ADMA is an endogenous competitive inhibitor of NO synthase and an independent marker of cardiovascular risk[38]. Elevated endogenous ADMA levels are associated with systemic manifestations of endothelial dysfunction in patients with cardiovascular risk factors[39]. The present study demonstrated that the serum ADMA level inversely correlated with ACh-induced percent change in CBF and eGFR levels and positively correlated with the serum UA level only in women. These data suggest that hyperuricemia-induced renal damage in women may be mediated via the accumulation of ADMA and secondary reductions in NO-dependent coronary endothelial function.

Relationship between Coronary Risk Factors and Hyperuricemia

The reason for the absence of correlation between UA and endothelial function in men remains unclear. Several epidemiological studies have reported a close relationship between hyperuricemia and hypertension, obesity, hyperlipidemia and diabetes[40-42]. The present study demonstrated that the mean UA concentration was significantly higher in obese than non-obese men. Moreover, serum UA levels positively correlated with BMI only in men.

Visceral fat obesity represents a greater risk for various diseases than subcutaneous fat obesity. The pathogenesis of hyperuricemia in patients with visceral fat is related to low urinary urate excretion and overproduction of UA[31]. Indeed, several studies have suggested that an excess flow of free fatty acid from accumulated visceral fat to the liver may increase the production of UA via de novo purine synthesis and the pentose phosphate pathway[32, 33]. Meanwhile, hyperinsulinemia associated with visceral fat obesity may reduce urinary urate excretion in parallel with a decrease in urinary sodium excretion[34]. However, in the present study, there was no correlation between serum UA levels and HOMA-R or the insulin level (data not shown). Matsuura and colleagues reported that hyperuricemia may not be caused by the low urinary urate excretion associated with hyperinsulinemia in Japanese patients due to genetic susceptibility to impaired insulin-secreting ability[31]. Therefore, UA elevation in men may be related to urate overproduction associated with obesity rather than decreased urate excretion. In this study, serum UA levels increased along with an increasing number of coronary risk factors in men. As several risk factors in men are multifactorially related to endothelial dysfunction and are suspected to overlap, UA alone is not a risk factor of endothelial dysfunction. Serum UA levels in women did not correlate with either the number of coronary risk factors or obesity but did correlate closely with renal dysfunction.

Study Limitations

Several limitations of this study must be considered when interpreting the results. First, this study was a retrospective analysis of coronary flow research; nevertheless, the present study does provide a preliminary framework for planning future prospective studies. Second, smoking was observed much more frequently in men than in women, which suggests that smoking may have influenced our results. Although smoking would be expected to have an unfavorable effect on endothelial function, there was no difference in coronary endothelial function between men and women in this study. Moreover, UA levels and the percent change in coronary blood flow induced by ACh were comparable in men regardless of smoking status (data not shown). Therefore, differences in smoking status should have no bearing on the interpretation of the present data. Third, many reports suggest that microalbuminuria is a predictor of cardiac events. Indeed, Cosson and colleagues reported that microalbuminuria correlated with coronary endothelial dysfunction[43]; however, microalbuminuria was not measured in the evaluation of renal function in the present study. Fourth, all study patients had angiographically normal or mildly diseased coronary arteries, limiting the generalization of these data to patients with advanced coronary artery disease. Finally, the study population was relatively small, and the resulting statistical power may have been insufficient to demonstrate differences in some parameters.

UA has both prooxidant and antioxidant properties. Hyperuricemia is associated with endothelial dysfunction and cardiovascular diseases; in contrast, UA administration increases serum antioxidant capacity in healthy volunteers[44] and improves endothelial function in patients with type 1 diabetes and in smokers[45]. Furthermore, higher serum UA concentrations are associated with elevated total serum antioxidant capacity among individuals with atherosclerosis, which is consistent with experimental evidence suggesting that hyperuricemia may be a compensatory mechanism to counteract oxidative damage related to atherosclero-

sis[46]. Indeed, high UA concentration might have a protective as well as non-protective role associated with increased cardiovascular risk[45]. These observations suggest that the protective role of UA deserve further investigation.

Conclusions

High UA concentration strongly correlates with coronary endothelial microvascular dysfunction in women. Further, in women, serum UA concentrations correlate with eGFR and ADMA. Thus, hyperuricemia-induced coronary endothelial dysfunction may be mediated via the accumulation of ADMA and renal damage in women.

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