Association between endothelial dysfunction and hyperuricaemia

Wan-Jing Ho¹, Wen-Pin Tsai², Kuang-Hui Yu², Pei-Kwei Tsay³, Chun-Li Wang¹, Tsu-Shiu Hsu¹ and Chi-Tai Kuo¹

Abstract

Objective. We used high-resolution peripheral vascular ultrasound imaging to assess endothelial function in hyperuricaemic patients.

Methods. Hyperuricaemia was defined as a serum uric acid concentration of >7.7 mg/dl in men or >6.6 mg/dl in women. Measurements of endothelium-dependent flow-mediated vasodilation (FMD) and endothelium-independent nitroglycerin-mediated vasodilation were performed in 46 hyperuricaemic patients and an equal number of healthy age- and gender-matched normal controls by high-resolution two-dimensional ultrasonographic imaging of the brachial artery. The serum levels of glucose, creatinine, alanine aminotransferase (ALT), lipid profiles and high-sensitivity CRP were measured for both the study groups.

Results. The serum uric acid levels averaged 9.24 (1.16) and 6.18 (0.99) mg/dl in the hyperuricaemic and control groups, respectively. Body weight and BMI were significantly higher in the hyperuricaemic group than in the control group. The serum levels of creatinine, ALT, triglyceride and high-sensitivity CRP were significantly different between the two groups. The FMD values were significantly lower in the hyperuricaemic patients than in the controls [4.45% (3.13%) vs 7.10% (2.48%); P < 0.001]. The FMD values were negatively associated with serum uric acid levels (r = −0.273; P = 0.009). Multivariate regression analysis showed that the presence of hyperuricaemia (β = −0.384; P < 0.001) and body weight (β = 0.215; P = 0.017) were independent determinants of low FMD values.

Conclusion. Hyperuricaemia is associated with endothelial dysfunction. Decreased nitric oxide bioavailability may be the main reason.

Key words: Hyperuricaemia, Endothelial dysfunction, Flow-mediated vasodilation, High-resolution ultrasound.

Introduction

The relationship between serum uric acid levels and cardiovascular diseases has been controversial [1, 2]. Most epidemiological evidence now suggests that serum uric acid level is a relevant independent risk factor for cardiovascular and renal diseases, particularly in patients with hypertension, heart failure or diabetes [3]. Furthermore, hyperuricaemia is an independent predictor of mortality in coronary artery disease, heart failure and stroke [3–5]. The mechanisms underlying the organ damage caused by uric acid are not completely understood, but many reports have indicated that endothelial dysfunction plays an important role in affecting cardiovascular and renal function, as well as the structure [6, 7].

A series of animal experiments revealed that in rats, hyperuricaemia induced by oxonic acid, a uricase inhibitor, causes hypertension and renal arteriolopathy and impairs nitric oxide (NO) generation [8–11]. Allopurinol, a xanthine oxidase inhibitor, has been reported to reverse the detrimental effects induced by hyperuricaemia [10]. Moreover, oxidative stress plays a crucial role in the vascular endothelial dysfunction of hyperuricaemia [12]. Uric acid has been shown to have antioxidant properties [13],...
and acute administration of uric acid improves endothelial function in smokers and diabetic patients but not in healthy subjects [14, 15]. Elevated serum uric acid levels may predict cardiac death, but it remains to be determined whether this marker is elevated in patients with cardiovascular diseases or actually a culprit in causing deleterious changes in vascular function.

High-resolution peripheral ultrasound imaging has been extensively used to measure flow-mediated vasodilation (FMD) for the evaluation of vascular endothelial function. It is a non-invasive method and is frequently used to assess the risk factors for coronary artery disease such as hypertension, diabetes mellitus, hypercholesterolaemia and obesity [16, 17]. The precise mechanism underlying brachial FMD is not completely understood; however, it is generally believed that brachial FMD is mainly mediated by NO [18].

To determine the effect of hyperuricaemia on endothelial function, we used peripheral ultrasound to study the endothelium-dependent FMD and endothelium-independent nitroglycerin-mediated vasodilatation (NMD) of the brachial artery in hyperuricaemic patients.

