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Original Article

Association of Serum Uric Acid Levels with Leg Ischemia in Patients with Peripheral Arterial Disease after Treatment

Yoko Sotoda[1], Shigeki Hirooka[1], Hiroyuki Orita[1] and Ichiro Wakabayashi[2]

[1]Department of Cardiovascular Surgery, Yamagata Saisei Hospital, Yamagata, Japan
[2]Department of Environmental and Preventive Medicine, Hyogo College of Medicine, Hyogo, Japan

***Aim*: We investigated the relationships of serum uric acid levels with the progression of atherosclerosis in patients with peripheral arterial disease (PAD) after treatment.**
***Methods*: Subjects were male patients diagnosed with PAD. Atherosclerosis at the common carotid artery was evaluated based on its intima−media thickness (IMT). Leg arterial flow was evaluated by measuring ankle−brachial index (ABI) and exercise-induced decrease in ABI.**
***Results*: Among various risk factors including age, blood pressure, adiposity, estimated glomerular filtration rate, and blood lipid, blood glucose, uric acid, fibrinogen and C-reactive protein levels, only uric acid levels showed significant correlations with ABI [Pearson's correlation coefficient, −0.292 ($p<0.01$)] and leg exercise-induced decrease in ABI [Pearson's correlation coefficient, 0.236 ($p<0.05$)]. However, there was no significant correlation between uric acid levels and maximum or mean IMT. Odds ratios of subjects with the 3rd tertile versus subjects with the 1st tertile for uric acid levels were significantly higher than the reference level of 1.00 for low ABI [4.44 (95% confidence interval, 1.45−13.65, $p<0.01$)] and for high % decrease in ABI after exercise [4.31 (95% confidence interval, 1.34−13.82, $p<0.05$)]. The associations of uric acid levels with the indicators of leg ischemia were also found after adjustment for age, history of revascularization therapy, diabetes, smoking, alcohol consumption, body mass index, triglyceride levels, and renal function.**
***Conclusion*: Uric acid levels are associated with the degree of leg ischemia in patients with PAD. Further interventional studies are needed to determine whether the correction of uric acid levels is effective in preventing the progression of PAD.**

Key words: Ankle−brachial index, Atherosclerosis, Diabetes mellitus, Peripheral arterial disease, Uric acid

Introduction

The pathogenesis of peripheral arterial disease (PAD) is based on atherosclerosis. Among various risk factors for atherosclerotic disease, smoking and diabetes are major risk factors for PAD[1, 2]. In addition, other atherosclerotic risk factors, including hypertension, dyslipidemia, hyperhomocysteinemia, elevated inflammatory marker levels, and chronic kidney disease, have been reported to be associated with an increased risk of PAD[1, 2]. Patients with PAD are known to have a high risk of cardiovascular events such as myocardial infarction and stroke, which determine the prognosis of patients[3-5]. Thus, modifying risk factors for PAD is important not only for preventing the progression of PAD but also for reducing mortality caused by cardiovascular complications. Therefore, smoking cessation and therapy for diabetes are strongly recommended for patients with PAD. Lipid-lowering agents, antihypertensive drugs, and anti-platelet drugs are used for patients with PAD[1, 2, 6-8]. However, limited information is available on whether the modification of other atherosclerotic risk factors including obesity, hyperuricemia, and renal dysfunction is effective for preventing the progression of PAD and other cardiovascular complications.

Hyperuricemia is a risk factor for atherosclerosis-related diseases[9] and accelerates atherosclerotic progression through the induction of inflammation and

Address for correspondence: Ichiro Wakabayashi, Department of Environmental and Preventive Medicine, Hyogo College of Medicine, Mukogawa-cho 1-1, Nishinomiya, Hyogo 663-8501, Japan
E-mail: wakabaya@hyo-med.ac.jp
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increase in oxidative stress resulting from the accumulation of uric acid and activation of xanthine oxidoreductase (XOR) in the arterial wall[10, 11]. Uric acid levels have been reported to be associated with the incidence and prevalence of PAD in the general population[12, 13] and in patients with diabetes[14] and with hypertension[15]. However, the significance of uric acid levels in patients with PAD is unknown. Therefore, the purpose of this study was to investigate the relationship of uric acid levels with PAD exacerbation after treatment.

In this study, the relationships of various atherosclerotic risk factors, including uric acid levels, with the degrees of atherosclerosis in patients with PAD were investigated. Intima−media thickness of common carotid arteries (IMT) and ankle−brachial index (ABI) were used to evaluate the degrees of atherosclerosis progression. Decrease in ABI induced by leg exercise loading, which is thought to be a good marker for asymptomatic PAD[16, 17], was also used to evaluate ischemia in the lower limbs.

