Unexpected high plasma xanthine oxidoreductase activity in female subjects with low levels of uric acid

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Abstract. Hypouricemia is a high-risk factor of exercise-induced acute kidney injury (EIAKI) probably through a lack of an antioxidant effect of uric acid. Xanthine oxidoreductase (XOR) is an enzyme that catalyzes the formation of uric acid from hypoxanthine and xanthine, leading to an increase in superoxide and reactive oxygen species. Activation of XOR has been proposed to promote oxidative stress-related tissue injury. We measured plasma XOR activity by a sensitive and accurate assay using a combination of liquid chromatography and triple quadrupole mass spectrometry in subjects with relatively low levels of uric acid ($\leq$4.0 mg/dL) who were recruited from 627 subjects (male/female: 292/335) in the Tanno-Sobetsu Study, a population-based cohort. The numbers of subjects with uric acid $\leq$4.0 mg/dL, $\leq$3.0 mg/dL and $\leq$2.0 mg/dL were 72 (11.5%, male/female: 5/67), 13 (2.1%, all females) and 2 (0.3%, both females), respectively. Plasma XOR activities in 5 male subjects were below the median value of the 292 male subjects. In 12 (17.9%) of the 67 female subjects with uric acid $\leq$4.0 mg/dL, plasma XOR activities were above the upper quartile value of the 335 female subjects. Eleven of the 12 female subjects with high plasma XOR activity and a low uric acid level had liver dysfunction and/or insulin resistance. In conclusion, unexpected high plasma XOR activities were found in some female subjects with relatively low levels of uric acid. Measurement of plasma XOR activity may help to identify hypouricemic patients with a high risk for EIAKI.

Key words: Xanthine oxidoreductase, Renal hypouricemia, Liver dysfunction, Insulin resistance, Exercise-induced acute kidney injury

URIC ACID is the end product of purine metabolism in humans [1]. Hyperuricemia is closely associated with various metabolic disorders and atherosclerotic cardiovascular diseases [2]. Therefore, elevation of uric acid level has been thought to be a surrogate marker of metabolic syndrome and cardiovascular diseases. On the other hand, hypouricemia per se is mostly asymptomatic, but the condition is well known to be a high risk for urolithiasis and exercise-induced acute kidney injury (EIAKI) [3]. Previous studies demonstrated that the prevalence of hypouricemia (uric acid $\leq$2.0 mg/dL) by unknown etiology was about 0.2–0.6%, being larger in female subjects than in male subjects, in the general population [4-6].

Renal hypouricemia results from an isolated renal tubular defect in reabsorption and/or secretion of uric acid that is genetically determined in an autosomal recessive inheritance pattern [7]. The main etiology of renal hypouricemia is a gene mutation of urate transporters including solute carrier family 22, member 12 (SLC22A12),
also known as urate transporter 1 (URAT1), and solute carrier family 2, member 9 (SLC2A9), also known as glucose transporter 9 (GLUT9) or voltage-driven urate efflux transporter (URATv1) [8-11]. The incidence of renal hypouricemia has been reported to range from 0.12% to 0.20% [12-14].

In 71 patients (male/female: 43/28) with renal hypouricemia based on genetic testing, it was reported that acute renal failure and urolithiasis as complications occurred in 15 patients (21.1%, male/female: 12/3) and 6 patients (8.5%, male/female: 5/1), respectively [15]. It has been proposed that hypouricemia causes EIAKI through oxidative stress-induced spasm of the renal artery, since uric acid acts as an antioxidant to protect endothelial function [16-18]. Interestingly, unlike renal hypouricemia caused by hyperexcretion of uric acid, hypouricemia caused by low production of uric acid in human xanthine oxidoreductase (XOR) deficiency (hereditary xanthinuria) has not been reported to cause EIAKI [14, 19, 20], suggesting that XOR activity may play a role in the development of EIAKI in hypouricemia.