Methods

Study subjects

We defined hyperuricaemia as a serum uric acid concentration of >7.7 mg/dl in men or >6.6 mg/dl in women, as measured by enzymatic-colorimetric methods [19, 20]. We recruited 46 consecutive patients with hyperuricaemia from the Rheumatology Clinic of Chang Gung Memorial Hospital (CGMH). Patients with no history of a gout attack for at least the previous 4 weeks and consumption of not more than 60 ml of alcohol per day were recruited. None of the patients was being treated with either uricosuric agents or xanthine oxidase inhibitors. Of the hyperuricaemic patients, 19.6% (9 out of 46) received colchicine for the prevention of acute gout attack; no other medications or vasoactive agents were taken. Patients with systemic diseases such as hypertension, diabetes, hypercholesterolaemia (≥240 mg/dl), coronary artery disease, congestive heart failure, peripheral vascular disease, stroke or any disease that predisposes to vasculitis or RP were excluded. We recruited 46 age- and gender-matched control subjects from a routine medical check-up group with normal physical examination results and no history of cardiovascular disease or rheumatological problems. None of the control subjects received any medication. Both groups were referred to the vascular laboratory for vascular ultrasound. Before participating in the study, all patients gave informed consent and the study was approved by the institutional review board of CGMH.

Biochemical measurements

Venous blood samples were collected after overnight fasting for 12 h. Serum uric acid level was measured by the uricase differential spectrophotometric method. The coefficient of variation was ≤1.8% for repeated measurements of serum uric acid levels performed throughout the year at the hospital laboratory on samples with previously determined uric acid content. Levels of serum cholesterol (desirable, <200 mg/dl), triglyceride (desirable, <150 mg/dl), creatinine (normal range: female 0.44–1.03 mg/dl; male 0.64–1.27 mg/dl) and alanine aminotransferase (ALT; normal range, 0–36 U/l) were enzymatically measured (Hitachi Automatic Analyzer model 7600; Hitachi, Tokyo, Japan). Fasting blood glucose levels (normal range, 70–105 mg/dl) were measured by the glucose oxidase method (Olympus AU640; Olympus, MA, USA). High-sensitivity CRP levels were determined by a high-sensitivity commercial assay kit (Daichi Pure Chemicals Co. Ltd, Tokyo, Japan; Analyzer: Hitachi 7600).

Measurement of vascular endothelial function

All study subjects were referred to the vascular laboratory in the morning after fasting overnight for at least 8 h. Cigarette smoking and the consumption of alcohol, coffee, tea and any medications were prohibited after midnight before the day of the examination. Brachial artery FMD was studied by high-resolution peripheral vascular ultrasonic imaging as described previously [21, 22]. Two-dimensional (2D) images of the left brachial artery and pulsed-Doppler flow velocity signals were obtained using an Acuson L7 7.5 MHz linear array transducer on an Acuson Aspen ultrasound system (Acuson, Mountain View, CA, USA). Imaging was performed in a dimly lit, quiet room with the room temperature maintained at 22–25 °C. Patients rested in a supine position for at least 10 min before the first scan and remained in the same position until the final recording was acquired under continuous ECG monitoring. Blood pressure measurement was taken from the right arm before imaging. Images were obtained ~3–5 cm above the antecubital fossa of the left arm. Baseline 2D images and pulsed-Doppler blood flow velocity were acquired. To induce hyperaemia, a 5.6-inch wide blood pressure cuff was inflated at the forearm to 200 mmHg. Arterial occlusion was maintained for 5 min, with the transducer positioned carefully in an identical position by a micrometer-adjustable stereostatic probe holder (probe-fixing unit: Aloka MP-PH0001; arm holder: Aloka MP-AH0001; Aloka, Tokyo, Japan). The cuff was then rapidly deflated, and pulsed-Doppler velocity signals were recorded for 15 s following deflation. Reactive hyperaemia was determined by the mosaic change in colour-flow imaging and increase in flow volume. At 60 s after cuff deflation, 2D images of the brachial artery were recorded for 15 s. To determine endothelium-independent NMD, a sublingual nitroglycerin spray (400 µg) was administered following another 10-min rest period, during which the vessel diameter restored to baseline. Brachial artery scans were performed at the same position 4 min after the nitroglycerin spray.

The FMD was expressed as the percentage of maximum vessel diameter change induced by hyperaemia. Similarly, the NMD was expressed as the percentage change in diameter in response to nitroglycerin sublingual spray. The intraobserver and interobserver coefficients of variation for the baseline arterial diameter measurement
were 1.6 and 2.1%, respectively, in our vascular laboratory (n = 15).

