Hyperuricemia is Independently Associated with Endothelial Dysfunction in Postmenopausal Women but not in Premenopausal Women

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Hyperuricemia is Independently Associated with Endothelial Dysfunction in Postmenopausal Women but not in Premenopausal Women

Brief title: Hyperuricemia and endothelial function in women

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Key words: uric acid, endothelial function, flow-mediated vasodilation, menopause

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Abstract

Objectives: The purpose of this study was to determine the relationships between uric acid, endothelial function, and cardiovascular risk factors in women without established cardiovascular disease.

Design: Cross-sectional study.

Setting: Three general hospitals in Japan.

Participants: 749 Japanese women aged 30 to 74 years recruited from people who underwent health-screening examinations with agreement for measurement of vascular function.

Measures: We measured serum concentrations of uric acid and flow-mediated vasodilation (FMD). Percent of FMD (Peak diameter-Baseline diameter/Baseline diameter) was used for analysis. Endothelial dysfunction was defined as FMD equal to or less than 4.90%, division point for the lowest tertile and the middle tertile of FMD. Menopause women were defined as subjects without menstruation for over 1 year or subjects with a history of hysterectomy or bilateral oophrectomy.

Results: Of the 749 subjects, 368 (49.1%) were premenopausal and 381 (50.9%) were postmenopausal women. Age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, estimated glomerular filtration, and Framingham risk score were significantly correlated with serum uric acid level. FMD showed a gradual decrease in accordance with the serum uric acid level (<4.0 mg/dL, 6.85±3.65%; 4.0 to <5.0 mg/dL, 6.79±3.60%; 5.0 to <6.0 mg/dL, 6.24±3.58%; ≥6.0 mg/dL, 5.27±3.18%; P=0.01). Multivariate analysis revealed that uric acid was a significantly independent risk factor for endothelial dysfunction in postmenopausal women (odds ratio; 8.04, 95% CI; 1.68-40.9), but not in premenopausal women.

Conclusions: These findings suggest that uric acid can be used as a risk marker of

endothelial dysfunction. As a causal cardiovascular risk factor, uric acid may be an independent risk factor for endothelial dysfunction in postmenopausal women, but not in premenopausal women.

Article summary

Article focus:

- To investigate the relationships between uric acid, endothelial function assessed by flow-mediated vasodilation (FMD), and cardiovascular risk factors in women without established cardiovascular disease.
- To examine whether menopausal status is associated with the relationship between uric acid and endothelial function in women without established cardiovascular disease.

Key messages

- Uric acid level was significantly associated with cardiovascular parameters and prevalence of cardiovascular risk factors.
- FMD showed a gradual decrease in accordance with the serum uric acid level.
- Uric acid was an independent risk factor for endothelial dysfunction even after adjustment for other cardiovascular risk factors in postmenopausal women, but not in premenopausal women.

Strengths and limitations of this study

- This study includes a large number of women who underwent flow-mediated vasodilation test.
- This study shows a difference in the relationship between uric acid and endothelial dysfunction depending on menopausal status in women without established cardiovascular disease.
- Residual confounding may exist for this cross-sectional study.
- Menopausal status was based on self-report.

Introduction

Several epidemiological studies have shown a relationship between serum uric acid level and subsequent cardiovascular disease.[1-10] In addition, increase in uric acid level is regarded as an independent marker of increased cardiovascular risk. However, it has remained controversial whether uric acid per se should be considered as a cardiovascular risk factor because of the difficulty in investigating the role of uric acid alone in the pathogenesis, development, and maintenance of atherosclerosis. There are significant relationships between elevated uric acid levels and established cardiovascular risk factors, such as hypertension,[11] metabolic syndrome[12] and kidney disease,[13] all of which are also well known as strong predictors of cardiovascular disease.[14 15] Some investigators have argued that elevated uric acid is not an independent risk factor but rather merely a marker of risk for cardiovascular disease.[1,6,7] However, recent epidemiological studies have demonstrated that uric acid is an independent risk factor for cardiovascular disease.[5 7-10] Although the mechanism by which uric acid causes cardiovascular disease is not fully understood, several lines of evidence suggest that elevated uric acid impairs endothelial function by inducing intracellular oxidative stress and inflammation.[16-18]

Endothelial dysfunction is established in the initial step of atherosclerosis and plays an important role in the development of atherosclerotic conditions, leading to cardiovascular outcomes.[19] Recently, measurement of flow-mediated vasodilation (FMD) as an index of endothelium-dependent vasodilation has been widely used as a method for assessing endothelial function.[20-24] In addition, growing evidence has shown that endothelial function assessed by FMD can serve as an independent predictor of cardiovascular events.[25-28] Recently, several investigators, including us, have shown a relationship between uric acid and endothelial function assessed by

FMD.[29-34] As for the relationship between uric acid and FMD in men, previous studies are consistent in demonstrating that uric acid is a significant independent risk factor for FMD.[29 34] However, there is little information on whether uric acid is an independent risk factor for endothelial dysfunction in women. In addition, it remains unclear whether menopausal status is associated with the relationship between uric acid and endothelial function. It is clinically important to confirm the role of uric acid per se in atherosclerosis. We therefore investigated the relationships between uric acid, FMD and cardiovascular risk factors in women without established cardiovascular diseases who underwent health-screening examinations.

Methods

Subjects

A total of 5321 Japanese adults aged 17 to 86 years who underwent health-screening examinations with agreement for measurement of vascular function were enrolled in the Flow-mediated Dilation Japan Registry between 1 April 2010 and 31 August 2012 at 3 general hospitals in Japan. All employees have an obligation to undergo health screening every year under the regulations of the Society-managed Health Insurance Union in Japan. In accordance with the regulations, we performed health-screening examinations. From the registry, 895 women aged 30 to 74 years were recruited for this study. Among the 895 subjects, information on serum uric acid levels, menopause, and phases of menstrual was available for 797 subjects. Subjects during menstruation (n=28), subjects receiving hormone replacement therapy (n=3), one subject who was pregnant, subjects with established cardiovascular diseases (n=15), and one subject who was being treated with an antihyperuricemic drug were excluded. Finally, 749 women without cardiovascular diseases were enrolled in this study. Hypertension was defined

as systolic blood pressure of ≥ 140 mm Hg or diastolic blood pressure of ≥ 90 mm Hg, in a sitting position, on at least 3 different occasions. Patients with secondary forms of hypertension were excluded in all patients with hypertension on the basis of complete history; physical examination; radiological and ultrasound examinations; urinalysis; plasma rennin activity; plasma aldosterone and norepinephrine concentrations; serum creatinine, potassium, calcium, and free throxine concentrations; and 24-hour urinary excretion of 17-hydroxycorticosteroids, 17-ketogenic steroids, and vanillymandelic acid. Diabetes was defined according to the American Diabetes Association.[35] Dyslipidemia was defined according to the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP-III).[36] We defined smokers as those who were current smokers. Measurement of FMD was performed without withholding medications. Framingham risk score was calculated by points of risk factors: age, total cholesterol level, high-density lipoprotein cholesterol level, systolic blood pressure, and smoking status.[37] Diagnosis of metabolic syndrome was made according to the criteria of NCEP ATP-III.[36] Thus, metabolic syndrome was diagnosed when 3 or more of the following risk determinants were present. (1) waist circumference of ≥88 cm, (2) triglyceride ≥1.7 mmol/L (150 mg/dL), (3) high-density lipoprotein cholesterol <1.29 mmol/L (50 mg/dL), (4) blood pressure ≥130 and/or 85 mm Hg, or (5) fasting blood glucose ≥6.11 mmol/L (110 mg/dL). The estimated glomerular filtration rate (eGFR) was calculated using the Japanese eGER equation.[38] Chronic kidney disease (CKD) was defined as eGFR <60 mL/min/1.73 m^2.[39] Menopause women were defined as subjects without menstruation for over 1 year or subjects with a history of hysterectomy or bilateral oophrectomy. The ethical committees of our institutions approved the study protocol. Written informed consent for participation in the study was

obtained from all subjects.

Study protocol

We measured vascular responses to reactive hyperemia in the brachial artery in all subjects. Subjects fasted the previous night for at least 12 hours. The study began at 8:30 AM. 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. After remaining in the supine position for 30 minutes, blood samples were obtained for measurement of basal fasting serum concentrations of total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, glucose, and uric acid. Then FMD was measured. The observers were blind to the form of examination.

Measurement of FMD

The subjects remained supine throughout the study. The 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. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 seconds after cuff deflation. Blood flow velocity was calculated from the Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 seconds, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 minutes. The longitudinal image of the artery was recorded continuously until 5 minutes after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 seconds at baseline and for 10 seconds immediately 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. Percentage of FMD (peak diameter-baseline diameter/baseline diameter) was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area ($-r^2$). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow.

Statistical analysis

Results are presented as mean±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. Relations between variables were determined by Spearman correlation coefficients analysis. Multivariate logistic

regression analyses were performed to identify factors associated with endothelial dysfunction in risk factors and laboratory data. The data were processed using the software package Stata version 9 (Stata Co., College Station, Texas, USA).

Results

Baseline clinical characteristics

The baseline clinical characteristics are summarized in Table 1. Of the 749 subjects, 112 (15.0%) had hypertension, 262 (35.0%) had dyslipidemia, 34 (4.5%) had diabetes mellitus, 59 (7.9%) were current smokers, 65 (8.7%) had metabolic syndrome, and 56 (8.4%) had chronic kidney disease. Mean Framingham risk score was 3.94±3.62%. The mean value of serum uric acid level was 4.44±1.09 mg/dL (median, 4.30 mg/dL; interquartile range, 3.80-5.00 mg/dL; range, 0.80 to 10.0 mg/dL). The mean value of FMD was 6.59±3.60% (range, -4.70 to 20.1%). Division point for the lowest tertile and the middle tertile of FMD was 4.90%. Therefore, endothelial dysfunction was defined as FMD equal or less than 4.90%.

Relationships between serum uric acid level and cardiovascular risk factors

Subjects were categorized according to serum uric acid levels (Table 2). Age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, and Framingham risk score were significantly increased and eGFR was significantly decreased with increase in serum uric acid level. As for the prevalence of cardiovascular risk factors, there were significant increases in the prevalence of hypertension, dyslipidemia, current smoking, metabolic syndrome, chronic kidney disease, and menopause in relation to increase in serum uric acid level.

Relationships between serum uric acid level and FMD

Subjects were categorized into 4 groups on the basis of serum uric acid level. FMD was significantly decreased with increase in serum uric acid level (<4.0 mg/dL, 6.85±3.65%; 4.0 to <5.0 mg/dL, 6.79±3.60%; 5.0 to <6.0 mg/dL, 6.24±3.58%; ≥6.0 mg/dL, 5.27±3.18%; P for trend = 0.01, Figure 1). Multiple logistic regression analysis revealed that serum uric acid level was significantly associated with endothelial dysfunction after adjustment for age (odds ratio, 5.23; 95% CI, 1.36 to 20.4) (Table 3). However, after adjustment for other risk factors, including body mass index, systolic blood pressure, total cholesterol, glucose, smoking status, eGFR, and brachial artery diameter, the significant association between serum uric acid level and endothelial dysfunction disappeared (odds ratio, 2.07; 95% CI, 0.47 to 9.23).

