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Abstract

Background: We developed a novel high-sensitive assay for plasma xanthine oxidoreductase (XOR) activity that is not affected by the original serum uric acid level. However, the association of plasma XOR activity with that level has not been fully examined.

Methods: This cross-sectional study included 191 subjects (91 males, 100 females) registered in the MedCity21 health examination registry. Plasma XOR activity was determined using our assay for plasma XOR activity with [$^{13}C_2$,$^{15}N_2$] xanthine and liquid chromatography/triple quadrupole mass spectrometry. Serum levels of uric acid and adiponectin, and visceral fat area (VFA) obtained by computed tomography were measured, and insulin resistance was determined based on the homeostasis model assessment (HOMA-IR) index.

Results: The median values for uric acid and plasma XOR activity were 333 μmol/L and 26.1 pmol/h/mL, respectively. Multivariable linear regression analysis showed a significant and positive association of serum uric acid level (coefficient: 26.503; 95% confidence interval: 2.06, 50.945; p = 0.035) with plasma XOR activity independent of VFA and HOMA-IR, and also age, gender, alcohol drinking habit, systolic blood pressure, estimated glomerular filtration rate (eGFR), glycated hemoglobin A_{1c}, triglyceride, and adiponectin levels. The "gender*XOR activity" interaction was not significant (p = 0.91), providing no evidence that gender modifies the relationship between plasma XOR activity and serum uric acid level.

Conclusions: Plasma XOR activity was found to be positively associated with serum uric acid level independent of other known confounding factors affecting that level, including gender difference, eGFR, adiponectin level, VFA, and HOMA-IR.

Keywords: insulin resistance; plasma XOR activity; uric acid; visceral obesity.

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Introduction

The serum level of uric acid is determined based on the balance of production and excretion of uric acid [1], the end-product of purine metabolism in humans [2]. Xanthine oxidoreductase (XOR), mainly expressed in the liver and intestines in humans [3, 4], is a rate-limiting enzyme involved in *in vivo* uric acid production that catalyzes oxidation from xanthine to uric acid [5], and hepatic XOR activity has been reported to be increased in gouty patients with overproduction of uric acid [6, 7]. It has been suggested that XOR leaks from cells into circulation as a result of physiologic cell turnover or consequence of pathological cell conditions [8].

We recently reported our novel method for determining the plasma XOR activity, which is unaffected by the serum uric acid level, as it utilizes an assay of stable isotope-labeled [$^{13}C_2$,$^{15}N_2$] xanthine with liquid chromatography (LC)/triple quadrupole mass spectrometry (TQMS) [9, 10]. With that method, we found that plasma XOR activity is influenced by gender, renal function, glycemic control status, and insulin resistance [11] (presently under consideration). In contrast, previously reported standard methods are influenced by the original level of uric acid in the sample [12], thus the association of plasma XOR activity with serum uric acid level has not been fully examined.

The purpose of the present study was to examine the association of plasma XOR activity with serum uric acid independent of visceral fat area (VFA), serum adiponectin level, and insulin resistance index, shown by the homeostatic model assessment (HOMA-IR) [13, 14], in a general population who participated in the MedCity21 health examination registry using our novel assay method.

Materials and methods

Study design

The MedCity21 health examination registry was instituted in April 2015 in a comprehensive manner to elucidate the causes of various diseases occurring in adults, including cancer, diabetes mellitus (DM), cardiovascular disease, cerebrovascular disease, mental disorders, dyslipidemia, hypertension, hyperuricemia, obesity, chronic respiratory disease, liver disease, digestive disease, gynecological diseases, and skin disease, and for the development of advanced diagnostic techniques, treatment methods, and prevention methods for affected patients. Individuals who underwent comprehensive medical examinations as part of MedCity21 at the Osaka City University Hospital Advanced Medical Center for Preventive Medicine (Osaka, Japan) were registered. The MedCity21 health examination registry protocol was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (approval No. 2927). Written informed consent was obtained from all subjects and the study was conducted in full accordance with the Declaration of Helsinki. The present study protocol was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (approval No. 3684) and performed with an opt-out option, explained in instructions on the website of the hospital. Results obtained from that study have been already submitted and the present study is a sub-analysis of the findings obtained.