Methods

Subjects

The subjects were 87 male outpatients with a mean age of 75.2±8.0 (47−92) years who had been diagnosed as having PAD defined as an ABI of <0.9 and/or a toe−brachial index (TBI) of <0.7[1, 18]. All subjects were patients with PAD who had been diagnosed by the criteria of low ABI (<0.9) and/or low TBI (<0.7). The subjects were patients who had already received therapy for PAD, and 75.9% of the subjects had received endovascular angioplasty and/or surgical treatment. The associations of uric acid levels with the incidence and prevalence of PAD in the general population have already been previously reported[12, 13]. The purpose of this clinical study was to investigate risk factors for the exacerbation of PAD after treatment in patients. Therefore, patients with a history of endovascular angioplasty and/or surgical treatment as well as those with a history of drug therapy (anti-platelet drugs) for PAD were included. This is the reason why more than 50% of the subjects showed an ABI of ≥0.9. The proportion of female patients with PAD was much lower than that of male patients (approximately 1:6), and the number of female patients with PAD was too small to perform proper statistical analysis of the data. Female patients were therefore not included.

The protocol of this study was approved by the Ethics Committee of Yamagata Saisei Hospital (approval number of 199 at the ethics committee in 2013), and all subjects provided informed consent. Histories of illness, medication, cigarette smoking, and alcohol consumption were surveyed by questionnaires. Subjects were divided into six groups by pack-year, which was calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person smoked, of cigarette consumption (nonsmokers, >0 and ≤10 pack-years, >10 and ≤20 pack-years, >20 and ≤40 pack-years, >40 and ≤60 pack-years, and >60 pack-years). Ex-smoker was not used as a category of smoking for analysis and was evaluated using pack-years. The frequency of habitual alcohol consumption was asked in the questionnaire. The frequency of weekly alcohol consumption was categorized as "5 days or more" (regular drinkers), "4 days or less" (occasional drinkers), and "never" (non-drinkers). In total, 77.0% and 54.0% of the subjects had histories of medication therapy for hypertension and dyslipidemia, respectively.

Measurements

Height and body weight were measured with light clothes on at the health checkup. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Waist circumference was measured at the navel level according to the recommendation of the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome[19].

Fasting blood was sampled from each subject in the morning. Serum triglyceride, HDL cholesterol and LDL cholesterol levels were measured by enzymatic methods using Determiner TG II, Metabolead HDL-C, and Metabolead LDL-C (Kyowa Medex Co., Ltd, Tokyo, Japan), respectively. The coefficients of variation for the reproducibility of measurement were ≤3% for triglyceride levels and ≤5% for HDL and LDL cholesterol levels. Hemoglobin A1c levels were measured using an automatic glyco-hemoglobin analyzer based on high-performance liquid chromatography (ADAMSTM A1c HA-8170, Sekisui Medical Co., Ltd, Tokyo, Japan). As the standards of hemoglobin A1c levels used for measurement are different in the National Glycohemoglobin Standardization Program (NGSP) and Japan Diabetes Society (JDS), hemoglobin A1c levels were calibrated using a formula proposed by the JDS[20]: hemoglobin A1c (NGSP) (%) = 1.02 × hemoglobin A1c (JDS) (%) + 0.25%. Subjects with diabetes were defined as those receiving drug therapy for diabetes and/or those showing high hemoglobin A1c levels (≥6.5%), according to the criteria for the diagnosis of diabetes by the American Diabetes Association[21]. Blood glucose, uric acid, and creatinine levels were measured by enzymatic methods using L-Type Wako Glu2, L-Type Wako UA M, and

Table 1. Characteristics of the subjects.

Variables	Means, medians or percentages
Age (years)	75.2 ± 8.0
Smokers (%)	current, 21.8%; ex, 74.7%
Cigarette consumption (pack-years)	40.0 (27.5, 55.0)
Alcohol drinkers (%)	non, 44.8; occasional, 16.1; regular 39.1
Diabetes (%)	44.8
Subjects receiving revascularization therapy (%)	75.9
Body mass index (kg/m^2)	22.8 ± 2.8
Waist circumference (cm)	81.6 ± 8.2
Waist-to-height ratio	0.502 ± 0.051
Systolic blood pressure (mmHg)	134.8 ± 15.0
Diastolic blood pressure (mmHg)	70.2 ± 11.1
Maximum IMT (mm)	3.20 ± 1.16
Mean IMT (mm)	1.22 ± 0.49
ABI	0.865 ± 0.168
Decrease in ABI after leg exercise (%)	19.1 ± 14.2
HDL cholesterol (mg/dl)	53.5 ± 17.6
LDL cholesterol (mg/dl)	109.0 ± 32.5
Triglycerides (mg/dl)	102 (76, 140)
Uric acid (mg/dl)	5.91 ± 1.44
Fasting blood glucose (mg/dl)	109.6 ± 30.0
Hemoglobin A1c (%)	6.33 ± 1.10
Fibrinogen (mg/dl)	293.2 ± 72.3
C-reactive protein (mg/dl)	0.08 (0.04, 0.21)
eGFR (ml/min/1.73 m^2)	61.5 ± 19.5

Means ± standard deviations, medians with 25 and 75 percentile values, or percentages of subjects are shown. IMT, intima-media thickness; ABI, ankle-brachial index; HDL, high-density lipoprotein; LDL; low-density lipoprotein; eGFR, estimated glomerular filtration rate.