Difference in the relationship between serum uric acid level and endothelial dysfunction according to menopausal status

Subjects were divided into two groups according to menopausal status to investigate the influence of menopause on the relationship between serum uric acid level and endothelial function (supplemental Table I). Of the 749 subjects, 368 (49.1%) were premenopausal and 381 (50.9%) were postmenopausal women. Postmenopausal women were significantly older than premenopausal women (58.6±6.3 vs. 41.5±6.1 years, P<0.001). The ages ranged from 30 to 54 years in premenopausal women and 31 to 74 years in postmenopausal women. Serum uric acid level was significantly higher in postmenopausal women than in premenopausal women (4.64±1.11 vs. 4.24±1.03 mg/dL, P<0.001). FMD was significantly impaired in postmenopausal women compared to that in premenopausal women (5.51±3.14 vs. 7.77±3.71%, P<0.001), and the prevalence of endothelial dysfunction was significantly higher in postmenopausal women than

premenopausal women (66.4% vs. 34.0%, P<0.001). In postmenopausal women, multiple logistic regression analysis revealed that serum uric acid level was significantly associated with endothelial dysfunction after adjustment for age (odds ratio, 7.33; 95% CI, 1.48 to 38.1) (Table 4). The significant association between serum uric acid level and endothelial dysfunction persisted after adjustment for other risk factors (odds ratio, 8.04; 95% CI, 1.68 to 40.9). In contrast, there was no significant association between serum uric acid level and endothelial dysfunction in premenopausal women (odds ratio, 2.98; 95% CI, 0.39 to 21.7) (Table 4). After adjustment for other risk parameters, the association between uric acid level and endothelial dysfunction remained insignificant (odds ratio, 0.13; 95% CI, 0.01 to 1.40).

Discussion

In the present study, we demonstrated that uric acid level was significantly associated with cardiovascular parameters and prevalence of cardiovascular risk factors. Although FMD showed a graded decrease according to serum uric acid level, uric acid was not an independent risk factor for endothelial dysfunction after adjustment for other cardiovascular risk parameters. However, when subjects were divided into two groups according to menopausal status, uric acid was a significantly independent risk factor for endothelial dysfunction even after adjustment for other cardiovascular risk parameters in postmenopausal women, but not in premenopausal women. To our knowledge, this is the first report showing a difference in the relationship between uric acid and endothelial dysfunction depending on menopausal status in women without established cardiovascular disease.

As a marker of atherosclerotic diseases, it has been demonstrated that serum uric acid level is associated with cardiovascular risk factors and subclinical atherosclerosis,

such as hypertension,[11] metabolic syndrome,[12] kidney disease,[13] coronary artery calcification,[40] and carotid atherosclerosis.[41] In the present study, we confirmed that serum uric acid level was significantly associated with age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, and eGFR, most of which are components of metabolic syndrome and CKD. Indeed, the prevalence of hypertension, dyslipidemia, metabolic syndrome, and CKD was linearly increased in relation to increase in serum uric acid level. An association between uric acid and metabolic syndrome has been demonstrated by the fact that uric acid level is often elevated in subjects with metabolic syndrome and that the prevalence of metabolic syndrome linearly increases in relation to uric acid level.[12 41] The mechanism behind the association between increase in serum uric acid level and metabolic syndrome is thought to be hyperinsulinemia, mediated by insulin resistance and visceral adiposity, leading to an increase in uric acid absorption in renal tubules.[42] Uric acid levels are also frequently elevated in patients with kidney disease as a result of reduction in GFR and renal urate excretion.[43] In addition, several epidemiological studies have shown a link between elevated serum uric acid level and subsequent cardiovascular events.[1-10] The association between serum uric acid level and cardiovascular disease has been reported to be generally stronger in women than in men.[9 44-46] These findings suggest that uric acid level is a biochemical marker of atherosclerotic disease and a useful predictor of the development of cardiovascular diseases.

Despite the association between elevated uric acid level and cardiovascular conditions, uric acid has not been established as an independent causal risk factor for cardiovascular disease because of the link between uric acid and established cardiovascular risk factors. Another possible explanation for the difficulty in determining the role of uric acid in cardiovascular disease is that uric acid may function

as an powerful antioxidant and as a scavenger of singlet oxygen and radicals.[47 48] In patients with cardiovascular disease, an increase in uric acid level, therefore, might be considered as a compensatory mechanism to counteract the oxidative stress induced in these conditions. Although it remains controversial whether uric acid is a causative factor or merely a marker of cardiovascular disease, some epidemiological studies have demonstrated that the association between uric acid and cardiovascular disease remains significant even after adjustment for concomitant risk factors, suggesting that uric acid per se is an independent risk factor for cardiovascular disease.

Although the precise mechanism behind the relationship between uric acid and cardiovascular disease remains to be elucidated, growing evidence indicates that endothelial dysfunction induced by uric acid plays an important role in the development of cardiovascular disease.[43] Several lines of evidence suggest that uric acid also has proinflammatory effects on endothelial cells, leading to reduction of endothelial nitric oxide bioavailability and consequent endothelial dysfunction.[17 18] Yu et al.[18] demonstrated that uric acid enhances the production of reactive oxygen species by activation of the local renin-angiotensin system, particularly angiotensin II, in human endothelial vascular cells and that probenecid, an inhibitor of urate transporter, inhibits uric acid-induced oxidative stress. Thus, uric acid is thought to mediate endothelial dysfunction by generating oxidative stress once absorbed into endothelial cells. In a clinical setting, measurement of FMD as an index of endothelium-dependent vasodilation in the brachial artery using high-resolution ultrasound has been widely used as a method for assessing vascular function.[20-23] Although several investigators have shown that uric acid inversely correlates with FMD and that uric acid is an independent predictor of FMD, the subjects in those studies were limited to a small number of subjects or highly selected subjects, such as patients with increased

cardiovascular risk,[30] hyperuricemia,[32] or nondiabetic CKD.[33] In addition, uric acid has been shown to be a significantly independent risk factor for endothelial dysfunction in men in an analysis of a large population,[34] but there is little information on the relationship between uric acid and FMD in women. As a risk marker, we demonstrated that serum uric acid level was significantly associated with FMD in women. FMD was significantly impaired with increase in serum uric acid level. FMD is known to be impaired as a consequence of cumulative cardiovascular risk factors.[21 49] Therefore, significant decrease in FMD according to serum uric acid level may reflect significant associations between uric acid and other cardiovascular risk factors, including hypertension, dyslipidemia, metabolic syndrome, and CKD, as a risk marker for cardiovascular disease in women. These findings provide a rationale for the relationships of uric acid with cardiovascular disease and mortality being generally strong in women.

As for the role of uric acid as a causal risk factor in endothelial function, multivariate analysis performed for the entire population indicated that uric acid was not an independent predictor of endothelial dysfunction after adjustment for other risk factors. However, when subjects were divided into two groups according to menopausal status, multivariate analyses revealed that uric acid was a significantly independent risk factor for endothelial dysfunction in postmenopausal women but not in premenopausal women. The incidence of cardiovascular diseases in women is lower than that in men until around the age of menopause, after which it markedly increases and becomes equal to that of their male counterparts, suggesting a protective effect of endogenous ovarian hormones on atherosclerosis.[50] Ovarian hormones, especially estradiol, have been suggested to have protective effects on endothelial function.[51 52] In addition to its protective effect on endothelial function, estradiol has been shown to lower uric acid

level through mechanisms involving renal clearance, secretion and reabsorption.[53-55] Therefore, endogenous estradiol may preserve endothelial function and lower serum uric acid level regardless of the presence or absence of cardiovascular risk factors, resulting in the weak relationship between uric acid and endothelial dysfunction in premenopausal women. Our findings in the analysis of premenopausal women are consistent with the results of a previous study demonstrating that uric acid was not an independent risk factor of FMD in young women aged 30 to 45 years.[56] In contrast to premenopausal women, uric acid remained a significantly independent risk for endothelial dysfunction even after adjustment for other risk factors in postmenopausal women. Although Maxwell et al.[29] reported that uric acid was not an independent determinant of FMD in women, the non-independent association between FMD and uric acid observed in their study may be, in part, attributed to inclusion of entire women in the analysis for the relationship between FMD and uric acid without taking into account menopausal status. The increased cardiovascular risk in postmenopausal women has been suggested to be associated with impairment of endothelial function after menopause.[57 58] There is a possibility that treatment of hyperuricemia in postmenopausal women will improve endothelial function, leading to a decrease in cardiovascular events.

In conclusion, uric acid is a useful marker for endothelial function in women. Significant relationships between serum uric acid level and cardiovascular risk factors, including hypertension, dyslipidemia, metabolic syndrome, and CKD, may result in the significant association between FMD and serum uric acid level, as a risk marker of atherosclerotic diseases. Uric acid, as a causal cardiovascular risk factor, may be an independent risk for endothelial dysfunction in postmenopausal women but not in premenopausal women. Further studies are needed to investigate whether treatment for

hyperuricemia improves endothelial function in postmenopausal women.

Table 1. Clinical Characteristics of the Subjects

Variables	Total (n=749)
Age, y	50.2±10.6
Body mass index, kg/m^2	22.0±3.4
Systolic blood pressure, mm Hg	121.1±19.0
Diastolic blood pressure, mm Hg	73.9±12.4
Heart rate, bpm	64.4±10.2
Total cholesterol, mmol/L	5.37±0.88
Triglycerides, mmol/L	1.07±0.71
HDL-cholesterol, mmol/L	1.80±0.42
LDL-cholesterol, mmol/L	3.11±0.77
Glucose, mmol/L	5.32±1.47
Uric acid, mg/dL	4.44±1.09
eGFR, mL/min/1.73 m^2	77.1±13.3
Framingham risk score, %	3.94±3.62
Hypertension, n (%)	112 (15.0)
Dyslipidemia, n (%)	262 (35.0)
Diabetes mellitus, n (%)	34 (4.5)
Smoking, n (%)	59 (7.9)
Metabolic syndrome, n (%)	65 (8.7)
Chronic kidney disease, n (%)	56 (8.4)
Menopause, n (%)	381 (50.9)
Flow-mediated vasodilation, %	6.59±3.60
Baseline brachial artery diameter, mm	3.40±0.47

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

Table 2. Clinical Characteristics according to Serum Uric Acid Levels

	Uric acid categories, mg/dL				
Variables	<4.0 (n=245)	4.0 to <5.0 (n=302)	5.0 to <6.0 (n=144)	6.0≤ (n=58)	P value
Uric acid, mg/dL	3.38±0.54	4.42±0.28	5.33±0.26	6.90±0.90	
Age, y	48.3±10.9	49.4±10.1	53.5±9.9	54.7±10.0	<0.001
Body mass index, kg/m^2	20.8±2.8	22.0±3.4	23.0±3.3	24.2±3.9	<0.001
Systolic blood pressure, mm Hg	117.2±17.6	120.4±18.3	125.9±20.4	129.2±20.5	<0.001
Diastolic blood pressure, mm Hg	71.5±11.4	73.6±12.1	77.4±13.4	77.0±12.5	<0.001
Heart rate, bpm	64.6±9.6	63.5±0.6	65.5±12.4	65.8±11.1	0.14
Total cholesterol, mmol/L	5.20±0.82	5.33±0.86	5.60±0.91	5.67±0.88	<0.001
Triglycerides, mmol/L	0.89±0.51	0.97±0.50	1.32±0.81	1.69±1.32	<0.001
HDL-cholesterol, mmol/L	1.83±0.38	1.83±0.41	1.69±0.45	1.69±0.54	0.001
LDL-cholesterol, mmol/L	3.00±0.70	3.10±0.77	3.31±0.80	3.19±0.96	0.006
Glucose, mmol/L	5.09±0.84	5.28±1.38	5.52±2.00	6.03±2.09	<0.001
eGFR, mL/min/1.73 m^2	80.9±13.7	76.8±12.1	74.4±13.0	67.7±13.8	<0.001
Baseline brachial artery diameter, mm	3.35±0.46	3.40±0.48	3.43±0.46	3.61±0.51	0.002
Framingham risk score, %	3.27±3.44	3.49±2.98	5.08±3.65	6.33±5.42	<0.001
Hypertension, n (%)	25 (10.2)	34 (11.3)	34 (23.6)	19 (32.8)	<0.001
Dyslipidemia, n (%)	59 (24.1)	90 (29.8)	72 (50.0)	41 (70.7)	<0.001
Diabetes mellitus, n (%)	9 (3.7)	8 (2.7)	9 (6.3)	8 (13.8)	0.008
Smoking, n (%)	12 (4.9)	23 (7.6)	15 (10.4)	9 (15.5)	0.04
Metabolic syndrome, n (%)	12 (4.9)	18 (6.0)	19 (13.2)	16 (28.1)	<0.001
Chronic kidney disease, n (%)	10 (4.6)	22 (7.9)	14 (10.9)	10 (24.4)	0.001
Menopause, n (%)	103 (42.0)	146 (48.3)	91 (63.2)	41 (70.7)	<0.001
Endothelial dysfunction, n (%)	75 (30.6)	87 (28.8)	50 (34.7)	32 (55.2)	0.002

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; P values for comparisons across the uric acid categories were performed with ANOVA for continuous variables and χ^2 test for categorical variables.