Participants

Using the MedCity21 health examination registry, we selected the final 200 sequential participants who participated in the lifestyle course of the advanced comprehensive medical examination between June 2015 and May 2017, designed to examine the status of lifestyle-related diseases, such as hypertension, diabetes, dyslipidemia, visceral obesity, hyperuricemia, atherosclerosis, and cerebrovascular disease. For our analysis, participants being treated with an XOR inhibitor (n = 4), sodium-glucose cotransporter 2 inhibitor (n = 2), uricosuric (n = 1) or insulin (n = 1) agents, or with missing data (n = 1) were excluded. As a result, 191 participants were enrolled as subjects in the present cross-sectional study.

Physical and laboratory measurements

Information regarding height, body weight, smoking and alcohol consumption habits, present and past illness, and use of medication was obtained. Blood pressure (BP) was determined using a conventional cuff method with an automatic sphygmomanometer after the subject had rested for at least 15 min. Body mass index was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood was drawn after an overnight fast. Biochemical parameters were analyzed using a standard laboratory method at the Central Laboratory of Osaka City University Hospital and the remaining blood samples were stored at −80 °C. Estimated glomerular filtration rate (eGFR) was calculated using an equation designed for Japanese subjects, as previously described [15]. Glycated hemoglobin A_{1c} (HbA_{1c}) levels were estimated as National Glycohemoglobin Standardization Program equivalent values (%) using the conversion formula established by the Japan Diabetes Society [16]. Serum adiponectin levels were measured using a latex particle-enhanced turbidimetric immunoassay (Otsuka Pharmaceutical Co., Tokyo, Japan) [17]. Serum immunoreactive insulin (IRI) levels were determined using an electrochemiluminescence immunoassay (Roche Diagnostics K.K., Tokyo, Japan). The HOMA-IR index was calculated according to the following formula: fasting IRI (IU/mL) × fasting plasma glucose (mmol/L)/22.5 [13, 14].

Measurement of visceral fat area

Using abdominal computed tomography images (Supria Grande, Hitachi, Ltd., Tokyo, Japan), we acquired a single 5-mm slice at the level of the umbilicus. VFA values were calculated using the fatPointer software package, version 2 (Hitachi, Ltd., Tokyo, Japan), as previously described (data presently under consideration).

Plasma XOR activity

Freshly frozen samples maintained at −80 °C until the time of the assay were used to determine the plasma XOR activity with our recently established novel method for assays of stable isotope-labeled [$^{13}C_2$,$^{15}N_2$] xanthine with LC/TQMS, as we previously described [9, 10]. Briefly, 100-μL aliquots of plasma were purified using a Sephadex G25 column, then mixed with Tris buffer (pH 8.5) containing [$^{13}C_2$,$^{15}N_2$] xanthine as a substrate, nicotinamide adenine dinucleotide$^+$, and [$^{13}C_3$,$^{15}N_3$] uric acid as an internal standard, with the mixtures then incubated at 37 °C for 90 min. Subsequently, they were combined with methanol (500 μL) and centrifuged at 2000 × *g* for 15 min at 4 °C. Supernatants were collected and transferred to new tubes, and then dried using a centrifugal evaporator. The residues were reconstituted in 150 μL of distilled water and filtered through an

ultrafiltration membrane, and then measurements were performed using LC/TQMS. Calibration standard samples were examined for the amount of [$^{13}C_2$,$^{15}N_2$] uric acid produced, which was calculated by the use of a calibration curve, with XOR activity expressed as that amount (pmol/h/mL). The intra- and inter-assay coefficients of variation of plasma XOR activity were 6.5% and 9.1%, respectively.

Statistical analysis

Values are expressed as number (%) or median (interquartile range). Serum triglycerides, adiponectin, HOMA-IR, and plasma XOR activity were logarithmically transformed before carrying out multivariable linear regression analyses due to the skewed distribution. Multivariable linear regression analyses were performed to determine whether plasma XOR activity was independently associated with serum uric acid level after adjustment using various clinical parameters, including adiponectin level, VFA, and HOMA-IR. In addition, we incorporated a two-factor interaction term (gender*plasma XOR activity) to assess the effect of gender on the relationship between plasma XOR activity and serum uric acid level. Variance inflation factor (VIF) was calculated to estimate multicollinearity for each predictor. The reliability of the final regression model was internally validated using the bootstrap method. One hundred and fifty sets of bootstrap samples were generated by resampling the original data and the level of optimism was estimated to determine the degree of overfitting. The R software package (version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria) and Statistical Package for the Social Sciences software package (PASW Statistics, version 22.0) were used for data analysis. All reported p-values are two-tailed and were considered to indicate statistical significance at $p < 0.05$.