L-Type Wako CRE M (Wako Pure Chemicals Industries, Osaka, Japan), respectively. Fibrinogen levels were measured by the thrombin time method using Thrombocheck-Fib (Sysmex, Kobe, Japan). C-reactive protein levels were measured by the latex agglutination method using LT Auto Wako CRP-HS (Wako Pure Chemicals Industries). The coefficients of variation for the reproducibility of measurements of hemoglobin A1c, glucose, uric acid, creatinine, and fibrinogen levels were ≤ 5% and that for the reproducibility of measurements of C-reactive protein levels was ≤ 10%. Estimated glomerular filtration rate (eGFR) was calculated using the following equation developed by the Japanese Society of Nephrology[22]: $eGFR = 194 \times Cre^{-1.094} \times age^{-0.287}$.

After each subject rested in the supine position, ABI was measured by an oscillometric method using an automatic ABI device (VaSera VS-1500, Fukuda Denshi, Tokyo, Japan) at rest and after stress loading. For load stress to the legs, fatigue in the gastrocnemius and soleus muscles was induced by an isotonic ankle plantar flexion exercise (100 pedals for each leg at 60 beats per minute on an alternate basis corresponding to 5.3 joules of work) using a leg loader (VSL-100A, Fukuda Denshi, Tokyo, Japan), a stress-loading device developed by Toribatake and Komine[23]. Lower and higher values measured at the right or left legs were used for the analysis of ABI and % decrease in ABI after stress loading, respectively. The cut-off values used for low ABI and high % decrease in ABI after stress loading were 0.9 and 20%, respectively. The exercise using the leg loader could not be performed by two subjects because of the weak power of their leg muscles. Arterial pressure of the right brachial artery was also recorded using CAVI-Vasera VS-1500 (Fukuda Denshi).

IMT was measured by ultrasonography in the supine position. Well-trained sonographers scanned high-resolution B-mode ultrasound images (Philips CX50, PHILIPS Electronics Japan, Tokyo, Japan) with an L12-3 MHz transducer. Three arterial wall segments in each common carotid artery were imaged from a fixed lateral transducer angle at the far wall. The far wall IMTs of both common carotid arteries

Table 2. Univariate linear regression analysis of relationships of each atherosclerotic risk factor with IMT and ABI.

	Maximum IMT	Mean IMT	ABI	Decrease in ABI after exercise
Age	0.088	−0.030	−0.208	−0.051
Body mass index	−0.038	−0.023	0.049	0.069
Waist-to-height ratio	−0.032	−0.050	−0.164	0.118
Systolic blood pressure	0.102	0.235[a]	0.104	0.067
Diastolic blood pressure	0.021	0.108	0.069	0.169
HDL cholesterol	−0.202	−0.186	0.142	−0.053
LDL cholesterol	−0.070	0.015	−0.018	0.205
Log (triglycerides)	0.096	0.167	−0.171	0.292[b]
Uric acid	0.039	−0.111	−0.292[b]	0.236[a]
Fasting blood glucose	0.163	0.235[a]	0.098	−0.089
Hemoglobin A1c	0.197	0.256[a]	0.048	0.099
Fibrinogen	0.114	0.121	0.004	−0.108
Log (C-reactive protein)	−0.005	0.044	−0.186	−0.007
eGFR	−0.059	−0.105	0.189	−0.085

Pearson's correlation coefficients between each pair of variables are shown. Asterisks denote significant correlation coefficients ([a], $P<.05$; [b], $P<.01$). IMT, intima-media thickness; ABI, ankle-brachial index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate. One subject with a CRP level of 0.00 mg/dl was excluded for analysis because log-transformed value could not be calculated.

were measured at three determinations (greatest thickness point and 1-cm upstream and downstream points from the greatest thickness point). The mean IMT over the six segments of both common carotid arteries was calculated. Higher values at the right or left carotid arteries were used for the analysis of maximum and mean IMTs. A mean IMT of less than 1.0 mm was regarded as normal[24]. The coefficients of variation for the inter-observer reproducibility of the maximum and mean IMT measurements were 7.4% and 4.8%, respectively, and the coefficients of variation for the intra-observer reproducibility of the maximum and mean IMT measurements were 7.7% and 8.4%, respectively.

Statistical Analysis

Statistical analyses were performed using a computer software program (SPSS version 16.0 J for Windows, Chicago IL, USA). Data are presented as means ± standard deviations or errors for variables (except for cigarette consumption, triglycerides and C-reactive protein) showing normal distributions and medians with 25 and 75 percentile values for variables (triglycerides and C-reactive protein) not showing normal distributions. In the linear regression analysis, Pearson's correlation coefficients and standardized regression coefficients were calculated. The uric acid levels were arranged in ascending order; then, the subjects were divided into three tertile groups of equal sizes. The means of each variable were compared among the groups of the 1st, 2nd, and 3rd tertiles for uric acid levels using ANOVA in the univariate analysis and ANCOVA followed by Student's *t*-test after Bonferroni correction in the multivariate analysis. In the logistic regression analysis, the odds ratios of subjects of the 2nd or 3rd tertile versus the 1st tertile for uric acid levels were estimated before and after adjustment for other explanatory variables. In the multivariate analyses, age, history of revascularization therapy, diabetes, smoking, alcohol consumption, BMI, and triglyceride levels were used as other explanatory variables in the regression analysis or covariates in ANCOVA. In some analyses, eGFR was added to the explanatory variables or covariates. As triglyceride and C-reactive protein levels did not show normal distributions, they were used for parametric analyses after log transformation. Probability values less than 0.05 were considered to be significant.