Table 3. Multivariate Analysis of the Relation Between Flow-mediated Vasodilation and Variables

Model	Variables	Odds ratio (95%CI)	P value
1	Uric acid (mg/dL)	11.2 (3.07 to 41.8)	<0.001
2	1+age	5.23 (1.36 to 20.4)	0.02
3	2+other variables	2.07 (0.47 to 9.23)	0.34

Other variables include body mass index, systolic blood pressure, total cholesterol, glucose, smoking status, eGFR, and brachial artery diameter.

Table 4. Multivariate Analysis of the Relation Between Endothelial Dysfunction and Variables

		Post-menopausal		Pre-menopausal	
Model	Covariates	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
1	Uric acid (mg/dL)	7.60 (1.55 to 39.2)	0.01	2.98 (0.39 to 21.7)	0.28
2	1+age	7.33 (1.48 to 38.1)	0.02	2.01 (0.24 to 16.5)	0.50
3	2+other variables	8.04 (1.68 to 40.9)	0.01	0.13 (0.01 to 1.40)	0.09

Other variables include body mass index, systolic blood pressure, total cholesterol, glucose, smoking status, eGFR, and brachial artery diameter.

Author Contributions

Tatsuya Maruhashi and Yukihito Higashi, drafting the article and conception of this study; Junko Soga, Noritaka Fujimura, Naomi Idei, Shinsuke Mikami,Yumiko Iwamoto, Masato Kajikawa, Takeshi Matsumoto, Takayuki Hidaka, Chikara Goto, Kensuke Noma, Ayumu Nakashima, Bonpei Takase, and Hirofumi Tomiyama, performing the ultrasonogarphy; Kazuaki Chayama, Yasuki Kihara, Akira Yamashina, revising the article critically for important intellectual content.

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Competing interests

None.

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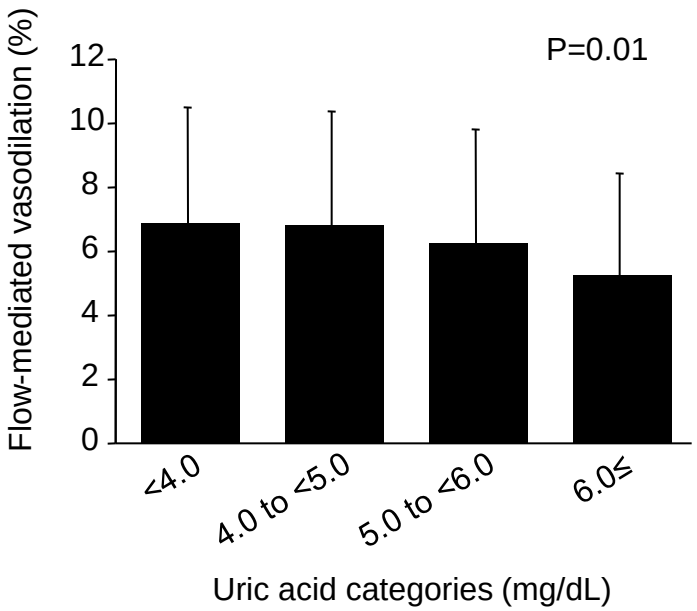
Figure legends

Figure 1. Bar graphs show flow-mediated vasodilation in women categorized according to serum uric acid level.


P=0.01
Flow-mediated vasodilation (%)
12
10
8
6
4
2
0
<4.0
4.0 to <5.0
5.0 to <6.0
6.0≤
Uric acid categories (mg/dL)

Supplemental data

Hyperuricemia is Independently Associated with Endothelial Dysfunction in Postmenopausal Women but not in Premenopausal Women

Brief title: Hyperuricemia and endothelial function in women

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Table I. Clinical Characteristics of Premenopause and Menopause Women

Variables	Premenopause (n=368)	Postmenopause (n=381)	P value
Age, y	41.5±6.1	58.6±6.3	<0.001
Body mass index, kg/m^2	21.4±3.5	22.5±3.2	<0.001
Systolic blood pressure, mm Hg	113.6±14.4	128.3±20.2	<0.001
Diastolic blood pressure, mm Hg	70.6±11.3	77.9±12.6	<0.001
Heart rate, bpm	63.5±9.6	65.2±10.6	0.02
Total cholesterol, mmol/L	5.05±0.80	5.67±0.84	<0.001
Triglycerides, mmol/L	0.87±0.59	1.25±0.76	<0.001
HDL-cholesterol, mmol/L	1.82±0.40	1.77±0.45	0.11
LDL-cholesterol, mmol/L	2.85±0.68	3.36±0.78	<0.001
Glucose, mmol/L	5.09±0.82	5.55±1.87	<0.001
Uric acid, mg/dL	4.24±1.03	4.64±1.11	<0.001
eGFR, mL/min/1.73 m^2	79.2±12.8	74.9±13.5	<0.001
Framingham risk score, %	1.67±1.36	6.13±3.77	<0.001
Hypertension, n (%)	12 (3.3)	100 (26.3)	<0.001
Dyslipidemia, n (%)	65 (17.7)	197 (51.7)	<0.001
Diabetes mellitus, n (%)	8 (2.2)	26 (6.8)	0.002
Smoking, n (%)	34 (9.2)	25 (6.6)	0.18
Metabolic syndrome, n (%)	17 (4.6)	48 (12.6)	<0.001
Chronic kidney disease, n (%)	18 (5.2)	38 (11.8)	0.002
Endothelial dysfunction, n (%)	125 (34.0)	253 (66.4)	<0.001
Flow-mediated vasodilation, %	7.77±3.71	5.51±3.14	<0.001
Baseline brachial artery diameter, mm	3.28±0.41	3.52±0.49	<0.001

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

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Hyperuricemia is Independently Associated with Endothelial Dysfunction in Postmenopausal Women but not in Premenopausal Women

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Primary Subject Heading:	Cardiovascular medicine

Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	VASCULAR MEDICINE, PATHOLOGY, General endocrinology < DIABETES & ENDOCRINOLOGY

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Key words: uric acid, endothelial function, flow-mediated vasodilation, menopause

Word count: 3612 words

Abstract

Objectives: The purpose of this study was to determine the relationships between uric acid, endothelial function, and cardiovascular risk factors and to investigate whether menopausal status was associated with the relationship between uric acid and endothelial function in women.

Design: Cross-sectional study.

Setting: Three general hospitals in Japan.

Participants: 749 Japanese women aged 30 to 74 years recruited from people who underwent health-screening examinations with agreement for measurement of vascular function.

Measures: We measured serum concentrations of uric acid and flow-mediated vasodilation (FMD). Percent of FMD (Peak diameter-Baseline diameter/Baseline diameter) was used for analysis. Endothelial dysfunction was defined as FMD equal to or less than 4.90%, division point for the lowest tertile and the middle tertile of FMD. Menopause women were defined as subjects without menstruation for over 1 year or subjects with a history of hysterectomy or bilateral oophrectomy.

Results: Of the 749 subjects, 368 (49.1%) were premenopausal and 381 (50.9%) were postmenopausal women. Age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, estimated glomerular filtration, and Framingham risk score were significantly correlated with serum uric acid level. FMD showed a gradual decrease in accordance with the serum uric acid level in the entire study population (<4.0 mg/dL, 6.85±3.65%; 4.0 to <5.0 mg/dL, 6.79±3.60%; 5.0 to <6.0 mg/dL, 6.24±3.58%; ≥6.0 mg/dL, 5.27±3.18%; P=0.01). Multivariate analysis revealed that uric acid was a significantly independent risk factor for endothelial dysfunction in postmenopausal women (odds ratio; 1.23, 95% confidence interval; 1.01-1.50), but not

in premenopausal women.

Conclusions: These findings suggest that uric acid can be used as a risk marker of endothelial dysfunction in a female population, and particularly as an independent risk factor in postmenopausal women but not in premenopausal women.

Registration number of the study: UMIN000003409

Article summary

Article focus:

- To investigate the relationships between uric acid, endothelial function assessed by flow-mediated vasodilation (FMD), and cardiovascular risk factors in women.
- To examine whether menopausal status is associated with the relationship between uric acid and endothelial function in women.

Key messages

- Uric acid level was significantly associated with cardiovascular parameters and prevalence of cardiovascular risk factors in the entire study population.
- FMD showed a gradual decrease in accordance with the serum uric acid level in the entire study population.
- Uric acid was an independent risk factor for endothelial dysfunction even after adjustment for other cardiovascular risk factors in postmenopausal women, but not in premenopausal women.

Strengths and limitations of this study

- This study includes a large number of women who underwent flow-mediated vasodilation test.
- This study shows a difference in the relationship between uric acid and endothelial dysfunction depending on menopausal status in women.
- Residual confounding may exist for this cross-sectional study.
- Menopausal status was based on self-report.

Introduction

Several epidemiological studies have shown a relationship between serum uric acid level and subsequent cardiovascular disease.[1-10] In addition, increase in uric acid level is regarded as an independent marker of increased cardiovascular risk. However, it has remained controversial whether uric acid per se should be considered as a cardiovascular risk factor because of the difficulty in investigating the role of uric acid alone in the pathogenesis, development, and maintenance of atherosclerosis. There are significant relationships between elevated uric acid levels and established cardiovascular risk factors, such as hypertension,[11] metabolic syndrome[12] and kidney disease,[13] all of which are also well known as strong predictors of cardiovascular disease.[14 15] Some investigators have argued that elevated uric acid is not an independent risk factor but rather merely a marker of risk for cardiovascular disease.[1,6,7] However, recent epidemiological studies have demonstrated that uric acid is an independent risk factor for cardiovascular disease.[5 7-10] Although the mechanism by which uric acid causes cardiovascular disease is not fully understood, several lines of evidence suggest that elevated uric acid impairs endothelial function by inducing intracellular oxidative stress and inflammation through activation of the local renin-angiotensin system, particularly angiontensin II, and the pro-oxidant effect of uric acid per se, once absorbed into endothelial cells.[16-18]

Endothelial dysfunction is established in the initial step of atherosclerosis and plays an important role in the development of atherosclerotic conditions, leading to cardiovascular outcomes.[19] Recently, measurement of flow-mediated vasodilation (FMD) as an index of endothelium-dependent vasodilation has been widely used as a method for assessing endothelial function.[20-24] In addition, growing evidence has shown that endothelial function assessed by FMD can serve as an independent predictor

of cardiovascular events.[25-28] Recently, several investigators, including us, have shown a relationship between uric acid and endothelial function assessed by FMD.[29-34] As for the relationship between uric acid and FMD in men, previous studies are consistent in demonstrating that uric acid is a significant independent risk factor for FMD.[29 34] However, there is little information on whether uric acid is an independent risk factor for endothelial dysfunction in women. In addition, it remains unclear whether menopausal status is associated with the relationship between uric acid and endothelial function. It is clinically important to confirm the role of uric acid per se in atherosclerosis. We therefore investigated the relationships between uric acid, FMD and cardiovascular risk factors and whether menopausal status was associated with the relationship between uric acid and endothelial function in women who underwent health-screening examinations.