Results

Clinical characteristics of subjects

The characteristics of the enrolled subjects are shown in Table 1. The median values for age, systolic BP (SBP), triglycerides, HbA_{1c}, eGFR, adiponectin, and VFA were 56 years, 121 mmHg, 1.00 mmol/L, 5.7% (39 mmol/mol), 76.8 mL/min/1.73 m^2, 8.8 μg/mL, and 69.5 cm^2, respectively, while those for uric acid and plasma XOR activity were 333 μmol/L and 26.1 pmol/h/mL, respectively.

Table 1: Clinical characteristics of subjects (n = 191).

Age, years	56 (47–67)
Males, n	91 (47.6)
Alcohol drinking habit, n	101 (52.9)
Body mass index, kg/m^2	22.5 (20.7–24.5)
Visceral fat area, m^2	69.5 (42.9–107.1)
Systolic blood pressure, mmHg	121 (110–132)
Diastolic blood pressure, mmHg	74 (66–82)
Total cholesterol, mmol/L	5.28 (4.68–5.83)
Triglycerides, mmol/L	1.00 (0.70–1.43)
HDL cholesterol, mmol/L	1.55 (1.29–1.81)
Fasting plasma glucose, mmol/L	5.56 (5.22–6.00)
HbA_{1c}, %	5.7 (5.5–6.0)
HbA_{1c}, mmol/mol	39 (37–42)
eGFR, mL/min/1.73 m^2	76.8 (66.3–86.6)
Adiponectin, μg/mL	8.8 (6.5–11.1)
HOMA-IR	1.4 (0.9–2.0)
Uric acid, μmol/L	333 (262–378)
XOR activity, pmol/h/mL	26.1 (15.3–55.7)

Data are expressed as median (interquartile range) or number (%). HDL, high-density lipoprotein; HbA_{1c}, glycated hemoglobin A_{1c}; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostatic model assessment of insulin resistance; XOR, xanthine oxidoreductase.

Association of plasma XOR activity with serum uric acid level independent of clinical factors, including adiposity, adiponectin, and insulin resistance

To examine whether plasma XOR activity is independently associated with serum uric acid after adjustment for other confounding factors, including age, gender, alcohol drinking habit, SBP, eGFR, HbA_{1c}, triglycerides, VFA, HOMA-IR, and adiponectin, multivariable linear regression analyses were performed (Table 2). Plasma XOR activity was significantly and independently associated with serum uric acid level (coefficient: 26.503; 95% confidence interval [CI]: 2.06, 50.945; $p = 0.035$) (Figure 1), whereas HOMA-IR, adiponectin level, SBP, and HbA_{1c} were not significantly associated with serum uric acid level (Table 2). A unit increase in logarithmic XOR activity was associated with an increase in serum uric acid level of approximately 26.5. VFA as well as age, gender, eGFR, and triglyceride level were significantly associated with serum uric acid level (coefficient: 0.432; 95% CI: 0.17, 0.694; $p = 0.037$) (Figure 2), while alcohol drinking habit showed a tendency to be associated with serum uric acid level. The "gender*plasma XOR activity" interaction was not significant ($p = 0.91$), providing no evidence that gender modifies the relationship between plasma XOR activity and serum uric acid level. VIF values for the predictors were less than 5 for each, showing no multicollinearity between the variables (Table 2). Furthermore, the regression model was internally validated and the estimated optimism level was 0.041, indicating no overfitting.

Discussion

The findings in the present study demonstrated that plasma XOR activity determined by stable isotope-labeled xanthine and LC/TQMS is positively associated

Table 2: Multivariable linear regression analysis of factors associated with serum uric acid level.

Independent variable	Coefficient	95% LCI	95% UCI	p-Value	VIF
Age, years	−1.382	−2.236	−0.528	0.002	1.98
Gender (male = 1, female = 0)	60.423	41.749	79.097	<0.001	1.46
Alcohol drinking habit (present = 1, absent = 0)	16.206	−0.522	32.934	0.059	1.17
Systolic blood pressure, mmHg	0.092	−0.482	0.667	0.753	1.40
eGFR, mL/min/1.73 m^2	−1.400	−2.082	−0.719	<0.001	1.57
HbA_{1c}, %	7.189	−13.623	28.002	0.499	1.49
Log triglycerides, mmol/L	46.632	3.215	90.048	0.037	1.64
Visceral fat area, m^2	0.432	0.17	0.694	0.001	2.40
Log HOMA-IR	7.337	−29.885	44.559	0.700	2.00
Log adiponectin, μg/mL	0.957	−52.999	54.913	0.972	1.99
Log XOR activity, pmol/h/mL	26.503	2.06	50.945	0.035	1.84

eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin A_{1c}; HOMA-IR, homeostatic model assessment of insulin resistance; XOR, xanthine oxidoreductase; LCI, lower confidence interval; UCI, upper confidence interval; VIF, variance inflation factor.