Results

Characteristics of Subjects

Table 1 shows the characteristics of subjects. The proportion of subjects aged 70 years or more was 77.0%. Three-fourths of the subjects received revascularization therapy (endovascular angioplasty and/or surgical treatment). The proportion of subjects with diabetes and the proportion of subjects who smoked (current or ex-smokers) were 44.8% and 96.6%, respectively. The proportion of subjects showing high

Table 3. Multivariate linear regression analysis of relationships of each variable with IMT and ABI.

	Maximum IMT	Mean IMT	ABI	Decrease in ABI after exercise
Uric acid	−0.077	−0.215	−0.368[b]	0.311[a]
Age	0.087	−0.048	−0.226	−0.009
History of revascularization therapy	0.206	0.081	0.121	−0.109
Diabetes	0.084	0.074	0.033	0.014
Smoking (pack-years)	0.087	0.042	−0.010	0.128
Alcohol drinking	0.167	−0.029	0.026	−0.129
Body mass index	−0.029	−0.017	0.255[a]	−0.166
Log (triglycerides)	0.088	0.177	−0.261[a]	0.305[a]
eGFR	−0.031	−0.170	0.008	0.040

Standardized regression coefficients between each pair of variables are shown. Asterisks denote significant correlation coefficients ([a], $P<.05$; [b], $P<.01$). IMT, intima-media thickness; ABI, ankle-brachial index; eGFR, estimated glomerular filtration rate.

mean IMTs (≥1.0 mm) was 59.8%. The proportions of subjects showing a low ABI (<0.9) and a high % decrease in ABI after leg exercise (≥20%) were 46.0% and 41.4%, respectively. One-fourth of the subjects (25.3%) had high uric acid levels in blood (≥7.0 mg/dl). The proportion of subjects taking uric acid-lowering drugs was 12.6%.

Univariate Linear Regression Analysis for Relationships of Atherosclerotic Risk Factors with Indicators of Atherosclerosis

Table 2 shows the Pearson's correlation coefficients of each atherosclerotic risk factor with maximum IMT, mean IMT, ABI, and exercise-induced decrease in ABI. Systolic blood pressure and fasting blood sugar and hemoglobin A1c levels showed significant correlations with mean IMT. Uric acid and log-transformed triglyceride levels showed significant correlations with exercise-induced decrease in ABI. There was also an inverse significant correlation between uric acid levels and ABI. However, uric acid levels showed no significant correlations with mean and maximum IMTs.

Multivariate Linear Regression Analysis for Relationships of Uric Acid Levels with Indicators of Atherosclerosis

As shown in **Table 3**, uric acid levels showed significant correlations with ABI and exercise-induced decrease in ABI after adjustment for age, history of revascularization therapy, diabetes, smoking, alcohol consumption, BMI, triglyceride levels, and eGFR. There was no significant correlation between uric acid levels and maximum or mean IMTs in the multivariate linear regression analysis. In the univariate linear regression analysis, blood pressure and LDL or HDL cholesterol levels showed no significant correlations with ABI and decrease in ABI after exercise (**Table 2**). Moreover, both blood pressure and HDL cholesterol levels are known to be associated with triglyceride levels, which showed significant associations with ABI and decrease in ABI after exercise (**Table 3**). Thus, blood pressure and cholesterol levels were not used as explanatory variables in multivariate analyses, although both are important risk factors for atherosclerosis. When LDL cholesterol-to-HDL cholesterol level ratio and mean arterial pressure were added to the explanatory variables in the multivariate linear regression analysis, uric acid levels showed significant correlations with ABI and decrease in ABI after exercise but not with maximum and mean IMTs [standardized regression coefficients of uric acid levels: −0.082 (with maximum IMT, $p=0.573$), −0.198 (with mean IMT, $p=0.180$), −0.374 (with ABI, $p=0.008$), and 0.337 (with decrease in ABI after exercise, $p=0.019$)].

Comparison of ABI and Exercise-Induced Decrease in ABI in Subjects with Different Uric Acid Levels

Table 4 shows mean ABI and % decrease in ABI after leg exercise in the subject groups of tertiles for uric acid levels. In the univariate analysis, ABI was significantly lower in the 3rd (highest) tertile group of uric acid levels than in the 1st (lowest) and 2nd (middle) tertile groups. In the multivariate analysis using age, history of revascularization therapy, diabetes, smoking, alcohol consumption, BMI, and triglyceride levels as covariates, there was still a significant difference in ABI of the 1st and 3rd tertile groups of uric acid levels. When eGFR was further added to the covariates, the difference in ABI of the 1st and 3rd tertile groups of uric acid levels was marginally significant ($p=0.076$). In the univariate and multivariate analyses, there were marginally significant differences

Table 4. Comparison of ABI and decrease in ABI after leg exercise among the tertile groups of uric acid.