Statistical analysis

Subject numbers were estimated in our pilot study. With 15 hyperuricaemic patients and 15 normal controls, the means (s.d.) of the FMD were 5.61% (2.82%) and 7.37% (2.74%) in the hyperuricaemic and control groups, respectively. Forty cases were determined to be an adequate sample size for each group, with 80% power at a 5% significance level.

All data are expressed as means (s.d.) for continuous variables and as percentages for categorical variables. Continuous variables were compared between the two groups using the unpaired Student’s t-test. Categorical variables were compared between the groups using either the chi-square test or, if the number of cases was fewer than five, Fisher’s exact test. Correlations between variables in hyperuricaemic patients and controls were calculated by Pearson’s coefficient. Additionally, multivariate analysis was performed by stepwise linear regression modelling to identify individual and joint factors influencing FMD. Values of \( P < 0.05 \) were considered to be statistically significant.

Results

Table 1 summarizes the demographic characteristics of the hyperuricaemic patients and the normal controls. The serum uric acid level was 9.24 (1.16) mg/dl in the hyperuricaemic patients and 6.18 (0.99) mg/dl in the controls. Body weight and BMI were significantly higher in the hyperuricaemic group than in the control group. Serum levels of creatinine, ALT, triglyceride and high-sensitivity CRP differed significantly between the two groups. However, no significant difference was noted between the two groups with regard to age, gender, waist circumference, heart rate, blood pressure and serum levels of fasting glucose, high-density lipoprotein, low-density lipoprotein and total cholesterol.

Table 2 lists the vascular parameters of the two groups. Figure 1 shows that the FMD values were significantly lower in the hyperuricaemic patients than in the controls [4.45% (3.13%) vs 7.10% (2.48%); \( P < 0.001 \)]. However, the difference in the NMD values between the two groups was insignificant [13.37% (5.44%) vs 15.21% (5.45%); \( P = 0.112 \)]. In the correlation study, FMD values were negatively associated with serum uric acid levels (\( r = -0.273; P = 0.009 \)), as shown in Fig. 2. Additionally, FMD values were significantly correlated with NMD (\( r = 0.527; P < 0.001 \)), baseline brachial artery diameter (\( r = -0.387; P < 0.001 \)) and triglyceride levels (\( r = -0.272; P = 0.009 \)).

To determine whether the low FMD values were independent of the demographic variables, we performed a multivariate regression analysis that included the full series of demographic variables and vascular parameters. In this analysis, NMD (\( j = 0.607; P < 0.001 \)), the presence of hyperuricaemia (\( j = -0.384; P < 0.001 \)) and body weight (\( j = 0.215; P = 0.017 \)) were independent determinants of impaired FMD with \( R^2 = 0.515 \) for the three variables.

Discussion

Our study shows that hyperuricaemia is associated with endothelial dysfunction. These observations suggest a possible and specific mechanism through which

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**Table 1** Demographic characteristics of the hyperuricaemic and control groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hyperuricaemia</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>46</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>43.00 (12.42)</td>
<td>44.98 (11.46)</td>
<td>0.429</td>
</tr>
<tr>
<td>Gender, male, %</td>
<td>87.0</td>
<td>89.1</td>
<td>0.748</td>
</tr>
<tr>
<td>Body height, cm</td>
<td>168.00 (6.21)</td>
<td>165.61 (6.76)</td>
<td>0.081</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>78.83 (12.16)</td>
<td>71.21 (9.56)</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.94 (4.18)</td>
<td>25.97 (3.17)</td>
<td>0.012</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>90.85 (9.18)</td>
<td>87.55 (7.61)</td>
<td>0.064</td>
</tr>
<tr>
<td>Cigarette smoking, %</td>
<td>15.2</td>
<td>15.2</td>
<td>1.000</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>73 (12)</td>
<td>73 (11)</td>
<td>0.978</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>124 (14)</td>
<td>119 (15)</td>
<td>0.153</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>79 (10)</td>
<td>76 (11)</td>
<td>0.139</td>
</tr>
<tr>
<td>Uric acid, mg/dl</td>
<td>9.24 (1.16)</td>
<td>6.18 (0.99); &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>1.13 (0.15)</td>
<td>1.04 (0.19); 0.013</td>
<td></td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>30 (19)</td>
<td>21 (8)</td>
<td>0.003</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>92 (9)</td>
<td>93 (8)</td>
<td>0.767</td>
</tr>
<tr>
<td>High-density lipoprotein, mg/dl</td>
<td>46 (10)</td>
<td>49 (10)</td>
<td>0.146</td>
</tr>
<tr>
<td>Low-density lipoprotein, mg/dl</td>
<td>118 (28)</td>
<td>115 (23)</td>
<td>0.587</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>194 (32)</td>
<td>188 (25)</td>
<td>0.318</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>150 (59)</td>
<td>119 (53)</td>
<td>0.011</td>
</tr>
<tr>
<td>High-sensitivity CRP, mg/l</td>
<td>1.96 (1.55)</td>
<td>1.22 (0.89); 0.007</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as means (s.d.) for continuous variables and as a percentage for categorical variables.
heightened uric acid levels might contribute to an increase in cardiovascular risk. We used high-frequency ultrasound imaging of the brachial artery to assess endothelium-dependent FMD. The hyperaemia induced by transient ischaemia of the forearm increases shear stress on the vascular wall; this causes the endothelial cells to release NO, resulting in blood vessel dilation. This process can be quantified as an index of vasomotor function [16]. More importantly, brachial FMD has become a gold standard for the non-invasive assessment of endothelial function in conduit arteries [17].