XOR is an enzyme that catalyzes the formation of uric acid from hypoxanthine and xanthine, leading to an increase in superoxide and reactive oxygen species [21]. Therefore, activation of XOR contributes to the development of oxidative stress-related tissue injury [22-24]. However, it has been difficult to accurately measure plasma XOR activity in humans since the activity is much lower in humans than in animals [25]. Recently, a novel, sensitive and accurate assay for plasma XOR activity in humans has been established using a combination of liquid chromatography and triple quadrupole mass spectrometry (LC/TQMS) to detect \(^{13}\text{C}_2, ^{15}\text{N}_2\) -uric acid using \(^{13}\text{C}_5, ^{15}\text{N}_2\)-xanthine as a substrate [26]. Plasma XOR activity has been reported to be associated with obesity, smoking, liver dysfunction, hyperuricemia, dyslipidemia, insulin resistance and adiponectin [27-29]. We hypothesized that plasma XOR activity plays a crucial role in the induction of complications in patients with renal hypouricemia. However, it is very difficult to recruit patients with renal hypouricemia and to prove our hypothesis, since the number of patients who have renal hypouricemia with and without complications is very small. Therefore, in the present study, we investigated plasma XOR activity in subjects with relatively low levels of serum uric acid in a general population as the first step for proving our hypothesis.

Materials and Methods

Study population

In the Tanno-Sobetsu Study, a study with a population-based cohort design in two rural towns, Tanno and Sobetsu, in Hokkaido, the northernmost island of Japan, a total of 627 Japanese subjects (male/female: 292/335, mean age: 65 ± 15 years) were recruited from residents of Sobetsu Town in 2016. The population was the same as that in our previous study for investigating plasma XOR activity [28]. Hypouricemia is conventionally defined as a serum uric acid concentration of ≤2 mg/dL [4-6, 12-14]. However, it has been reported that levels of uric acid are more than 3 mg/dL in some patients with renal hypouricemia who have heterozygote mutations of SLC22A12/URAT1 or SLC2A9/GLUT9/URATv1 [15, 30]. Therefore, subjects with relatively low levels of uric acid (≤4.0 mg/dL) were enrolled in the present study. Subjects diagnosed with diabetes mellitus (HbA1c ≥6.5% and fasting glucose ≥126 mg/dL) and subjects being treated with antidiabetic drugs, XOR inhibitors including allopurinol, febuxostat and topiroxostat, and some angiotensin II receptor blockers (losartan and irbesartan) were excluded because of possible reduction of uric acid level. This study conformed to the principles outlined in the Declaration of Helsinki and was performed with the approval of the Ethical Committee of Sapporo Medical University. Written informed consent was received from all of the study subjects.

Medical checkups were performed between 06:00 h and 09:00 h after an overnight fast. After measuring anthropometric parameters, blood pressure was measured twice consecutively on the upper arm using an automated sphygmomanometer (HEM-907, Ommor Co., Kyoto, Japan) with subjects in a seated resting position, and average blood pressure was used for analysis. Body mass index (BMI) was calculated as body weight (in kilograms) divided by the square of body height (in meters). After physical examination, peripheral venous blood samples were obtained from the subjects for complete blood count and biochemical analyses. Samples of serum and plasma were analyzed immediately or stored at −80°C until biochemical analyses.

Measurements

Plasma glucose was determined by the glucose oxidase method. Fasting plasma insulin was measured by a chemiluminescent enzyme immunoassay method. Hemoglobin A1c (HbA1c) was determined by a latex
coagulation method and was expressed in National Glycohemoglobin Standardization Program (NGSP) scale. Creatinine, blood urea nitrogen (BUN), uric acid, aspartate transaminase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γGTP) and lipid profiles, including total cholesterol, HDL cholesterol and triglycerides, were determined by enzymatic methods. LDL cholesterol level was calculated by the Friedewald equation. Homeostasis model assessment of insulin resistance (HOMA-R), an index of insulin resistance, was calculated by the previously reported formula: HOMA-R = insulin (µU/mL) × glucose (mg/dL)/405. As an index of renal function, estimated glomerular filtration rate (eGFR) was calculated by an equation for Japanese [31]: eGFR (mL/min/1.73 m²) = 194 × creatinine^{–1.094} × age^{–0.287} × 0.739 (if female).