	1st tertile of uric acid (3.7−4.9 mg/dl, $n=27$)	2nd tertile of uric acid (5.0−6.5 mg/dl, $n=31$)	3rd tertile of uric acid (6.7−10.0 mg/dl, $n=29$)
ABI			
Univariate	0.898 ± 0.032 [a]	0.907 ± 0.028 [a]	0.790 ± 0.031
Multivariate (Adjustment 1)	0.915 ± 0.035 [a]	0.892 ± 0.030 [c]	0.789 ± 0.032
Multivariate (Adjustment 2)	0.914 ± 0.035 [d]	0.891 ± 0.030	0.792 ± 0.034
Decrease in ABI after exercise			
Univariate	14.0 ± 1.8 [e]	19.8 ± 2.6	23.2 ± 3.0
Multivariate (Adjustment 1)	13.7 ± 3.0 [f]	19.4 ± 2.6	23.8 ± 2.8
Multivariate (Adjustment 2)	13.6 ± 3.0 [g]	19.4 ± 2.6	23.9 ± 2.9

Means ± standard errors of each variable are shown. Adjustment 1: for age, history of revascularization therapy, diabetes, smoking, alcohol drinking, body mass index and log-transformed triglycerides; Adjustment 2: for estimated glomerular filtration rate in addition to the variables used in Adjustment 1. Asterisks denote significant differences from the 3rd tertile ([a], $P<.05$). Marginally significant differences from the 3rd tertile: [c], $P=.067$; [d], $P=.076$; [e], $P=.052$; [f], $P=.063$; [g], $P=.081$. ABI, ankle-brachial index.

in exercise-induced decrease in ABI between the 1st and 3rd tertile groups of uric acid levels. ABI and exercise-induced % decrease in ABI tended to be lower and higher, respectively, with an increase in tertile in the multivariate analysis.

Logistic Regression Analysis for the Relationship of Uric Acid Levels with Leg Ischemia

Table 5 shows odds ratios for leg ischemia of the 2nd or 3rd tertile versus the 1st tertile for the levels of uric acid and prevalence of abnormal ABI in each tertile. The prevalences of low ABI and high % decrease in ABI after exercise were significantly higher in the 3rd tertile of uric acid levels than in the 1st tertile, and the prevalence of high % decrease in ABI after exercise was marginally significantly higher in the 2nd tertile of uric acid levels than in the 1st tertile. The crude odds ratios of the 3rd versus the 1st tertile groups of uric acid levels for low ABI and high % decrease in ABI after leg exercise were significantly higher than the reference level of 1.00. The odds ratio of the 3rd versus the 1st tertiles for low ABI was still significant after adjustment for age, history of revascularization therapy, diabetes, smoking, alcohol consumption, BMI, and triglyceride levels but was not significant after adjustment for eGFR in addition to the above explanatory variables. In the multivariate analyses with adjustment for the above explanatory variables including or excluding eGFR, odds ratios of the 3rd versus the 1st tertile groups of uric acid levels for high % decrease in ABI after exercise were significantly higher than the reference level. Crude and adjusted odds ratios of the 2nd versus the 1st tertile groups of uric acid levels for high % decrease in ABI after exercise were marginally significantly higher than the reference level. Odds ratios for low ABI and high % decrease in ABI after exercise tended to be higher with an increase in tertile.

Discussion

In the linear regression analysis, among the various atherosclerotic risk factors tested, only serum uric acid levels showed significant correlations with both ABI and leg exercise-induced % decrease in ABI. These associations were confirmed by other analyses including ANOVA, ANCOVA, and logistic regression analysis and were found after adjustment for age, history of revascularization therapy, diabetes, smoking, alcohol consumption, BMI, triglyceride levels, and eGFR. Therefore, uric acid levels are related to leg ischemia independently of these possible confounding factors. To the best of our knowledge, this is the first study showing an association of uric acid levels with leg ischemia in patients with PAD. The findings of the present study may be reasonable as there have been some studies showing an association of the levels of uric acid with the incidence or prevalence of PAD in the general population[12, 13] and in patients with diabetes[14] and with hypertension[15], although the significance of uric acid levels in patients with PAD is unknown. Thus, uric acid is thought to be a risk factor for both onset and progression of PAD. The prognosis of PAD is determined by complicated coronary heart disease and stroke[3-5]. Therefore, the present findings agree with the findings of recent studies that high serum uric acid levels are associated with increased cardiovascular disease mortality in Japanese men and women[25] and with subclinical atherosclerosis evaluated based on IMT in Chinese men and women[26]. Moreover, a recent clinical study demonstrated that serum uric acid levels are associated with coronary lipid-rich plaques in Japanese men and

Table 5. Logistic regression analysis for low ABI and high % decrease in ABI after leg exercise in the tertile subject groups for uric acid.