Methods

Subjects

A total of 5321 Japanese adults aged 17 to 86 years who underwent health-screening examinations with agreement for measurement of vascular function were enrolled in the Flow-mediated Dilation Japan Registry between 1 April 2010 and 31 August 2012 at 3 general hospitals in Japan. All employees have an obligation to undergo health screening every year under the regulations of the Society-managed Health Insurance Union in Japan. In accordance with the regulations, we performed health-screening examinations. From the registry, 895 women aged 30 to 74 years were recruited for this study. Among the 895 subjects, information on serum uric acid levels, menopause, and phases of menstrual was available for 797 subjects. Subjects during menstruation (n=28), subjects receiving hormone replacement therapy (n=3), one subject who was

pregnant, subjects who had been previously diagnosed with cardiovascular diseases (n=15), and one subject who was being treated with an antihyperuricemic drug were excluded. Finally, 749 women without cardiovascular diseases were enrolled in this study. Hypertension was defined as systolic blood pressure of ≥ 140 mm Hg or diastolic blood pressure of ≥ 90 mm Hg, in a sitting position, on at least 3 different occasions. [35] Patients with secondary forms of hypertension were excluded in all patients with hypertension on the basis of complete history; physical examination; radiological and ultrasound examinations; urinalysis; plasma rennin activity; plasma aldosterone and norepinephrine concentrations; serum creatinine, potassium, calcium, and free throxine concentrations; and 24-hour urinary excretion of 17-hydroxycorticosteroids, 17-ketogenic steroids, and vanillymandelic acid. Diabetes was defined according to the American Diabetes Association.[36] Dyslipidemia was defined according to the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP-III).[37] We defined smokers as those who were current smokers. Measurement of FMD was performed without withholding medications. Framingham risk score was calculated by points of risk factors: age, total cholesterol level, high-density lipoprotein cholesterol level, systolic blood pressure, and smoking status.[38] Diagnosis of metabolic syndrome was made according to the criteria of NCEP ATP-III.[37] Thus, metabolic syndrome was diagnosed when 3 or more of the following risk determinants were present. (1) waist circumference of ≥88 cm, (2) triglyceride ≥1.7 mmol/L (150 mg/dL), (3) high-density lipoprotein cholesterol <1.29 mmol/L (50 mg/dL), (4) blood pressure ≥130 and/or 85 mm Hg, or (5) fasting blood glucose ≥6.11 mmol/L (110 mg/dL). The estimated glomerular filtration rate (eGFR) was calculated using the Japanese eGER equation.[39] Chronic kidney disease (CKD) was defined as eGFR <60

mL/min/1.73 m^2.[40] Menopause women were defined as subjects without menstruation for over 1 year or subjects with a history of hysterectomy or bilateral oophrectomy.[41] The ethical committees of our institutions approved the study protocol. Written informed consent for participation in the study was obtained from all subjects.

Study protocol

We measured vascular responses to reactive hyperemia in the brachial artery in all subjects. Subjects fasted the previous night for at least 12 hours. The study began at 8:30 AM. 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. After remaining in the supine position for 30 minutes, blood samples were obtained for measurement of basal fasting serum concentrations of total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, glucose, and uric acid. Then FMD was measured. The observers were blind to the form of examination. Serum uric acid levels were measured by the uricase-peroxidase method (JCA-BM6010; JEOL Ltd., Tokyo, Japan). Levels of serum total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and creatinine were enzymatically measured (JCA-BM6010; JEOL Ltd., Tokyo, Japan). Glucose levels were measured by the glucose oxidase immobilezed oxygen electrode method (GA08II; A&T, Yokohama, Japan).

Measurement of FMD

The subjects remained supine throughout the study. The 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. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 seconds after cuff deflation. Blood flow velocity was calculated from the Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 seconds, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 minutes. The longitudinal image of the artery was recorded continuously until 5 minutes after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 seconds at baseline and for 10 seconds immediately 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. Percentage of FMD (peak diameter-baseline diameter/baseline diameter) was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the

angle) by heart rate and vessel cross-sectional area (-r^2). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow.

Statistical analysis

Results are presented as mean±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. Comparisons between the groups categorized according to serum uric acid level were carried out using P for trend analysis with Bonferroni's test for post-hoc comparisons. Relations between variables were determined by Spearman correlation coefficients analysis. Stepwise multivariate logistic regression analyses were performed to identify factors associated with endothelial dysfunction among potential confounders (P<0.20) in univariate analysis. Using variables associated with endothelial dysfunction in stepwise multiple regression analyses, we performed the final logistic regression analyses for which results are presented in the results section. The data were processed using the software package Stata version 9 (Stata Co., College Station, Texas, USA).

Results

Baseline clinical characteristics

The baseline clinical characteristics are summarized in Table 1. Of the 749 subjects, 112 (15.0%) had hypertension, 262 (35.0%) had dyslipidemia, 34 (4.5%) had diabetes mellitus, 59 (7.9%) were current smokers, 65 (8.7%) had metabolic syndrome, and 56 (8.4%) had chronic kidney disease. Mean Framingham risk score was 3.94±3.62%. The mean value of serum uric acid level was 4.44±1.09 mg/dL (median, 4.30 mg/dL;

interquartile range, 3.80-5.00 mg/dL; range, 0.80 to 10.0 mg/dL). The mean value of FMD was 6.59±3.60% (range, -4.70 to 20.1%). Division point for the lowest tertile and the middle tertile of FMD was 4.90%. Therefore, endothelial dysfunction was defined as FMD equal or less than 4.90%.

Relationships between serum uric acid level and cardiovascular risk factors

Univariate regression analysis revealed that serum uric acid level significantly correlated with age (r=0.21, P<0.001), body mass index (r=0.26, P<0.001), systolic blood pressure (r=0.17, P<0.001), diastolic blood pressure (r=0.17, P<0.001), total cholesterol (r=0.23, P<0.001), triglycerides (r=0.25, P<0.001), high-density lipoprotein cholesterol (r=-0.13, P=0.002), low-density lipoprotein cholesterol (r=0.22, P<0.001), glucose (r=0.12, P=0.004), and eGFR (r=-0.27, P<0.001). Subjects were categorized according to serum uric acid levels (Table 2). Age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, and Framingham risk score were significantly increased and eGFR was significantly decreased with increase in serum uric acid level. As for the prevalence of cardiovascular risk factors, there were significant increases in the prevalence of hypertension, dyslipidemia, current smoking, metabolic syndrome, chronic kidney disease, and menopause in relation to increase in serum uric acid level.

Relationships between serum uric acid level and FMD

Subjects were categorized into 4 groups on the basis of serum uric acid level. FMD was significantly decreased with increase in serum uric acid level (<4.0 mg/dL, 6.85±3.65%; 4.0 to <5.0 mg/dL, 6.79±3.60%; 5.0 to <6.0 mg/dL, 6.24±3.58%; ≥6.0 mg/dL, 5.27±3.18%; P for trend = 0.01, Figure 1). Multiple logistic regression analysis revealed

that serum uric acid level was significantly associated with endothelial dysfunction after adjustment for age (odds ratio, 1.20; 95% confident interval [CI], 1.03 to 1.39) (Table 3). However, after adjustment for other risk factors, including high-density lipoprotein cholesterol, glucose, and smoking status, the significant association between serum uric acid level and endothelial dysfunction disappeared (odds ratio, 1.13; 95% CI, 0.97 to 1.32).

Difference in the relationship between serum uric acid level and endothelial dysfunction according to menopausal status

Subjects were divided into two groups according to menopausal status to investigate the influence of menopause on the relationship between serum uric acid level and endothelial function (supplemental Table I). Of the 749 subjects, 368 (49.1%) were premenopausal and 381 (50.9%) were postmenopausal women. Postmenopausal women were significantly older than premenopausal women (58.6±6.3 vs. 41.5±6.1 years, P<0.001). The ages ranged from 30 to 54 years in premenopausal women and 31 to 74 years in postmenopausal women. Serum uric acid level was significantly higher in postmenopausal women than in premenopausal women (4.64±1.11 vs. 4.24±1.03 mg/dL, P<0.001). FMD was significantly impaired in postmenopausal women compared to that in premenopausal women (5.51±3.14 vs. 7.77±3.71%, P<0.001), and the prevalence of endothelial dysfunction was significantly higher in postmenopausal women than premenopausal women (66.4% vs. 34.0%, P<0.001). In postmenopausal women, multiple logistic regression analysis revealed that serum uric acid level was significantly associated with endothelial dysfunction after adjustment for age (odds ratio, 1.26; 95% CI, 1.05 to 1.53) (Table 4). The significant association between serum uric acid level and endothelial dysfunction persisted after adjustment for other risk factors (odds ratio,

1.23; 95% CI, 1.01 to 1.50). In contrast, there was no significant association between serum uric acid level and endothelial dysfunction in premenopausal women (odds ratio, 1.14; 95% CI, 0.90 to 1.44) (Table 4). After adjustment for other risk parameters, the association between uric acid level and endothelial dysfunction remained insignificant (odds ratio, 0.98; 95% CI, 0.75 to 1.26).

Discussion

In the present study, we demonstrated that uric acid level was significantly associated with cardiovascular parameters and prevalence of cardiovascular risk factors. Although FMD showed a graded decrease according to serum uric acid level, uric acid was not an independent risk factor for endothelial dysfunction after adjustment for other cardiovascular risk parameters. However, when subjects were divided into two groups according to menopausal status, uric acid was a significantly independent risk factor for endothelial dysfunction even after adjustment for other cardiovascular risk parameters in postmenopausal women, but not in premenopausal women. To our knowledge, this is the first report showing a difference in the relationship between uric acid and endothelial dysfunction depending on menopausal status in women.