**Plasma XOR activity**

The modified assay for plasma XOR activity in humans, which was established on the basis of assays for XOR activity in mice [32, 33], was performed as previously reported [26, 28]. In brief, 100 µL of each plasma sample was purified by removing small molecules, including hypoxanthine, xanthine and uric acid, using a Sephadex G25 column and was mixed with 16 µM [15C2, 15N2]-xanthine as a substrate, 16 µM NAD+ and 1 µM [15C3, 15N2]-uric acid as an internal standard in 250 µL Tris buffer (pH 8.5). Each of the mixtures was incubated at 37°C for 90 min, mixed with 500 µL methanol, and centrifuged at 2,000 × g for 15 min at 4°C. The supernatants were transferred to new tubes and dried by a centrifugal evaporator. The residues were reconstituted with 150 µL of distilled water, filtered through an ultrafiltration membrane, and subjected to LC/TQMS using a Nano Space SI-2 LC system (Shiseido, Ltd., Tokyo, Japan) and a TSQ-Quantum triple quadrupole mass spectrometer (TQMS, Thermo Fisher Scientific, Bremen, Germany) equipped with an ESI interface. The amounts of [15C2, 15N2]-uric acid produced were quantitated from the calibration curve, and XOR activities were expressed as [15C2, 15N2]-uric acid in pmol/h/mL plasma. The lower detection limit was 6.67 pmol/h/mL plasma, and intra- and inter-assay coefficients of variation of pooled human plasma XOR activity were 6.5% and 9.1%, respectively [26].

**Statistical analysis**

Numeric variables are expressed as means ± SD for normal distributions or medians (interquartile ranges) for skewed variables. The distribution of each parameter was tested for its normality using the Shapiro-Wilk W test. Comparison between two groups was done with Student’s t test for parametric parameters and the Mann-Whitney U test for nonparametric parameters. Intergroup differences in percentages of demographic parameters were examined by the chi-square test. A p value of less than 0.05 was considered statistically significant. All data were analyzed by using JMP 9 for Macintosh (SAS Institute, Cary, NC).

**Results**

**Basal characteristics of the recruited subjects**

Uric acid levels in male subjects (n = 292) and female subjects (n = 335) of the 627 subjects were 6.0 ± 1.2 and 4.8 ± 1.1 mg/dL, respectively (Fig. 1A, B). The number of subjects with a low level of uric acid (≤4.0 mg/dL) in the 627 subjects was 89 (male/female: 10/79). After exclusion of subjects diagnosed with diabetes mellitus (n = 3) and subjects being treated with antidiabetic drugs (n = 11), XOR inhibitors (n = 0), losartan (n = 2) or irbesartan (n = 1), 72 subjects (male/female: 5/67) were recruited in the present study. Characteristics of the recruited subjects with low levels of uric acid are shown in Table 1. Mean age, BMI and waist circumference of the recruited subjects were 63 ± 16 years, 21.9 ± 3.0 kg/m² and 82.3 ± 10.4 cm, respectively. The numbers of subjects with smoking and drinking habits were 10 (13.9%) and 16 (22.2%), respectively. Of the 72 subjects, 20 (27.8%) and 11 (15.3%) subjects were taking antihypertensive and antidyislipidemic drugs, respectively. A significantly larger percentage of male subjects had a drinking habit, and male subjects had a significantly higher creatinine level and significantly lower level of insulin. No significant differences in other parameters were found between the male and female subjects. The numbers of subjects with uric acid ≤4.0 mg/dL, ≤3.0 mg/dL and ≤2.0 mg/dL were 72 (11.5%, male/female: 5/67), 13 (2.1%, all females) and 2 (0.3%, both females), respectively (Table 2).

**Plasma XOR activities in the recruited subjects with low levels of uric acid**

In the 627 subjects (male/female: 292/335), plasma XOR activities [median (interquartile ranges)] in the male and female subjects were 43.4 (24.4–86.3) and 32.3 (19.8–53.4) pmol/h/mL plasma, respectively (Fig. 1C, D), as shown in our previous study [28]. Plasma XOR...
Fig. 1  Plasma XOR activities in subjects with low levels of uric acid.
A–D. Frequency distribution analyses of uric acid (mg/dL) (A, male; B, female) and plasma xanthine oxidoreductase (XOR) activity (pmol/h/mL plasma) (C, male; D, female) in the 627 recruited subjects (male/female: 292/335). E, F. Plasma XOR activity was plotted against uric acid level in each subject with a relatively low level of uric acid (≤4.0 mg/dL) (E, male, n = 5; F, female, n = 67). The quartiles of plasma XOR activity in male (n = 292) and female (n = 335) Japanese subjects were from a previous report (Ref. 28). Q1, the first (lower) quartile of the activity; Q2, the second (median) quartile of the activity; Q3, the third (upper) quartile of the activity. Open triangles: male subjects with activity below of the Q3 value (n = 5), Open and closed circles: female subjects with activity below of the Q3 value (n = 55) and with activity of the Q3 value or more (n = 12), respectively.
Unexpected high plasma XOR activity in some subjects with low levels of uric acid