	1st tertile of uric acid (3.7−4.9 mg/dl, $n=27$)	2nd tertile of uric acid (5.0−6.5 mg/dl, $n=31$)	3rd tertile of uric acid (6.7−10.0 mg/dl, $n=29$)
Low ABI			
Number	9	11	20
Prevalence (%)	33.3	35.5	69.0[a]
OR (Crude)	1.00	1.10 (0.37-3.26)	4.44 (1.45-13.65)[b]
OR (Adjustment 1)	1.00	1.96 (0.44-8.63)	5.56 (1.24-24.90)[a]
OR (Adjustment 2)	1.00	2.07 (0.43-10.02)	3.69 (0.72-18.97)
High % decrease in ABI after exercise			
Number	6	14	16
Prevalence (%)	22.2	45.2[c]	55.2[a]
OR (Crude)	1.00	2.88 (0.91-9.11)[d]	4.31 (1.34-13.82)[a]
OR (Adjustment 1)	1.00	3.73 (0.78-17.76)[e]	6.93 (1.40-34.27)[a]
OR (Adjustment 2)	1.00	3.73 (0.78-17.80)[e]	8.08 (1.38-47.44)[a]

Odds ratios (ORs) with their 95% confidence intervals are shown. Adjustment 1: for age, history of revascularization therapy, diabetes, smoking, alcohol drinking, body mass index and log-transformed triglycerides; Adjustment 2: for estimated glomerular filtration rate (eGFR) in addition to the variables used in Adjustment 1. Asterisks denote significant differences from the 1st tertile or the reference level of 1.00 ([a], $P<.05$; [b], $P<.01$). Marginally significant differences from the reference level: [c], $P=0.097$; [d], $P=0.071$; [e], $P=0.098$. ABI, ankle-brachial arterial pressure index.

women[27].

To confirm no confounding by history of revascularization therapy for the association of uric acid levels with leg ischemia, multiple linear regression analysis and logistic regression analysis were performed using only subjects who underwent revascularization therapy. Among subjects with a history of revascularization therapy, serum uric acid levels showed a significant correlation with ABI ($r=-0.398$, $p<0.05$) but not with maximum IMT ($r=-0.110$, $p=0.542$) or mean IMT ($r=-0.107$, $p=0.538$). These findings agree with the findings for overall subjects (**Table 3**). However, the correlation coefficient between uric acid levels and % decrease in ABI after exercise in subjects with a history of revascularization therapy did not reach a significant level ($r=0.233$, $p=0.185$). In the logistic regression analysis using subjects with a history of revascularization therapy, crude odds ratios for high % decrease in ABI after exercise of the 2nd and 3rd versus the 1st tertile groups for uric acid levels were significantly higher ($p<0.05$) than the reference level of 1.00 [2nd tertile, 6.23 (1.40−27.84); 3rd tertile, 6.14 (1.43−26.35]). These odds ratios were significantly or marginally significantly higher than the reference level in the multivariate analysis using age, history of diabetes, smoking, alcohol consumption, BMI, log-transformed triglyceride levels, and eGFR as other explanatory variables (data not shown). The crude odds ratio for uric acid levels of the 3rd versus the 1st tertile groups for low ABI was marginally significantly higher ($p=0.057$) than the reference level of 1.00 [3.30 (0.97−11.29)), but the odds ratio was not significantly different from the reference level in the multivariate analysis (data not shown). The prevalence of low ABI was significantly higher ($p<0.05$) in the 3rd tertile group for uric acid levels than in its 1st tertile group (64.0% vs. 35.0%), and the prevalence of high % decrease in ABI after exercise was significantly higher ($p<0.05$) in the 2nd and 3rd tertile groups for uric acid levels than in its 1st tertile group [15.0% (1st tertile) vs. 52.4% (2nd tertile) vs. 52.0% (3rd tertile)]. Thus, trends for the relationships of uric acid levels with ABI and decrease in ABI after exercise were similar in the analysis using overall subjects and the analysis using only subjects with a history of revascularization therapy. Therefore, it is unlikely that revascularization therapy confounded the association of uric acid levels with leg ischemia in this study.

The purpose of this cross-sectional study was to investigate the relationship of the uricemic status with ischemia of the lower extremities. The current uricemic status depends on whether medication therapy is being received. Therefore, we did not add medication use for hyperuricemia as a covariate or an explanatory variable in the multivariate analyses. When medication use for hyperuricemia was added as an explanatory variable in the multivariate linear regression analysis, uric acid levels showed significant negative and positive correlations with ABI ($\beta=-0.350$, $p<0.05$) and decrease in ABI after exercise ($\beta=0.299$, $p<0.05$), respectively, although absolute values of the correlation coefficients became smaller by the inclusion of medication use for hyperuricemia to the explanatory variables. Thus, the significant associations of uric acid

levels with ABI and % decrease in ABI after exercise were found even after adjustment for therapy for hyperuricemia.

It remains to be clarified whether anti-hyperuricemic therapy retards the progression of PAD. A previous randomized controlled trial showed that treatment with allopurinol did not prolong the exercise duration in patients with PAD[28]. Although this study is cross-sectional in its design and thus no causality can be discussed by the findings of this study, there is a possibility that the symptoms and prognosis of PAD are improved by therapy for hyperuricemia. Future randomized controlled studies are needed to prove this hypothesis.