As a marker of atherosclerotic diseases, it has been demonstrated that serum uric acid level is associated with cardiovascular risk factors and subclinical atherosclerosis, such as hypertension,[11] metabolic syndrome,[12] kidney disease,[13] coronary artery calcification,[42] and carotid atherosclerosis.[43] In the present study, we confirmed that serum uric acid level was significantly associated with age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, and eGFR, most of which are components of metabolic syndrome and CKD. Indeed, the prevalence of hypertension, dyslipidemia, metabolic syndrome, and CKD was linearly increased in

relation to increase in serum uric acid level. An association between uric acid and metabolic syndrome has been demonstrated by the fact that uric acid level is often elevated in subjects with metabolic syndrome and that the prevalence of metabolic syndrome linearly increases in relation to uric acid level.[12 43] The mechanism behind the association between increase in serum uric acid level and metabolic syndrome is thought to be hyperinsulinemia, mediated by insulin resistance and visceral adiposity, leading to an increase in uric acid absorption in renal tubules.[44] Uric acid levels are also frequently elevated in patients with kidney disease as a result of reduction in GFR and renal urate excretion.[45] In addition, several epidemiological studies have shown a link between elevated serum uric acid level and subsequent cardiovascular events.[1-10] The association between serum uric acid level and cardiovascular disease has been reported to be generally stronger in women than in men.[9 46-48] These findings suggest that uric acid level is a biochemical marker of atherosclerotic disease and a useful predictor of the development of cardiovascular diseases.

Despite the association between elevated uric acid level and cardiovascular conditions, uric acid has not been established as an independent causal risk factor for cardiovascular disease because of the link between uric acid and established cardiovascular risk factors. Another possible explanation for the difficulty in determining the role of uric acid in cardiovascular disease is that uric acid may function as an powerful antioxidant and as a scavenger of singlet oxygen and radicals.[49 50] In patients with cardiovascular disease, an increase in uric acid level, therefore, might be considered as a compensatory mechanism to counteract the oxidative stress induced in these conditions. Although it remains controversial whether uric acid is a causative factor or merely a marker of cardiovascular disease, some epidemiological studies have demonstrated that the association between uric acid and cardiovascular disease remains

significant even after adjustment for concomitant risk factors, suggesting that uric acid per se is an independent risk factor for cardiovascular disease.[5 7-10]

Although the precise mechanism behind the relationship between uric acid and cardiovascular disease remains to be elucidated, growing evidence indicates that endothelial dysfunction induced by uric acid plays an important role in the development of cardiovascular disease.[45] Several lines of evidence suggest that uric acid also has proinflammatory effects on endothelial cells, leading to reduction of endothelial nitric oxide bioavailability and consequent endothelial dysfunction.[17 18] Yu et al.[18] demonstrated that uric acid enhances the production of reactive oxygen species by activation of the local renin-angiotensin system, particularly angiotensin II, in human endothelial vascular cells and that probenecid, an inhibitor of urate transporter, inhibits uric acid-induced oxidative stress. Thus, uric acid is thought to mediate endothelial dysfunction by generating oxidative stress once absorbed into endothelial cells. In a clinical setting, measurement of FMD as an index of endothelium-dependent vasodilation in the brachial artery using high-resolution ultrasound has been widely used as a method for assessing vascular function.[20-23] Although several investigators have shown that uric acid inversely correlates with FMD and that uric acid is an independent predictor of FMD, the subjects in those studies were limited to a small number of subjects or highly selected subjects, such as patients with increased cardiovascular risk,[30] hyperuricemia,[32] or nondiabetic CKD.[33] In addition, uric acid has been shown to be a significantly independent risk factor for endothelial dysfunction in men in an analysis of a large population,[34] but there is little information on the relationship between uric acid and FMD in women. As a risk marker, we demonstrated that serum uric acid level was significantly associated with FMD in women. FMD was significantly impaired with increase in serum uric acid level. FMD is

known to be impaired as a consequence of cumulative cardiovascular risk factors.[21 51] Therefore, significant decrease in FMD according to serum uric acid level may reflect significant associations between uric acid and other cardiovascular risk factors, including hypertension, dyslipidemia, metabolic syndrome, and CKD, as a risk marker for cardiovascular disease in women. These findings provide a rationale for the relationships of uric acid with cardiovascular disease and mortality being generally strong in women.

As for the role of uric acid as a causal risk factor in endothelial function, multivariate analysis performed for the entire population indicated that uric acid was not an independent predictor of endothelial dysfunction after adjustment for other risk factors. However, when subjects were divided into two groups according to menopausal status, multivariate analyses revealed that uric acid was a significantly independent risk factor for endothelial dysfunction in postmenopausal women but not in premenopausal women. The incidence of cardiovascular diseases in women is lower than that in men until around the age of menopause, after which it markedly increases and becomes equal to that of their male counterparts, suggesting a protective effect of endogenous ovarian hormones on atherosclerosis.[52] Ovarian hormones, especially estradiol, have been suggested to have protective effects on endothelial function.[53 54] In addition to its protective effect on endothelial function, estradiol has been shown to lower uric acid level through mechanisms involving renal clearance, secretion and reabsorption.[55-57] Therefore, endogenous estradiol may preserve endothelial function and lower serum uric acid level regardless of the presence or absence of cardiovascular risk factors, resulting in the weak relationship between uric acid and endothelial dysfunction in premenopausal women. Our findings in the analysis of premenopausal women are consistent with the results of a previous study demonstrating that uric acid was not an

independent risk factor of FMD in young women aged 30 to 45 years.[58] In contrast to premenopausal women, uric acid remained a significantly independent risk for endothelial dysfunction even after adjustment for other risk factors in postmenopausal women. Although Maxwell et al.[29] reported that uric acid was not an independent determinant of FMD in women, the non-independent association between FMD and uric acid observed in their study may be, in part, attributed to inclusion of entire women in the analysis for the relationship between FMD and uric acid without taking into account menopausal status. The increased cardiovascular risk in postmenopausal women has been suggested to be associated with impairment of endothelial function after menopause.[59 60] There is a possibility that treatment of hyperuricemia in postmenopausal women will improve endothelial function, leading to a decrease in cardiovascular events.

There were some limitations in this study. The cross-sectional design did not allow us to establish a definitive causal relationship between hyperuricemia and endothelial dysfunction. In addition, multiple factors, such as vitamins, phosphate and statin use, that are known to influence FMD and residual unrecognized confounding factors were not taken into account in this study. Future prospective and interventional studies are certainly warranted to obtain more specific conclusions as to whether hyperuricemia should be treated, which subgroup should be treated, and whether treatment of hyperuricemia improves endothelial function with subsequent decrease in cardiovascular events.

In conclusion, uric acid is a useful marker for endothelial function in women. Significant relationships between serum uric acid level and cardiovascular risk factors, including hypertension, dyslipidemia, metabolic syndrome, and CKD, may result in the significant association between FMD and serum uric acid level, as a risk marker of

atherosclerotic diseases. Uric acid, as a causal cardiovascular risk factor, may be an independent risk for endothelial dysfunction in postmenopausal women but not in premenopausal women. Further studies are needed to investigate whether treatment for hyperuricemia improves endothelial function in postmenopausal women.

Table 1. Clinical Characteristics of the Subjects

Variables	Total (n=749)
Age, y	50.2±10.6
Body mass index, kg/m^2	22.0±3.4
Systolic blood pressure, mm Hg	121.1±19.0
Diastolic blood pressure, mm Hg	73.9±12.4
Heart rate, bpm	64.4±10.2
Total cholesterol, mmol/L	5.37±0.88
Triglycerides, mmol/L	1.07±0.71
HDL-cholesterol, mmol/L	1.80±0.42
LDL-cholesterol, mmol/L	3.11±0.77
Glucose, mmol/L	5.32±1.47
Uric acid, mg/dL	4.44±1.09
eGFR, mL/min/1.73 m^2	77.1±13.3
Framingham risk score, %	3.94±3.62
Hypertension, n (%)	112 (15.0)
Dyslipidemia, n (%)	262 (35.0)
Diabetes mellitus, n (%)	34 (4.5)
Smoking, n (%)	59 (7.9)
Metabolic syndrome, n (%)	65 (8.7)
Chronic kidney disease, n (%)	56 (8.4)
Menopause, n (%)	381 (50.9)
Flow-mediated vasodilation, %	6.59±3.60
Baseline brachial artery diameter, mm	3.40±0.47

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

Table 2. Clinical Characteristics according to Serum Uric Acid Levels *†§

	Uric acid categories, mg/dL				
Variables	<4.0 (n=245)	4.0 to <5.0 (n=302)	5.0 to <6.0 (n=144)	6.0≤ (n=58)	P value
Uric acid, mg/dL	3.38±0.54	4.42±0.28	5.33±0.26	6.90±0.90	
Age, y	48.3±10.9	49.4±10.1	53.5±9.9*†	54.7±10.0*†	<0.001
Body mass index, kg/m^2	20.8±2.8	22.0±3.4*	23.0±3.3*†	24.2±3.9*†	<0.001
Systolic blood pressure, mm Hg	117.2±17.6	120.4±18.3	125.9±20.4*†	129.2±20.5*†	<0.001
Diastolic blood pressure, mm Hg	71.5±11.4	73.6±12.1	77.4±13.4*†	77.0±12.5*	<0.001
Heart rate, bpm	64.6±9.6	63.5±0.6	65.5±12.4	65.8±11.1	0.14
Total cholesterol, mmol/L	5.20±0.82	5.33±0.86	5.60±0.91*†	5.67±0.88*†	<0.001
Triglycerides, mmol/L	0.89±0.51	0.97±0.50	1.32±0.81*†	1.69±1.32*†§	<0.001
HDL-cholesterol, mmol/L	1.83±0.38	1.83±0.41	1.69±0.45*†	1.69±0.54	0.001
LDL-cholesterol, mmol/L	3.00±0.70	3.10±0.77	3.31±0.80*	3.19±0.96	0.006
Glucose, mmol/L	5.09±0.84	5.28±1.38	5.52±2.00*	6.03±2.09*†	<0.001
eGFR, mL/min/1.73 m^2	80.9±13.7	76.8±12.1*	74.4±13.0*	67.7±13.8*†§	<0.001
Flow-mediated vasodilation, %	6.85±3.65	6.79±3.60	6.24±3.58	5.27±3.18*†	0.01
Baseline brachial artery diameter, mm	3.35±0.46	3.40±0.48	3.43±0.46	3.61±0.51*†	0.002
Framingham risk score, %	3.27±3.44	3.49±2.98	5.08±3.65*†	6.33±5.42*†	<0.001
Hypertension, n (%)	25 (10.2)	34 (11.3)	34 (23.6)*†	19 (32.8)*†	<0.001
Dyslipidemia, n (%)	59 (24.1)	90 (29.8)	72 (50.0)*†	41 (70.7)*†§	<0.001
Diabetes mellitus, n (%)	9 (3.7)	8 (2.7)	9 (6.3)	8 (13.8)*†	0.008
Smoking, n (%)	12 (4.9)	23 (7.6)	15 (10.4)	9 (15.5)	0.04
Metabolic syndrome, n (%)	12 (4.9)	18 (6.0)	19 (13.2)*	16 (28.1)*†	<0.001
Chronic kidney disease, n (%)	10 (4.6)	22 (7.9)	14 (10.9)	10 (24.4)*†	0.001
Menopause, n (%)	103 (42.0)	146 (48.3)	91 (63.2)*†	41 (70.7)*†	<0.001
Endothelial dysfunction, n (%)	75 (30.6)	87 (28.8)	50 (34.7)	32 (55.2)*†§	0.002

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; P values for comparisons across the uric acid categories were performed with ANOVA for continuous variables and χ^2 test for categorical variables.

*P<0.05 vs. serum uric acid lever<4.0 mg/dL group, †P<0.05 vs. serum uric acid level 4.0 to <5.0 mg/dL group, §P<0.05 vs. serum uric acid level 5.0 to <6.0 mg/dL group.