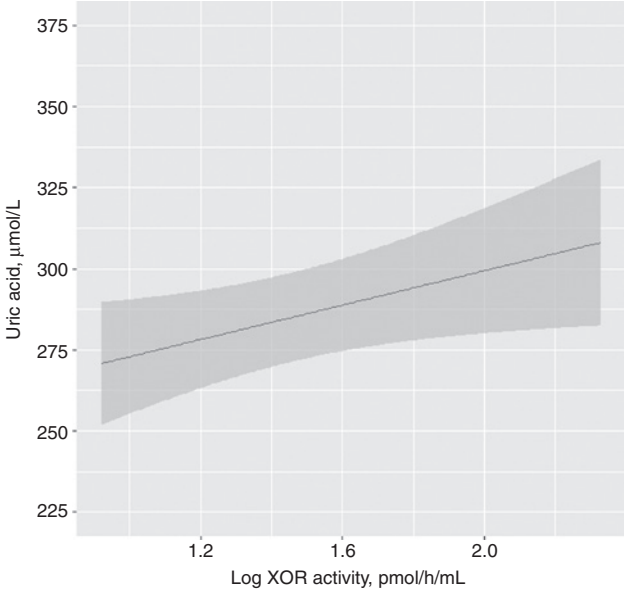

Figure 1: Plasma XOR activity and serum uric acid.
Plasma XOR activity was independently associated with serum uric acid level. The best fitted line and 95% confidence interval are shown by a solid line and gray band, respectively. Serum uric acid level was adjusted to the median values for age (56 years), gender (female), alcohol drinking habit (presence), systolic blood pressure (121 mmHg), eGFR (76.8 mL/min/1.73 m^2), HbA_{1c} (5.7% [39 mmol/mol]), log triglycerides (0.00 mmol/L), VFA (69.5 m^2), log HOMA-IR (0.15), and log adiponectin (0.94 μg/mL). XOR, xanthine oxidoreductase; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin A_{1c}; VFA, visceral fat area; HOMA-IR, homeostatic model assessment of insulin resistance.

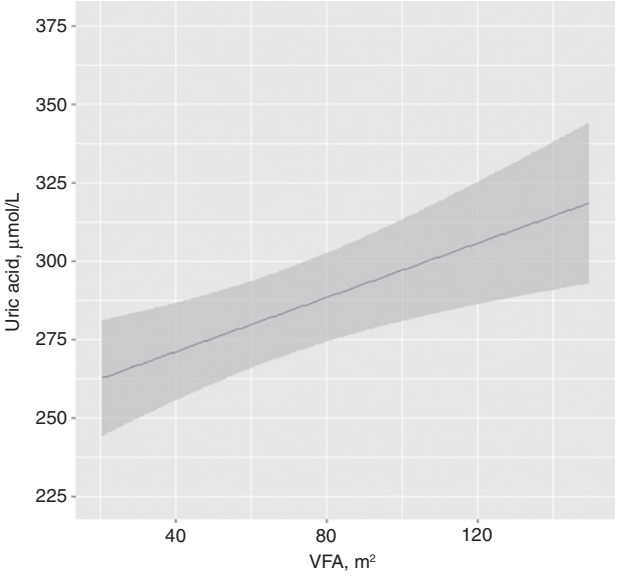

Figure 2: VFA and serum uric acid.
VFA was independently associated with serum uric acid level. The best fitted line and 95% confidence interval are shown by a solid line and gray band, respectively. Serum uric acid level was adjusted to the median values for age (56 years), gender (female), alcohol drinking habit (presence), systolic blood pressure (121 mmHg), eGFR (76.8 mL/min/1.73 m^2), HbA_{1c} (5.7% [39 mmol/mol]), log triglycerides (0.00 mmol/L), log HOMA-IR (0.15), log adiponectin level (0.94 μg/mL), and log plasma XOR activity (1.42 pmol/h/mL). VFA, visceral fat area; XOR, xanthine oxidoreductase; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin A_{1c}; HOMA-IR, homeostatic model assessment of insulin resistance.

with serum uric acid level, independent of other known confounding factors affecting that level, including gender difference, eGFR, adiponectin level, VFA, and HOMA-IR [18–22].