Endothelial dysfunction is a risk factor for cardiovascular diseases [23]. Recent data from experimental animal models directly implicated the role of uric acid in endothelial dysfunction [10, 11]. In rats, hyperuricaemia induced by oxonic acid decreased NO production [10]. Additionally, allopurinol, which lowers uric acid levels, can reverse the uric acid-induced endothelial dysfunction [10]. The findings of the study performed by Sánchez-Lozada et al. [11] are also impressive; they reported that L-arginine, a NO substrate, prevented glomerular hypertrophy and pre-glomerular arteriopathy in hyperuricaemic rats. Few studies on hyperuricaemia and endothelial dysfunction have been conducted in humans, and the data that are available are controversial [15, 24, 25]. Waring et al. [15] showed that acute uric acid administration caused no change in haemodynamic parameters, forearm blood flow or NO-dependent endothelial function in healthy individuals. However, impaired FMD was found in hyperuricaemic patients, who were at increased risk of cardiovascular diseases. Furthermore, allopurinol treatment for 3 months improved FMD in hyperuricaemic patients but not in normal subjects [25]. In a small-scale study, the FMD values of 17 male hyperuricaemic patients were significantly lower than those of 9 control subjects [24]. This is in line with the findings of our study. Furthermore, we performed the study with more patients and included women. Hyperuricaemia was defined as a serum uric acid concentration of >7.7 mg/dl in men or >6.6 mg/dl in women in this study according to previous nationwide population-based studies in our country [19, 20]. Although it is true that men tend to have higher serum urate levels than women, it is possible that the choice of a lower serum urate level for women may have resulted in a smaller difference between the hyperuricaemic and control groups vis-à-vis FMD, thus leading us to underestimate the actual effect.

**TABLE 2** Vascular parameters of the hyperuricaemic and control groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hyperuricaemia</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>46</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Baseline vessel diameter, mm</td>
<td>4.45 (0.53)</td>
<td>4.36 (0.53)</td>
<td>0.428</td>
</tr>
<tr>
<td>Hyperaemia vessel diameter, mm</td>
<td>4.64 (0.50)</td>
<td>4.66 (0.53)</td>
<td>0.833</td>
</tr>
<tr>
<td>NTG vessel diameter, mm</td>
<td>5.03 (0.49)</td>
<td>5.01 (0.51)</td>
<td>0.854</td>
</tr>
<tr>
<td>Reactive hyperaemic flow, %</td>
<td>265 (90)</td>
<td>280 (117)</td>
<td>0.842</td>
</tr>
<tr>
<td>FMD, %</td>
<td>4.45 (3.13)</td>
<td>7.10 (2.48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NMD, %</td>
<td>13.37 (5.44)</td>
<td>15.21 (5.45)</td>
<td>0.112</td>
</tr>
</tbody>
</table>

Data are expressed as means (s.d.). NTG: nitroglycerin.
The impact of oxidative stress on vascular endothelial function is well recognized. Xanthine oxidase forms uric acid, which has been shown to be a marker of cardiovascular risk [12]. However, it is not clearly understood whether uric acid itself is actually a culprit or whether elevated uric acid level is a surrogate marker for identifying vascular endothelial dysfunction. In a different aspect related to the vascular effects of blocking xanthine oxidase, allopurinol inhibits xanthine oxidase and thereby not only reduces uric acid but also decreases the formation of reactive oxygen species in the same reaction that produces uric acid [12]. George et al. [26] have shown that high-dose allopurinol is an effective antioxidant, and the improvement in endothelial function is caused by reducing vascular oxidative stress, not by lowering uric acid in stable heart failure patients.