Table 3. Multivariate Analysis of the Relation Between Endothelial Dysfunction and Variables

Model	Variables	Odds ratio (95%CI)	P value
1	Uric acid (mg/dL)	1.30 (1.13 to 1.50)	<0.001
2	1+age	1.20 (1.03 to 1.39)	0.02
3	2+other variables	1.13 (0.97 to 1.32)	0.11

Initial factors included in the model 3 were body mass index, systolic blood pressure, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose, eGFR, and smoking. Using stepwise multiple regression analysis, we selected high-density lipoprotein cholesterol, glucose, and smoking as independent factors for endothelial dysfunction (other variables).

Table 4. Multivariate Analysis of the Relation Between Endothelial Dysfunction and Variables

		Post-menopausal		Pre-menopausal	
Model	Covariates	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
1	Uric acid (mg/dL)	1.27(1.05 to 1.53)	0.01	1.14 (0.90 to 1.44)	0.28
2	1+age	1.26 (1.05 to 1.53)	0.02	1.12 (0.85 to 1.39)	0.50
3	2+other variables	1.23 (1.01 to 1.50)	0.04	0.98 (0.75 to 1.26)	0.85

Other variables: high-density lipoprotein cholesterol, glucose, and smoking.

Author Contributions

Tatsuya Maruhashi and Yukihito Higashi, drafting the article and conception of this study; Junko Soga, Noritaka Fujimura, Naomi Idei, Shinsuke Mikami,Yumiko Iwamoto, Masato Kajikawa, Takeshi Matsumoto, Takayuki Hidaka, Chikara Goto, Kensuke Noma, Ayumu Nakashima, Bonpei Takase, and Hirofumi Tomiyama, performing the ultrasonogarphy; Kazuaki Chayama, Yasuki Kihara, Akira Yamashina, revising the article critically for important intellectual content.

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Competing interests

None.

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Data sharing

There is no additional unpublished data from the study.

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Figure legends

Figure 1. Bar graphs show flow-mediated vasodilation in women categorized according to serum uric acid level.

Hyperuricemia is Independently Associated with Endothelial Dysfunction in Postmenopausal Women but not in Premenopausal Women

Brief title: Hyperuricemia and endothelial function in women

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Abstract

Objectives: The purpose of this study was to determine the relationships between uric acid, endothelial function, and cardiovascular risk factors and to investigate whether menopausal status was associated with the relationship between uric acid and endothelial function in women.

Design: Cross-sectional study.

Setting: Three general hospitals in Japan.

Participants: 749 Japanese women aged 30 to 74 years recruited from people who underwent health-screening examinations with agreement for measurement of vascular function.

Measures: We measured serum concentrations of uric acid and flow-mediated vasodilation (FMD). Percent of FMD (Peak diameter-Baseline diameter/Baseline diameter) was used for analysis. Endothelial dysfunction was defined as FMD equal to or less than 4.90%, division point for the lowest tertile and the middle tertile of FMD. Menopause women were defined as subjects without menstruation for over 1 year or subjects with a history of hysterectomy or bilateral oophrectomy.

Results: Of the 749 subjects, 368 (49.1%) were premenopausal and 381 (50.9%) were postmenopausal women. Age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, estimated glomerular filtration, and Framingham risk score were significantly correlated with serum uric acid level. FMD showed a gradual decrease in accordance with the serum uric acid level in the entire study population (<4.0 mg/dL, 6.85±3.65%; 4.0 to <5.0 mg/dL, 6.79±3.60%; 5.0 to <6.0 mg/dL, 6.24±3.58%; ≥6.0 mg/dL, 5.27±3.18%; P=0.01). Multivariate analysis revealed that uric acid was a significantly independent risk factor for endothelial dysfunction in postmenopausal women (odds ratio; 1.23, 95% confidence interval; 1.01-1.50), but not

in premenopausal women.

Conclusions: These findings suggest that uric acid can be used as a risk marker of endothelial dysfunction in a female population, and particularly as an independent risk factor in postmenopausal women but not in premenopausal women.

Registration number of the study: UMIN000003409

Article summary

Article focus:

- To investigate the relationships between uric acid, endothelial function assessed by flow-mediated vasodilation (FMD), and cardiovascular risk factors in women.
- To examine whether menopausal status is associated with the relationship between uric acid and endothelial function in women.

Key messages

- Uric acid level was significantly associated with cardiovascular parameters and prevalence of cardiovascular risk factors in the entire study population.
- FMD showed a gradual decrease in accordance with the serum uric acid level in the entire study population.
- Uric acid was an independent risk factor for endothelial dysfunction even after adjustment for other cardiovascular risk factors in postmenopausal women, but not in premenopausal women.

Strengths and limitations of this study

- This study includes a large number of women who underwent flow-mediated vasodilation test.
- This study shows a difference in the relationship between uric acid and endothelial dysfunction depending on menopausal status in women.
- Residual confounding may exist for this cross-sectional study.
- Menopausal status was based on self-report.

Introduction

Several epidemiological studies have shown a relationship between serum uric acid level and subsequent cardiovascular disease.[1-10] In addition, increase in uric acid level is regarded as an independent marker of increased cardiovascular risk. However, it has remained controversial whether uric acid per se should be considered as a cardiovascular risk factor because of the difficulty in investigating the role of uric acid alone in the pathogenesis, development, and maintenance of atherosclerosis. There are significant relationships between elevated uric acid levels and established cardiovascular risk factors, such as hypertension,[11] metabolic syndrome[12] and kidney disease,[13] all of which are also well known as strong predictors of cardiovascular disease.[14 15] Some investigators have argued that elevated uric acid is not an independent risk factor but rather merely a marker of risk for cardiovascular disease.[1,6,7] However, recent epidemiological studies have demonstrated that uric acid is an independent risk factor for cardiovascular disease.[5 7-10] Although the mechanism by which uric acid causes cardiovascular disease is not fully understood, several lines of evidence suggest that elevated uric acid impairs endothelial function by inducing intracellular oxidative stress and inflammation through activation of the local renin-angiotensin system, particularly angiontensin II, and the pro-oxidant effect of uric acid per se, once absorbed into endothelial cells.[16-18]

Endothelial dysfunction is established in the initial step of atherosclerosis and plays an important role in the development of atherosclerotic conditions, leading to cardiovascular outcomes.[19] Recently, measurement of flow-mediated vasodilation (FMD) as an index of endothelium-dependent vasodilation has been widely used as a method for assessing endothelial function.[20-24] In addition, growing evidence has shown that endothelial function assessed by FMD can serve as an independent predictor

of cardiovascular events.[25-28] Recently, several investigators, including us, have shown a relationship between uric acid and endothelial function assessed by FMD.[29-34] As for the relationship between uric acid and FMD in men, previous studies are consistent in demonstrating that uric acid is a significant independent risk factor for FMD.[29 34] However, there is little information on whether uric acid is an independent risk factor for endothelial dysfunction in women. In addition, it remains unclear whether menopausal status is associated with the relationship between uric acid and endothelial function. It is clinically important to confirm the role of uric acid per se in atherosclerosis. We therefore investigated the relationships between uric acid, FMD and cardiovascular risk factors and whether menopausal status was associated with the relationship between uric acid and endothelial function in women who underwent health-screening examinations.

Methods

Subjects

A total of 5321 Japanese adults aged 17 to 86 years who underwent health-screening examinations with agreement for measurement of vascular function were enrolled in the Flow-mediated Dilation Japan Registry between 1 April 2010 and 31 August 2012 at 3 general hospitals in Japan. All employees have an obligation to undergo health screening every year under the regulations of the Society-managed Health Insurance Union in Japan. In accordance with the regulations, we performed health-screening examinations. From the registry, 895 women aged 30 to 74 years were recruited for this study. Among the 895 subjects, information on serum uric acid levels, menopause, and phases of menstrual was available for 797 subjects. Subjects during menstruation (n=28), subjects receiving hormone replacement therapy (n=3), one subject who was

pregnant, subjects who had been previously diagnosed with cardiovascular diseases (n=15), and one subject who was being treated with an antihyperuricemic drug were excluded. Finally, 749 women without cardiovascular diseases were enrolled in this study. Hypertension was defined as systolic blood pressure of ≥ 140 mm Hg or diastolic blood pressure of ≥ 90 mm Hg, in a sitting position, on at least 3 different occasions. [35] Patients with secondary forms of hypertension were excluded in all patients with hypertension on the basis of complete history; physical examination; radiological and ultrasound examinations; urinalysis; plasma rennin activity; plasma aldosterone and norepinephrine concentrations; serum creatinine, potassium, calcium, and free throxine concentrations; and 24-hour urinary excretion of 17-hydroxycorticosteroids, 17-ketogenic steroids, and vanillymandelic acid. Diabetes was defined according to the American Diabetes Association.[36] Dyslipidemia was defined according to the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP-III).[37] We defined smokers as those who were current smokers. Measurement of FMD was performed without withholding medications. Framingham risk score was calculated by points of risk factors: age, total cholesterol level, high-density lipoprotein cholesterol level, systolic blood pressure, and smoking status.[38] Diagnosis of metabolic syndrome was made according to the criteria of NCEP ATP-III.[37] Thus, metabolic syndrome was diagnosed when 3 or more of the following risk determinants were present. (1) waist circumference of ≥88 cm, (2) triglyceride ≥1.7 mmol/L (150 mg/dL), (3) high-density lipoprotein cholesterol <1.29 mmol/L (50 mg/dL), (4) blood pressure ≥130 and/or 85 mm Hg, or (5) fasting blood glucose ≥6.11 mmol/L (110 mg/dL). The estimated glomerular filtration rate (eGFR) was calculated using the Japanese eGER equation.[39] Chronic kidney disease (CKD) was defined as eGFR <60

mL/min/1.73 m^2.[40] Menopause women were defined as subjects without menstruation for over 1 year or subjects with a history of hysterectomy or bilateral oophrectomy.[41] The ethical committees of our institutions approved the study protocol. Written informed consent for participation in the study was obtained from all subjects.

Study protocol

We measured vascular responses to reactive hyperemia in the brachial artery in all subjects. Subjects fasted the previous night for at least 12 hours. The study began at 8:30 AM. 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. After remaining in the supine position for 30 minutes, blood samples were obtained for measurement of basal fasting serum concentrations of total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, glucose, and uric acid. Then FMD was measured. The observers were blind to the form of examination. Serum uric acid levels were measured by the uricase-peroxidase method (JCA-BM6010; JEOL Ltd., Tokyo, Japan). Levels of serum total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and creatinine were enzymatically measured (JCA-BM6010; JEOL Ltd., Tokyo, Japan). Glucose levels were measured by the glucose oxidase immobilezed oxygen electrode method (GA08II; A&T, Yokohama, Japan).

Measurement of FMD

The subjects remained supine throughout the study. The 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. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 seconds after cuff deflation. Blood flow velocity was calculated from the Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 seconds, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 minutes. The longitudinal image of the artery was recorded continuously until 5 minutes after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 seconds at baseline and for 10 seconds immediately 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. Percentage of FMD (peak diameter-baseline diameter/baseline diameter) was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the

angle) by heart rate and vessel cross-sectional area (-r^2). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow.

Statistical analysis

Results are presented as mean±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. Comparisons between the groups categorized according to serum uric acid level were carried out using P for trend analysis with Bonferroni's test for post-hoc comparisons. Relations between variables were determined by Spearman correlation coefficients analysis. Stepwise multivariate logistic regression analyses were performed to identify factors associated with endothelial dysfunction among potential confounders (P<0.20) in univariate analysis. Using variables associated with endothelial dysfunction in stepwise multiple regression analyses, we performed the final logistic regression analyses for which results are presented in the results section. The data were processed using the software package Stata version 9 (Stata Co., College Station, Texas, USA).