Characteristics of the 12 female subjects with a low level of uric acid and high plasma XOR activity are shown in Table 3. Two of those subjects had a smoking habit. Subject 6 had been treated with antihypertensive and antidyslipidemic drugs. Subjects 1–3, 5 and 9 had

### Table 1  Characteristics of the 72 recruited subjects with low level of uric acid (≤4.0 mg/dL)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>72</td>
<td>5</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>63 ± 16</td>
<td>67 ± 13</td>
<td>62 ± 16</td>
<td>0.479</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.9 ± 3.0</td>
<td>23.1 ± 2.8</td>
<td>21.8 ± 3.0</td>
<td>0.332</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82.3 ± 10.4</td>
<td>85.3 ± 11.7</td>
<td>82.1 ± 10.4</td>
<td>0.513</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>131 ± 23</td>
<td>139 ± 18</td>
<td>130 ± 10</td>
<td>0.375</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73 ± 9</td>
<td>74 ± 4</td>
<td>72 ± 10</td>
<td>0.776</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>70 ± 9</td>
<td>67 ± 9</td>
<td>70 ± 10</td>
<td>0.436</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>10 (13.9)</td>
<td>1 (20.0)</td>
<td>9 (13.4)</td>
<td>0.693</td>
</tr>
<tr>
<td>Drinking habit</td>
<td>16 (22.2)</td>
<td>4 (80.0)</td>
<td>12 (17.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antihypertensive drugs</td>
<td>20 (27.8)</td>
<td>3 (60.0)</td>
<td>17 (25.4)</td>
<td>0.095</td>
</tr>
<tr>
<td>Antidyslipidemic drugs</td>
<td>11 (15.3)</td>
<td>0 (0)</td>
<td>11 (16.4)</td>
<td>0.325</td>
</tr>
<tr>
<td>Biochemical data</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AST (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>21 (18–25)</td>
<td>23 (21–40)</td>
<td>21 (17–25)</td>
<td>0.131</td>
</tr>
<tr>
<td>γGTP (IU/L)</td>
<td>17 (13–24)</td>
<td>24 (19–28)</td>
<td>17 (13–24)</td>
<td>0.118</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>3.4 ± 0.5</td>
<td>3.5 ± 0.3</td>
<td>3.4 ± 0.5</td>
<td>0.645</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>214 ± 33</td>
<td>191 ± 31</td>
<td>216 ± 33</td>
<td>0.107</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>125 ± 28</td>
<td>112 ± 27</td>
<td>126 ± 28</td>
<td>0.314</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>68 ± 15</td>
<td>57 ± 13</td>
<td>68 ± 15</td>
<td>0.117</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>80 (67–107)</td>
<td>89 (61–103)</td>
<td>80 (66–111)</td>
<td>0.991</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>88 (83–95)</td>
<td>88 (54–95)</td>
<td>88 (83–95)</td>
<td>0.974</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>7.1 (3.1–15.6)</td>
<td>2.5 (1.1–7.4)</td>
<td>7.3 (3.5–15.8)</td>
<td>0.046</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>1.42 (0.76–3.46)</td>
<td>0.55 (0.21–1.76)</td>
<td>1.44 (0.78–3.60)</td>
<td>0.051</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.4 (5.1–5.6)</td>
<td>5.2 (5.1–5.4)</td>
<td>5.4 (5.2–5.6)</td>
<td>0.166</td>
</tr>
<tr>
<td>XOR (pmol/h/mL plasma)</td>
<td>23 (18–44)</td>
<td>19 (18–29)</td>
<td>25 (18–46)</td>
<td>0.347</td>
</tr>
</tbody>
</table>

Variables are expressed as number (%), means ± SD or medians (interquartile ranges).
AST, aspartate transaminase; ALT, alanine transaminase; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; γGTP, γ-glutamyl transpeptidase; HOMA-R, homeostasis model assessment of insulin resistance; XOR, xanthine oxidoreductase.