Although endothelial dysfunction has been reported as the factor responsible for hyperuricaemia in cardiovascular diseases, other related hormonal and cytokine effects of uric acid-mediated pro-inflammation and proliferation on vascular smooth muscle cells (SMCs) are also important [27]. All the subjects in our study had no history of a gout attack for at least the previous 4 weeks and lacked any clinical evidence of infection; the hyperuricaemic patients had higher levels of CRP than the controls. The increased CRP levels are a risk factor for cardiovascular diseases [28]. Uric acid-induced expression of CRP has been observed previously in human vascular SMCs and endothelial cells [29]. More importantly, uric acid was found to be associated with several inflammatory markers, including CRP and IL-6, in a population-based study [30]. Thus, these results imply that uric acid may induce endothelial dysfunction and vascular inflammation reaction, which play pivotal roles in the pathogenesis of atherosclerosis [23, 27].

We also studied endothelium-independent vasodilation by administering nitroglycerin, a NO donor, to hyperuricaemic patients and normal subjects. There was no significant difference between the two groups in an independent analysis [13.37% (5.44%) vs 15.21% (5.45%); P = 0.112]. However, in a secondary analysis by multivariate linear regression, NMD is an independent determinant of low FMD values. This may be caused by the positive correlation between FMD and NMD (r = 0.527; P = 0.001). It could be seen as the effect of NO on the brachial artery, which has a poor response to endogenous NO (i.e. endothelium dependent and flow mediated) and also responds poorly to exogenous NO (i.e. endothelium independent and nitroglycerin mediated) [16, 18].

Many epidemiological studies confirm an association between hyperuricaemia and cardiovascular diseases, but it remains uncertain whether serum uric acid level is an independent or dependent risk factor for cardiovascular diseases [3]. The question is particularly complex because hyperuricaemic patients frequently have multiple comorbidities (e.g. hypertension, glucose intolerance, hyperlipidaemia, renal insufficiency and obesity). The independent association between serum uric acid and adverse cardiovascular outcome is more convincing for high-risk patients than for healthy subjects, suggesting that adjusting studies for these risk factors may be biologically inappropriate [31]. In this study, we excluded many known cardiac comorbidities in order to achieve a potentially more convincing result on the effect of serum uric acid on endothelial function. Our rigorous selection of the study population may have led to an underestimation of a genuine effect of uric acid.

In our study, the average body weights of the hyperuricaemic patients were greater than those of the controls. Hyperuricaemia is commonly found in obese patients [32]. This may also explain the higher levels of serum triglyceride and ALT in the hyperuricaemic group than in the control group.

In addition, we found higher serum creatinine levels among the hyperuricaemic patients than in the control subjects. This could indicate that uric acid may have effects on renal function. Uric acid has been previously proved to influence renal vascular function and structures [8, 11]. Mazzali et al. [8] showed hyperuricaemia-induced renal arteriopathy, independent of blood pressure. In addition, it has been reported that elevated uric acid levels induce a decrease in NO synthesis and an increase in blood pressure via the stimulation of the renin–angiotensin system [9]. In a community-based screening study, hyperuricaemia has been shown to be a risk factor for developing renal failure [33]. Thus, both experimental and clinical studies suggest the possibility that an elevation in serum uric acid levels can be detrimental to renal function.

The findings of our study have important clinical implications for the way we view hyperuricaemia and for further therapeutic interventions. Uric acid may be considered to be a risk factor for cardiovascular diseases.

Our study has a few limitations. First, this is a cross-sectional study, and it does not allow the establishment of a direct causal role of hyperuricaemia in endothelial dysfunction. Secondly, the patients recruited in this study were referred from a rheumatology clinic; they represent a selected population that is not representative of primary care.

Conclusions

Hyperuricaemia is associated with endothelial dysfunction, and decreased NO bioavailability may be the main reason for this dysfunction. However, large-scale clinical trials will help to clarify the mechanism underlying the role of uric acid in the development of cardiovascular diseases.

Rheumatology key messages

- Hyperuricaemia is associated with vascular endothelial dysfunction.
- Elevated serum uric acid level is a risk factor of cardiovascular diseases.
Acknowledgements

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