Various plasma XOR activity assays have been basically developed based on the rate of production of uric acid from its substrate, xanthine, determined with ultraviolet (UV) detectors [23] or LC/UV [24]. However, those

methods are influenced by the original level of uric acid in the sample [12]; thus, the association of plasma XOR activity with serum uric acid has not been fully investigated. In contrast, our novel method for determining plasma XOR activity, which utilizes an assay of stable isotope-labeled xanthine with LC/TQMS, does not require subtraction of the level of uric acid contained in the original sample and is not influenced by uric acid contained therein [9, 10].

Some previous studies that used our XOR assay method have found a positive correlation of plasma XOR activity with serum uric acid level [9, 25, 26], though those findings were confirmed only with an unadjusted model. Additionally, other studies have noted either positive findings or no association of that level with plasma XOR activity [11, 27, 28]. The latter results were confirmed with an adjusted model using serum uric acid level as a covariate but not an independent variable, or with plasma XOR activity used as an independent variable but not a covariate. Therefore, whether plasma XOR activity is independently associated with serum uric acid level remained unclear. The present study is the first to demonstrate an independent and positive association of plasma XOR activity with serum uric acid level, based on the findings obtained using multivariable linear regression analyses, in which plasma XOR activity was used as a covariate and serum uric acid level as an independent variable (Table 2, Figure 1).

XOR is mainly expressed in the liver and intestines in humans [3, 4], and hepatic XOR activity, though not in the intestine, was reported to be increased in gouty patients with overproduction of uric acid [6, 7]. Thus, the level of serum uric acid is known to be strongly influenced by hepatic XOR activity. Although we did not measure hepatic XOR activity, those previous studies along with the present results suggest that plasma XOR activity reflects systemic, especially hepatic, XOR activity.

Consistent with a previous report showing a generally higher uric acid level in males as compared to females [18], male subjects in the present study also showed significantly and independently higher levels of serum uric acid. Although gender does not have an effect on the relationship between plasma XOR activity and serum uric acid level, we previously found that male subjects had a significantly higher plasma XOR activity than female subjects (presently under consideration), demonstrating that elevated XOR activity in part contributes to the increase in the level of serum uric acid in males.

Other important findings obtained in the present study include an association of VFA with serum uric acid level, independent of plasma XOR activity and HOMA-IR (Table 2, Figure 2). Numerous studies have shown that hyperuricemia is frequently complicated by obesity in individuals with visceral fat accumulation [20, 29]. Hyperuricemia is considered to be caused by reduced renal excretion of uric acid as a result of insulin resistance caused by increased visceral adiposity [22]. Recently, we reported that insulin resistance also contributes to stimulated XOR activity, which explains, at least in part, the higher uric acid level seen in individuals with visceral fat accumulation (presently under consideration). However, VFA was associated with serum uric acid independent of plasma XOR activity and HOMA-IR in the present study, suggesting that visceral fat accumulation contributes to hyperuricemia by various mechanisms in addition to other processes, such as reduced renal excretion of uric acid through insulin resistance and/or overproduction of uric acid through increased XOR activity. Increased free fatty acid [30] and/or hypoxanthine derived from visceral fat [31], as well as lifestyle habits such as excessive intake of fructose or purine-rich foods [32–34], may be potential factors.

Limitations

Our findings have several limitations. First, because of the study design, we did not measure the intake of dietary purine or urinary excretion of uric acid, which are known to be important factors related to serum uric acid level. Second, this was a cross-sectional study, thus even though relationships were explored in predictive terms, the results cannot be interpreted to show causal relationships. Finally, we did not measure hepatic XOR activity, thus were not able to analyze a possible direct association between hepatic and plasma XOR activity. Nevertheless, the findings obtained are the first to demonstrate that plasma XOR activity is independently associated with serum uric acid.

Conclusions

The results determined by our novel assay showed that plasma XOR activity is positively associated with serum uric acid level in a manner independent of visceral adiposity, adipocytokines, and insulin resistance, suggesting that such activity in plasma reflects systemic, especially hepatic, XOR activity.

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