Results

Baseline clinical characteristics

The baseline clinical characteristics are summarized in Table 1. Of the 749 subjects, 112 (15.0%) had hypertension, 262 (35.0%) had dyslipidemia, 34 (4.5%) had diabetes mellitus, 59 (7.9%) were current smokers, 65 (8.7%) had metabolic syndrome, and 56 (8.4%) had chronic kidney disease. Mean Framingham risk score was 3.94±3.62%. The mean value of serum uric acid level was 4.44±1.09 mg/dL (median, 4.30 mg/dL;

interquartile range, 3.80-5.00 mg/dL; range, 0.80 to 10.0 mg/dL). The mean value of FMD was 6.59±3.60% (range, -4.70 to 20.1%). Division point for the lowest tertile and the middle tertile of FMD was 4.90%. Therefore, endothelial dysfunction was defined as FMD equal or less than 4.90%.

Relationships between serum uric acid level and cardiovascular risk factors

Univariate regression analysis revealed that serum uric acid level significantly correlated with age (r=0.21, P<0.001), body mass index (r=0.26, P<0.001), systolic blood pressure (r=0.17, P<0.001), diastolic blood pressure (r=0.17, P<0.001), total cholesterol (r=0.23, P<0.001), triglycerides (r=0.25, P<0.001), high-density lipoprotein cholesterol (r=-0.13, P=0.002), low-density lipoprotein cholesterol (r=0.22, P<0.001), glucose (r=0.12, P=0.004), and eGFR (r=-0.27, P<0.001). Subjects were categorized according to serum uric acid levels (Table 2). Age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, and Framingham risk score were significantly increased and eGFR was significantly decreased with increase in serum uric acid level. As for the prevalence of cardiovascular risk factors, there were significant increases in the prevalence of hypertension, dyslipidemia, current smoking, metabolic syndrome, chronic kidney disease, and menopause in relation to increase in serum uric acid level.

Relationships between serum uric acid level and FMD

Subjects were categorized into 4 groups on the basis of serum uric acid level. FMD was significantly decreased with increase in serum uric acid level (<4.0 mg/dL, 6.85±3.65%; 4.0 to <5.0 mg/dL, 6.79±3.60%; 5.0 to <6.0 mg/dL, 6.24±3.58%; ≥6.0 mg/dL, 5.27±3.18%; P for trend = 0.01, Figure 1). Multiple logistic regression analysis revealed

that serum uric acid level was significantly associated with endothelial dysfunction after adjustment for age (odds ratio, 1.20; 95% confident interval [CI], 1.03 to 1.39) (Table 3). However, after adjustment for other risk factors, including high-density lipoprotein cholesterol, glucose, and smoking status, the significant association between serum uric acid level and endothelial dysfunction disappeared (odds ratio, 1.13; 95% CI, 0.97 to 1.32).

Difference in the relationship between serum uric acid level and endothelial dysfunction according to menopausal status

Subjects were divided into two groups according to menopausal status to investigate the influence of menopause on the relationship between serum uric acid level and endothelial function (supplemental Table I). Of the 749 subjects, 368 (49.1%) were premenopausal and 381 (50.9%) were postmenopausal women. Postmenopausal women were significantly older than premenopausal women (58.6±6.3 vs. 41.5±6.1 years, P<0.001). The ages ranged from 30 to 54 years in premenopausal women and 31 to 74 years in postmenopausal women. Serum uric acid level was significantly higher in postmenopausal women than in premenopausal women (4.64±1.11 vs. 4.24±1.03 mg/dL, P<0.001). FMD was significantly impaired in postmenopausal women compared to that in premenopausal women (5.51±3.14 vs. 7.77±3.71%, P<0.001), and the prevalence of endothelial dysfunction was significantly higher in postmenopausal women than premenopausal women (66.4% vs. 34.0%, P<0.001). In postmenopausal women, multiple logistic regression analysis revealed that serum uric acid level was significantly associated with endothelial dysfunction after adjustment for age (odds ratio, 1.26; 95% CI, 1.05 to 1.53) (Table 4). The significant association between serum uric acid level and endothelial dysfunction persisted after adjustment for other risk factors (odds ratio,

1.23; 95% CI, 1.01 to 1.50). In contrast, there was no significant association between serum uric acid level and endothelial dysfunction in premenopausal women (odds ratio, 1.14; 95% CI, 0.90 to 1.44) (Table 4). After adjustment for other risk parameters, the association between uric acid level and endothelial dysfunction remained insignificant (odds ratio, 0.98; 95% CI, 0.75 to 1.26).

Discussion

In the present study, we demonstrated that uric acid level was significantly associated with cardiovascular parameters and prevalence of cardiovascular risk factors. Although FMD showed a graded decrease according to serum uric acid level, uric acid was not an independent risk factor for endothelial dysfunction after adjustment for other cardiovascular risk parameters. However, when subjects were divided into two groups according to menopausal status, uric acid was a significantly independent risk factor for endothelial dysfunction even after adjustment for other cardiovascular risk parameters in postmenopausal women, but not in premenopausal women. To our knowledge, this is the first report showing a difference in the relationship between uric acid and endothelial dysfunction depending on menopausal status in women.

As a marker of atherosclerotic diseases, it has been demonstrated that serum uric acid level is associated with cardiovascular risk factors and subclinical atherosclerosis, such as hypertension,[11] metabolic syndrome,[12] kidney disease,[13] coronary artery calcification,[42] and carotid atherosclerosis.[43] In the present study, we confirmed that serum uric acid level was significantly associated with age, body mass index, systolic blood pressure, total cholesterol, triglycerides, glucose, and eGFR, most of which are components of metabolic syndrome and CKD. Indeed, the prevalence of hypertension, dyslipidemia, metabolic syndrome, and CKD was linearly increased in

relation to increase in serum uric acid level. An association between uric acid and metabolic syndrome has been demonstrated by the fact that uric acid level is often elevated in subjects with metabolic syndrome and that the prevalence of metabolic syndrome linearly increases in relation to uric acid level.[12 43] The mechanism behind the association between increase in serum uric acid level and metabolic syndrome is thought to be hyperinsulinemia, mediated by insulin resistance and visceral adiposity, leading to an increase in uric acid absorption in renal tubules.[44] Uric acid levels are also frequently elevated in patients with kidney disease as a result of reduction in GFR and renal urate excretion.[45] In addition, several epidemiological studies have shown a link between elevated serum uric acid level and subsequent cardiovascular events.[1-10] The association between serum uric acid level and cardiovascular disease has been reported to be generally stronger in women than in men.[9 46-48] These findings suggest that uric acid level is a biochemical marker of atherosclerotic disease and a useful predictor of the development of cardiovascular diseases.

Despite the association between elevated uric acid level and cardiovascular conditions, uric acid has not been established as an independent causal risk factor for cardiovascular disease because of the link between uric acid and established cardiovascular risk factors. Another possible explanation for the difficulty in determining the role of uric acid in cardiovascular disease is that uric acid may function as an powerful antioxidant and as a scavenger of singlet oxygen and radicals.[49 50] In patients with cardiovascular disease, an increase in uric acid level, therefore, might be considered as a compensatory mechanism to counteract the oxidative stress induced in these conditions. Although it remains controversial whether uric acid is a causative factor or merely a marker of cardiovascular disease, some epidemiological studies have demonstrated that the association between uric acid and cardiovascular disease remains

significant even after adjustment for concomitant risk factors, suggesting that uric acid per se is an independent risk factor for cardiovascular disease.[5 7-10]

Although the precise mechanism behind the relationship between uric acid and cardiovascular disease remains to be elucidated, growing evidence indicates that endothelial dysfunction induced by uric acid plays an important role in the development of cardiovascular disease.[45] Several lines of evidence suggest that uric acid also has proinflammatory effects on endothelial cells, leading to reduction of endothelial nitric oxide bioavailability and consequent endothelial dysfunction.[17 18] Yu et al.[18] demonstrated that uric acid enhances the production of reactive oxygen species by activation of the local renin-angiotensin system, particularly angiotensin II, in human endothelial vascular cells and that probenecid, an inhibitor of urate transporter, inhibits uric acid-induced oxidative stress. Thus, uric acid is thought to mediate endothelial dysfunction by generating oxidative stress once absorbed into endothelial cells. In a clinical setting, measurement of FMD as an index of endothelium-dependent vasodilation in the brachial artery using high-resolution ultrasound has been widely used as a method for assessing vascular function.[20-23] Although several investigators have shown that uric acid inversely correlates with FMD and that uric acid is an independent predictor of FMD, the subjects in those studies were limited to a small number of subjects or highly selected subjects, such as patients with increased cardiovascular risk,[30] hyperuricemia,[32] or nondiabetic CKD.[33] In addition, uric acid has been shown to be a significantly independent risk factor for endothelial dysfunction in men in an analysis of a large population,[34] but there is little information on the relationship between uric acid and FMD in women. As a risk marker, we demonstrated that serum uric acid level was significantly associated with FMD in women. FMD was significantly impaired with increase in serum uric acid level. FMD is

known to be impaired as a consequence of cumulative cardiovascular risk factors.[21 51] Therefore, significant decrease in FMD according to serum uric acid level may reflect significant associations between uric acid and other cardiovascular risk factors, including hypertension, dyslipidemia, metabolic syndrome, and CKD, as a risk marker for cardiovascular disease in women. These findings provide a rationale for the relationships of uric acid with cardiovascular disease and mortality being generally strong in women.

As for the role of uric acid as a causal risk factor in endothelial function, multivariate analysis performed for the entire population indicated that uric acid was not an independent predictor of endothelial dysfunction after adjustment for other risk factors. However, when subjects were divided into two groups according to menopausal status, multivariate analyses revealed that uric acid was a significantly independent risk factor for endothelial dysfunction in postmenopausal women but not in premenopausal women. The incidence of cardiovascular diseases in women is lower than that in men until around the age of menopause, after which it markedly increases and becomes equal to that of their male counterparts, suggesting a protective effect of endogenous ovarian hormones on atherosclerosis.[52] Ovarian hormones, especially estradiol, have been suggested to have protective effects on endothelial function.[53 54] In addition to its protective effect on endothelial function, estradiol has been shown to lower uric acid level through mechanisms involving renal clearance, secretion and reabsorption.[55-57] Therefore, endogenous estradiol may preserve endothelial function and lower serum uric acid level regardless of the presence or absence of cardiovascular risk factors, resulting in the weak relationship between uric acid and endothelial dysfunction in premenopausal women. Our findings in the analysis of premenopausal women are consistent with the results of a previous study demonstrating that uric acid was not an

independent risk factor of FMD in young women aged 30 to 45 years.[58] In contrast to premenopausal women, uric acid remained a significantly independent risk for endothelial dysfunction even after adjustment for other risk factors in postmenopausal women. Although Maxwell et al.[29] reported that uric acid was not an independent determinant of FMD in women, the non-independent association between FMD and uric acid observed in their study may be, in part, attributed to inclusion of entire women in the analysis for the relationship between FMD and uric acid without taking into account menopausal status. The increased cardiovascular risk in postmenopausal women has been suggested to be associated with impairment of endothelial function after menopause.[59 60] There is a possibility that treatment of hyperuricemia in postmenopausal women will improve endothelial function, leading to a decrease in cardiovascular events.