Activities in 5 male subjects were below the second quartile (median) value (Q2, 43.4 pmol/h/mL plasma) of the 292 male subjects (Fig. 1E). In 12 (17.9%) of the 67 female subjects with uric acid ≤4.0 mg/dL, plasma XOR activities were above the third (upper) quartile value (Q3, 53.4 pmol/h/mL plasma) of the 335 female subjects (Fig. 1F).
liver dysfunction defined as above a cut-off level of AST, ALT or γGTP. In subjects 3–9, 11 and 12, HOMA-R levels were more than 2.00 as a cut-off level of insulin resistance. All of the subjects except for subject 10 had liver dysfunction and/or insulin resistance.

Discussion

The present study revealed for the first time that 12 (17.9%) of the 67 female subjects with a relatively low level of uric acid had unexpected high plasma XOR activities. Eleven of the 12 female subjects had liver dysfunction and/or insulin resistance. Plasma XOR activity has been reported to be independently associated with obesity, smoking, hyperuricemia, dyslipidemia, liver dysfunction, insulin resistance and adiponectin in a general population [27-29]. XOR is abundantly expressed in the liver and leaks into the blood without non-specific membrane damage [21, 34]. Strong correlations between plasma XOR activity and liver enzymes, including AST

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Uric acid level and XOR activity in the 72 recruited subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n = 627)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>UA ≤4 mg/dL</td>
<td>72 (11.5)</td>
</tr>
<tr>
<td>UA ≤3 mg/dL</td>
<td>13 (2.1)</td>
</tr>
<tr>
<td>UA ≤2 mg/dL</td>
<td>2 (0.3)</td>
</tr>
</tbody>
</table>

Variables are expressed as number, number (%), or medians (interquartile ranges).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Characteristics of the 12 subjects with low uric acid level and high plasma XOR activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject No.</td>
<td>1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>74</td>
</tr>
<tr>
<td>Gender</td>
<td>F</td>
</tr>
<tr>
<td>XOR (pmol/h/mL plasma)</td>
<td>197.0</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>3.9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.7</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>79.0</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>50</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>39</td>
</tr>
<tr>
<td>γGTP (IU/L)</td>
<td>30</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>74.2</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>60</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>96</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>87</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>3.5</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>0.76</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5</td>
</tr>
<tr>
<td>Antihypertensive drugs</td>
<td>–</td>
</tr>
<tr>
<td>Antidyslipidemic drugs</td>
<td>–</td>
</tr>
<tr>
<td>Habitual smoking</td>
<td>–</td>
</tr>
</tbody>
</table>

eGFR, estimated glomerular filtration rate; γGTP, γ-glutamyl transpeptidase; HOMA-R, homeostasis model assessment of insulin resistance; XOR, xanthine oxidoreductase.
thought to be protection of renal vasoconstriction follow- 
ing mechanisms of prevention of EIAKI onset were 
denied.

a low level of uric acid in the present study, though the 
possibility that increased plasma XOR activity was a 
result of low level of uric acid cannot be completely 
denied.

Uric acid has a powerful antioxidant effect [36] and 
plays a protective role in the kidney. The decreased anti- 
oxidant potential in renal hypouricemia may lead to kid- 
ney injury caused by oxidative stress [16-18]. Oxidative 
imbalance with increased oxidative stress after exercise 
and decreased antioxidant capacity in patients with renal 
hypouricemia may cause EIAKI [18]. Renal perfusion in 
patients with renal hypouricemia would be diminished 
during exercise, and reperfusion after exercise may lead 
to renal damage through ischemia-reperfusion injury. 
Unlike renal hypouricemia caused by hyperexcretion of 
uric acid, hypouricemia caused by low production of uric 
acid in hereditary xanthinuria (XOR deficiency) has not 
been reported to cause EIAKI [14, 19, 20], suggesting 
that XOR activity may play a role in the induction of 
EIAKI in patients with hypouricemia. Measurement of 
XOR activity may help to identify hypouricemic patients 
with a high risk of EIAKI.

It was previously reported that pretreatment with allo- 
purinol, an XOR inhibitor, could prevent the onset of 
EIAKI in a subject with renal hypouricemia [37]. Poten- 
tial mechanisms of prevention of EIAKI onset were 
thought to be protection of renal vasoconstriction follow- 
ing depletion of free radical scavengers including uric 
acid and reduction of increased free radicals with isch- 
emia reperfusion injury [30, 38, 39]. It has also been sug- 
gested that high plasma XOR activity is a metabolic 
parameter that is superior to uric acid level, and adequate 
inhibition of plasma XOR activity, not just lowering uric 
ad level, would be a novel therapeutic strategy for met- 
abolic and cardiovascular diseases [28]. Therefore, even 
in hypouricemia, plasma XOR activity would be impor- 
tant for the development of oxidative stress-mediated 
diseases. To prove our hypothesis that plasma XOR 
activity plays a crucial role in the induction of EIAKI in 
patients with renal hypouricemia, further study is needed 
to elucidate plasma XOR activities at rest and in exercise 
conditions in subjects with renal hypouricemia.