Hyperuricemia is a risk factor for atherosclerosis-related diseases[9]. However, there was no significant association of uric acid levels with maximum or mean IMT in patients with PAD. Serum uric acid levels also showed no association with cardio–ankle vascular index, a good indicator of arterial stiffness of the aorta[29] (data not shown). Thus, uric acid levels were related not to the degree of atherosclerosis in carotid arteries and the aorta but to that in the lower limb arteries in patients with PAD. The reason for this dissociation of the results by the locus of the artery is unknown. One possible explanation for the dissociation is a stronger effect of hyperuricemia on the lower limb arteries than on arteries at other loci, which could be examined in future basic experiments. The expression and activity of XOR, a rate-limiting enzyme in uric acid synthesis, are increased under hypoxia by tissue ischemia following ischemic heart disease and heart failure[10, 11, 30-32]. Therefore, in patients with PAD, XOR expression and activity are expected to be increased in the ischemic lower limb muscles, and this might explain the higher susceptibility of the lower limb arteries to hyperuricemia than that of other arteries.

There are limitations in this study. The population size was small, and the present findings should be confirmed by further studies using larger populations. Data for only male subjects were analyzed, and the possibility of a gender-related difference in the association of uric acid levels with leg ischemia needs to be investigated in the future. Three-fourths of the subjects received revascularization therapy, and approximately half of the subjects showed normal ABIs, which were 0.9 or higher. Thus, therapy for PAD is a possible confounder for the uric acid level–ABI association, although the association was also found when the history of therapy for revascularization was used as an explanatory variable or a covariate in the multivariate analyses. The design of this study was cross-sectional, and further interventional studies are therefore needed to determine whether the correction of uric acid levels in the blood is useful for improving leg arterial flow in patients with diabetes.

In conclusion, serum uric acid levels were associated with leg ischemia in patients with PAD independently of other atherosclerotic risk factors including age, history of revascularization therapy, diabetes, smoking, alcohol consumption, obesity, triglyceride levels, and renal function. Further studies are required to determine the usefulness of the correction of uric acid levels for an improvement in the prognosis of PAD.

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Conflicts of Interest

The authors have no conflicts of interest.

References

1) Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG; TASC II Working Group. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). J Vasc Surg, 2007; 45 Suppl S: S5-67
2) Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, Hiratzka LF, Murphy WR, Olin JW, Puschett JB, Rosenfield KA, Sacks D, Stanley JC, Taylor LM Jr, White CJ, White J, White RA, Antman EM, Smith SC Jr, Adams CD, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Hunt SA, Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B; American Association for Vascular Surgery; Society for Vascular Surgery; Society for Cardiovascular Angiography and Interventions; Society for Vascular Medicine and Biology; Society of Interventional Radiology; ACC/AHA Task Force on Practice Guidelines Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease; American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; Vascular Disease Foundation; ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart,

Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. Circulation, 2006; 113: e463-654
3) Hirsch AT, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JW, Krook SH, Hunninghake DB, Comerota AJ, Walsh ME, McDermott MM, Hiatt WR. Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA, 2001; 286: 1317-1324
4) Haugen S, Casserly IP, Regensteiner JG, Hiatt WR. Risk assessment in the patient with established peripheral arterial disease. Vasc Med, 2007; 12: 343-350
5) Newman AB, Siscovick DS, Manolio TA, Polak J, Fried LP, Borhani NO, Wolfson SK. Ankle-arm index as a marker of atherosclerosis in the Cardiovascular Health Study. Cardiovascular Heart Study (CHS) Collaborative Research Group. Circulation, 1993; 88: 837-845
6) Flu HC, Tamsma JT, Lindeman JH, Hamming JF, Lardenoye JH. A systematic review of implementation of established recommended secondary prevention measures in patients with PAOD. Eur J Vasc Endovasc Surg, 2010; 39: 70-86
7) Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefèbvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Korányi L, Laakso M, Mokán M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Schernthaner G, Schmitz O, Skrha J, Smith U, Taton J; PROactive investigators. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. Lancet, 2005; 366: 1279-1289
8) ADVANCE Collaborative Group, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med, 2008; 358: 2560-2572
9) Borghi C, Rosei EA, Bardin T, Dawson J, Dominiczak A, Kielstein JT, Manolis AJ, Perez-Ruiz F, Mancia G. Serum uric acid and the risk of cardiovascular and renal disease. J Hypertens, 2015; 33: 1729-1741
10) Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol, 2004; 555: 589-606
11) Battelli MG, Polito L, Bolognesi A. Xanthine oxidoreductase in atherosclerosis pathogenesis: not only oxidative stress. Atherosclerosis, 2014; 237: 562-567
12) Baker JF, Schumacher HR, Krishnan E. Serum uric acid level and risk for peripheral arterial disease: analysis of data from the multiple risk factor intervention trial. Angiology, 2007; 58: 450-457
13) Shankar A, Klein BE, Nieto FJ, Klein R. Association between serum uric acid level and peripheral arterial disease. Atherosclerosis, 2008; 196: 749-755
14) Tseng CH. Independent association of uric acid levels with peripheral arterial disease in Taiwanese patients with Type 2 diabetes. Diabet Med, 2004; 21: 724-729
15) Langlois M, De Bacquer D, Duprez D, De Buyzere M, Delanghe J, Blaton V. Serum uric acid in hypertensive patients with and without peripheral arterial disease. Atherosclerosis, 2003; 168: 163-168
16) Aboyans V, Criqui MH, Abraham P, Allison MA, Creager MA, Diehm C, Fowkes FG, Hiatt WR, Jönsson B, Lacroix P, Marin B, McDermott MM, Norgren L, Pande RL, Preux PM, Stoffers HE, Treat-Jacobson D; American Heart Association Council on Peripheral Vascular Disease; Council on Epidemiology and Prevention; Council on Clinical Cardiology; Council on Cardiovascular Nursing; Council on Cardiovascular Radiology and Intervention, and Council on Cardiovascular Surgery and Anesthesia; Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association. Circulation, 2012; 126: 2890-2909
17) Mahe G, Pollak AW, Liedl DA, Cohoon KP, Mc Carter C, Rooke TW, Wennberg PW. Discordant Diagnosis of Lower Extremity Peripheral Artery Disease Using American Heart Association Postexercise Guidelines. Medicine (Baltimore), 2015; 94: e1277
18) Høyer C, Sandermann J, Petersen LJ. The toe-brachial index in the diagnosis of peripheral arterial disease. J Vasc Surg, 2013; 58: 231-238
19) Anonymous. Metabolic Syndrome-Definition and Diagnostic Criteria in Japan. J Jpn Soc Int Med, 2005; 94: 794-809 (in Japanese)
20) Kashiwagi A, Kasuga M, Araki E, Oka Y, Hanafusa T, Ito H, Tominaga M, Oikawa S, Noda M, Kawamura T, Sanke T, Namba M, Hashiramoto M, Sasahara T, Nishio Y, Kuwa K, Ueki K, Takei I, Umemoto M, Murakami M, Yamakado M, Yatomi Y, Ohashi H; Committee on the Standardization of Diabetes Mellitus-Related Laboratory Testing of Japan Diabetes Society. International clinical harmonization of glycated hemoglobin in Japan: From Japan Diabetes Society to National Glycohemoglobin Standardization Program values. J Diabetes Investig, 2012; 3: 39-40
21) Anonymous: American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33 (Suppl 1): S62-69
22) The Japanese Society of Nephrology. Clinical practice guidebook for diagnosis and treatment of chronic kidney disease 2012. Nihon Jinzo Gakkai Shi, 2012; 54: 1034-1191
23) Toribatake Y, Komine N. Usefulness of stress-loading test for ankle brachial index using an originally developed exercise device to detect peripheral arterial disease. Int Angiol, 2009; 28: 100-105
24) Handa N, Matsumoto M, Maeda H, Hougaku H, Ogawa S, Fukunaga R, Yoneda S, Kimura K, Kamada T. Ultrasonic evaluation of early carotid atherosclerosis. Stroke, 1990; 21: 1567-1572
25) Zhang W1, Iso H, Murakami Y, Miura K, Nagai M, Sugiyama D, Ueshima H, Okamura T; EPOCH-JAPAN GROUP. Serum uric acid and mortality form cardiovascular disease: EPOCH-JAPAN Study. J Atheroscler Thromb, 2016; 23: 692-703
26) Chen Y, Xu B, Sun W, Sun J, Wang T, Xu Y, Xu M, Lu J, Li X, Bi Y, Wang W, Ning G. Impact of the serum uric acid level on subclinical atherosclerosis in middle-aged

and elderly chinese. J Atheroscler Thromb. 2015; 22: 823-832
27) Ando K, Takahashi H, Watanabe T, Daidoji H, Otaki Y, Nishiyama S, Arimoto T, Shishido T, Miyashita T, Miyamoto T, Kubota I. Impact of serum uric acid levels on coronary plaque stability evaluated using integrated backscatter intravascular ultrasound in patients with coronary artery disease. J Atheroscler Thromb. 2016; 23: 932-939
28) Robertson AJ, Struthers AD. A randomized controlled trial of allopurinol in patients with peripheral arterial disease. Can J Cardiol, 2016; 32: 190-196
29) Yambe T, Yoshizawa M, Saijo Y, Yamaguchi T, Shibata M, Konno S, Nitta S, Kuwayama T. Brachio-ankle pulse wave velocity and cardio-ankle vascular index (CAVI). Biomed Pharmacother, 2004; 58 Suppl 1: S95-98
30) Terada LS, Guidot DM, Leff JA, Willingham IR, Hanley ME, Piermattei D, Repine JE. Hypoxia injures endothelial cells by increasing endogenous xanthine oxidase activity. Proc Natl Acad Sci U S A, 1992; 89: 3362-3366
31) Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, Marbán E, Hare JM. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation, 2001; 104: 2407-2411
32) Landmesser U, Spiekermann S, Dikalov S, Tatge H, Wilke R, Kohler C, Harrison DG, Hornig B, Drexler H. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. Circulation, 2002; 106: 3073-3078