There were some limitations in this study. The cross-sectional design did not allow us to establish a definitive causal relationship between hyperuricemia and endothelial dysfunction. In addition, multiple factors, such as vitamins, phosphate and statin use, that are known to influence FMD and residual unrecognized confounding factors were not taken into account in this study. Future prospective and interventional studies are certainly warranted to obtain more specific conclusions as to whether hyperuricemia should be treated, which subgroup should be treated, and whether treatment of hyperuricemia improves endothelial function with subsequent decrease in cardiovascular events.

In conclusion, uric acid is a useful marker for endothelial function in women. Significant relationships between serum uric acid level and cardiovascular risk factors, including hypertension, dyslipidemia, metabolic syndrome, and CKD, may result in the significant association between FMD and serum uric acid level, as a risk marker of

atherosclerotic diseases. Uric acid, as a causal cardiovascular risk factor, may be an independent risk for endothelial dysfunction in postmenopausal women but not in premenopausal women. Further studies are needed to investigate whether treatment for hyperuricemia improves endothelial function in postmenopausal women.

Table 1. Clinical Characteristics of the Subjects

Variables	Total (n=749)
Age, y	50.2±10.6
Body mass index, kg/m^2	22.0±3.4
Systolic blood pressure, mm Hg	121.1±19.0
Diastolic blood pressure, mm Hg	73.9±12.4
Heart rate, bpm	64.4±10.2
Total cholesterol, mmol/L	5.37±0.88
Triglycerides, mmol/L	1.07±0.71
HDL-cholesterol, mmol/L	1.80±0.42
LDL-cholesterol, mmol/L	3.11±0.77
Glucose, mmol/L	5.32±1.47
Uric acid, mg/dL	4.44±1.09
eGFR, mL/min/1.73 m^2	77.1±13.3
Framingham risk score, %	3.94±3.62
Hypertension, n (%)	112 (15.0)
Dyslipidemia, n (%)	262 (35.0)
Diabetes mellitus, n (%)	34 (4.5)
Smoking, n (%)	59 (7.9)
Metabolic syndrome, n (%)	65 (8.7)
Chronic kidney disease, n (%)	56 (8.4)
Menopause, n (%)	381 (50.9)
Flow-mediated vasodilation, %	6.59±3.60
Baseline brachial artery diameter, mm	3.40±0.47

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

Table 2. Clinical Characteristics according to Serum Uric Acid Levels *†§

	Uric acid categories, mg/dL				
Variables	<4.0 (n=245)	4.0 to <5.0 (n=302)	5.0 to <6.0 (n=144)	6.0≤ (n=58)	P value
Uric acid, mg/dL	3.38±0.54	4.42±0.28	5.33±0.26	6.90±0.90	
Age, y	48.3±10.9	49.4±10.1	53.5±9.9*†	54.7±10.0*†	<0.001
Body mass index, kg/m^2	20.8±2.8	22.0±3.4*	23.0±3.3*†	24.2±3.9*†	<0.001
Systolic blood pressure, mm Hg	117.2±17.6	120.4±18.3	125.9±20.4*†	129.2±20.5*†	<0.001
Diastolic blood pressure, mm Hg	71.5±11.4	73.6±12.1	77.4±13.4*†	77.0±12.5*	<0.001
Heart rate, bpm	64.6±9.6	63.5±0.6	65.5±12.4	65.8±11.1	0.14
Total cholesterol, mmol/L	5.20±0.82	5.33±0.86	5.60±0.91*†	5.67±0.88*†	<0.001
Triglycerides, mmol/L	0.89±0.51	0.97±0.50	1.32±0.81*†	1.69±1.32*†§	<0.001
HDL-cholesterol, mmol/L	1.83±0.38	1.83±0.41	1.69±0.45*†	1.69±0.54	0.001
LDL-cholesterol, mmol/L	3.00±0.70	3.10±0.77	3.31±0.80*	3.19±0.96	0.006
Glucose, mmol/L	5.09±0.84	5.28±1.38	5.52±2.00*	6.03±2.09*†	<0.001
eGFR, mL/min/1.73 m^2	80.9±13.7	76.8±12.1*	74.4±13.0*	67.7±13.8*†§	<0.001
Flow-mediated vasodilation, %	6.85±3.65	6.79±3.60	6.24±3.58	5.27±3.18*†	0.01
Baseline brachial artery diameter, mm	3.35±0.46	3.40±0.48	3.43±0.46	3.61±0.51*†	0.002
Framingham risk score, %	3.27±3.44	3.49±2.98	5.08±3.65*†	6.33±5.42*†	<0.001
Hypertension, n (%)	25 (10.2)	34 (11.3)	34 (23.6)*†	19 (32.8)*†	<0.001
Dyslipidemia, n (%)	59 (24.1)	90 (29.8)	72 (50.0)*†	41 (70.7)*†§	<0.001
Diabetes mellitus, n (%)	9 (3.7)	8 (2.7)	9 (6.3)	8 (13.8)*†	0.008
Smoking, n (%)	12 (4.9)	23 (7.6)	15 (10.4)	9 (15.5)	0.04
Metabolic syndrome, n (%)	12 (4.9)	18 (6.0)	19 (13.2)*	16 (28.1)*†	<0.001
Chronic kidney disease, n (%)	10 (4.6)	22 (7.9)	14 (10.9)	10 (24.4)*†	0.001
Menopause, n (%)	103 (42.0)	146 (48.3)	91 (63.2)*†	41 (70.7)*†	<0.001
Endothelial dysfunction, n (%)	75 (30.6)	87 (28.8)	50 (34.7)	32 (55.2)*†§	0.002

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; P values for comparisons across the uric acid categories were performed with ANOVA for continuous variables and χ^2 test for categorical variables.

*P<0.05 vs. serum uric acid lever<4.0 mg/dL group, †P<0.05 vs. serum uric acid level 4.0 to <5.0 mg/dL group, §P<0.05 vs. serum uric acid level 5.0 to <6.0 mg/dL group.

Table 3. Multivariate Analysis of the Relation Between Endothelial Dysfunction and Variables

Model	Variables	Odds ratio (95%CI)	P value
1	Uric acid (mg/dL)	1.30 (1.13 to 1.50)	<0.001
2	1+age	1.20 (1.03 to 1.39)	0.02
3	2+other variables	1.13 (0.97 to 1.32)	0.11

Initial factors included in the model 3 were body mass index, systolic blood pressure, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose, eGFR, and smoking. Using stepwise multiple regression analysis, we selected high-density lipoprotein cholesterol, glucose, and smoking as independent factors for endothelial dysfunction (other variables).

Table 4. Multivariate Analysis of the Relation Between Endothelial Dysfunction and Variables

		Post-menopausal		Pre-menopausal	
Model	Covariates	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
1	Uric acid (mg/dL)	1.27(1.05 to 1.53)	0.01	1.14 (0.90 to 1.44)	0.28
2	1+age	1.26 (1.05 to 1.53)	0.02	1.12 (0.85 to 1.39)	0.50
3	2+other variables	1.23 (1.01 to 1.50)	0.04	0.98 (0.75 to 1.26)	0.85

Other variables: high-density lipoprotein cholesterol, glucose, and smoking.

Author Contributions

Tatsuya Maruhashi and Yukihito Higashi, drafting the article and conception of this study; Junko Soga, Noritaka Fujimura, Naomi Idei, Shinsuke Mikami,Yumiko Iwamoto, Masato Kajikawa, Takeshi Matsumoto, Takayuki Hidaka, Chikara Goto, Kensuke Noma, Ayumu Nakashima, Bonpei Takase, and Hirofumi Tomiyama, performing the ultrasonogarphy; Kazuaki Chayama, Yasuki Kihara, Akira Yamashina, revising the article critically for important intellectual content.

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Competing interests

None.

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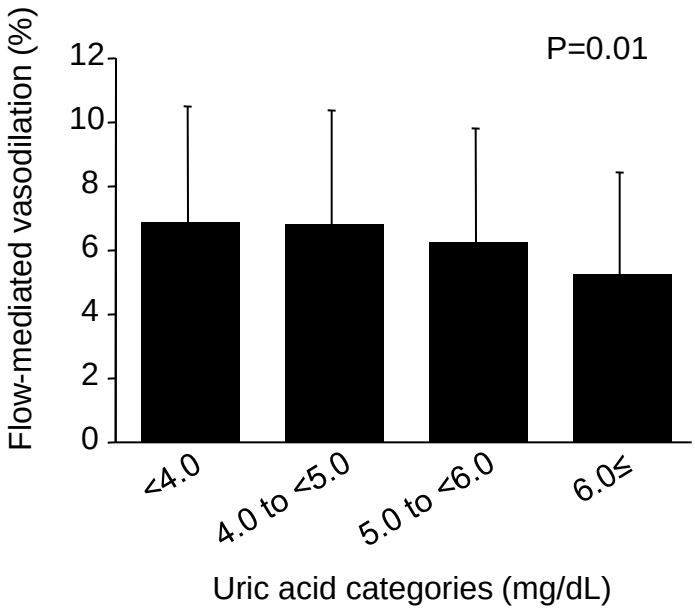
Figure legends

Figure 1. Bar graphs show flow-mediated vasodilation in women categorized according to serum uric acid level.


Flow-mediated vasodilation (%)
12
10
8
6
4
2
0
P=0.01
<4.0
4.0 to <5.0
5.0 to <6.0
6.0≤
Uric acid categories (mg/dL)

Supplemental data

Hyperuricemia is Independently Associated with Endothelial Dysfunction in Postmenopausal Women but not in Premenopausal Women

Brief title: Hyperuricemia and endothelial function in women

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Table I. Clinical Characteristics of Premenopause and Menopause Women

Variables	Premenopause (n=368)	Postmenopause (n=381)	P value
Age, y	41.5±6.1	58.6±6.3	<0.001
Body mass index, kg/m^2	21.4±3.5	22.5±3.2	<0.001
Systolic blood pressure, mm Hg	113.6±14.4	128.3±20.2	<0.001
Diastolic blood pressure, mm Hg	70.6±11.3	77.9±12.6	<0.001
Heart rate, bpm	63.5±9.6	65.2±10.6	0.02
Total cholesterol, mmol/L	5.05±0.80	5.67±0.84	<0.001
Triglycerides, mmol/L	0.87±0.59	1.25±0.76	<0.001
HDL-cholesterol, mmol/L	1.82±0.40	1.77±0.45	0.11
LDL-cholesterol, mmol/L	2.85±0.68	3.36±0.78	<0.001
Glucose, mmol/L	5.09±0.82	5.55±1.87	<0.001
Uric acid, mg/dL	4.24±1.03	4.64±1.11	<0.001
eGFR, mL/min/1.73 m^2	79.2±12.8	74.9±13.5	<0.001
Framingham risk score, %	1.67±1.36	6.13±3.77	<0.001
Hypertension, n (%)	12 (3.3)	100 (26.3)	<0.001
Dyslipidemia, n (%)	65 (17.7)	197 (51.7)	<0.001
Diabetes mellitus, n (%)	8 (2.2)	26 (6.8)	0.002
Smoking, n (%)	34 (9.2)	25 (6.6)	0.18
Metabolic syndrome, n (%)	17 (4.6)	48 (12.6)	<0.001
Chronic kidney disease, n (%)	18 (5.2)	38 (11.8)	0.002
Endothelial dysfunction, n (%)	125 (34.0)	253 (66.4)	<0.001
Flow-mediated vasodilation, %	7.77±3.71	5.51±3.14	<0.001
Baseline brachial artery diameter, mm	3.28±0.41	3.52±0.49	<0.001

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

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.