The prevalence of subjects with uric acid ≤2.0 mg/dL 
in the present study was 0.3%, which was similar to the 
prevalence of hypouricemia (0.2–0.4%) in previous stud- 
ies using Japanese subjects [4-6]. The number of subjects 
with a relatively low level of uric acid was larger in 
female subjects than in male subjects in the present 
study. As a possible reason, female hormones, estrogen 
and progesterone, have been suggested to decrease uric 
acid level [40]. Since uric acid level is generally lower in 
females than in males [28, 41], the prevalence of hypour- 
icemia would be higher in females than that in males. In 
fact, the incidences of renal hypouricemia were reported 
to be 0.16% and 0.23% in normal male and female 
adults, respectively [42]. It has also been reported that 3 
(0.24%) of 1,249 male subjects and 7 (0.56%) of 1,256 
female subjects had uric acid <2.0 mg/dL among healthy 
Japanese children [43].

Conversely, it has been reported that acute renal fail- 
ure probably due to EIAKI in subjects with renal hypour- 
icemia based on genetic testing occurred in 12 (27.9%) 
of 43 male patients and 3 (10.7%) of 28 female patients 
[15]. Male predominance of EIAKI seems to be unrelat- 
ed to the incidence of renal hypouricemia. Strenuous and 
prolonged exercise causes a sequential increase in oxy- 
gen free radical levels [44-46], linking to the pathogene- 
sis of ischemic acute kidney injury [47]. Patients with 
renal hypouricemia may be at risk of free radical-induced 
damage to the kidney during exercise. Exercise also 
causes increased levels of several mediators including 
noradrenaline, angiotensin II, arginine vasopressin, endo- 
toxin, endothelin, cytokines and leukotrienes [46], lead- 
ing to facilitation of renal ischemia. One possible reason 
for the male predominance of EIAKI is the probability of 
strenuous and prolonged exercise performed by males. It 
has also been reported that estrogen has potent anti- 
oxidant properties and may influence the degree of 
exercise-induced muscle damage and repair processes 
[48]. This may be another explanation for the gender 
difference in the incidence of EIAKI. Measurement of 
plasma XOR activity, especially during exercise toler- 
ance test, may be useful to predict the onset of EIAKI in 
both males and females with hypouricemia.

The present study has several limitations. First, since 
the present study was a cross-sectional design, it is 
unclear whether hypouricemia was transient or persist- 
et. It has been reported that hypouricemia was transient 
in 40% of outpatients because hypouricemia is a heterol- 
ogous disease composed of renal hypouricemia, xanthi-
uraria and secondary hypouricemia frequently caused either by drug administration or systemic diseases [12]. Differential diagnosis of hypouricemia was not completely performed in the present study, though subjects with conditions that may affect serum uric acid levels, including subjects diagnosed with diabetes mellitus and subjects treated with antidiabetic drugs, XOR inhibitors, and some angiotensin II receptor blockers (losartan and irbesartan), were excluded. Second, the number of subjects enrolled in the present study was small. A longitudinal study with larger numbers of subjects is necessary for confirming the prevalence and impact of high plasma XOR activity in subjects with relatively low levels of uric acid. Third, complications including urinary stones and EIAKI were not investigated in the present study. Fourth, because the recruited subjects were only Japanese people, it is unclear whether the present findings can be generalized to other ethnicities. Lastly, plasma XOR activity measurement is not standardized across laboratories. Therefore, values of plasma XOR activity measured in the present study are not directly comparable to those measured in another laboratory using a different method.

In conclusion, unexpected high plasma XOR activities possibly associated with liver dysfunction and insulin resistance were found in some female subjects with a relatively low level of uric acid in a general population. Measurement of XOR activity may help to identify hypouricemic patients with a high risk of EIAKI. Further study is needed to elucidate the link between plasma XOR activity and EIAKI in both male and female subjects with hypouricemia.

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Disclosures

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