Plasma xanthine oxidoreductase activity in Japanese patients with type 2 diabetes across hospitalized treatment

Yusuke Kawachi1, Yuya Fujishima1, Hitoshi Nishizawa1*, Hirofumi Nagao1, Takashi Nakamura2, Seigo Akari2, Takayo Murase2, Naohiro Tayama1, Kazuo Omori1, Akimitsu Miyake3, Shiro Fukuda1, Mitsuyoshi Takahara1,4, Shunbun Kita1,5, Naoto Katakami1,6, Norikazu Maeda1,6, Ichiro Shimomura1

Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan, 2Sanwa Kagaku Kenkyusho Co., Ltd, Inabe, Mie, Japan, 3Department of Medical Innovation, Osaka University Hospital, Suita, Osaka, Japan, 4Department of Diabetes Care Medicine, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan, 5Department of Adipose Management, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan, and 6Department of Metabolism and Atherosclerosis, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan

Keywords
Liver transaminases, Type 2 diabetes mellitus, Xanthine oxidoreductase

*Correspondence
Hitoshi Nishizawa
Tel: +81-6-6879-3732
Fax: +81-6-6879-3739
E-mail address: hitoshin1127@endmet.med.osaka-u.ac.jp

J Diabetes Investig 2021; 12: 1512–1520
doi: 10.1111/jdi.13467

ABSTRACT
Aims/Introduction: Xanthine oxidoreductase (XOR) is an enzyme that catalyzes hypoxanthine and xanthine to xanthine and uric acid, respectively. Plasma XOR activity has recently been measured in humans. However, limited information is known about plasma XOR activity in patients with type 2 diabetes mellitus, and its changes after short-term glycemic control treatment.

Materials and Methods: We enrolled 28 Japanese patients (10 men/18 women) with type 2 diabetes mellitus who were hospitalized to undergo medical treatment for diabetes. Plasma XOR activity, quantified using triple quadrupole mass spectrometry and liquid chromatography, and other clinical parameters were examined at admission and 2 weeks after treatment during hospitalization. Changes in plasma XOR activity after treatment during hospitalization and associated clinical parameters were assessed.

Results: At the time of admission, the median plasma XOR activity was 83.1 pmol/h/mL, with a wide range of 14.4–1150 pmol/h/mL. Multiple regression analysis identified serum aspartate transaminase and alanine transaminase levels as significant and independent factors correlating with the baseline plasma XOR. Two weeks after treatment during hospitalization was associated with a significant decrease in plasma XOR activity. Changes in serum aspartate transaminase were also the only significant and independent factor correlating with changes in plasma XOR activity.

Conclusions: A close relationship was observed between plasma XOR activity and liver transaminases in patients with type 2 diabetes mellitus, cross-sectionally, and also across treatment during hospitalization.

INTRODUCTION
Hyperuricemia is closely associated with visceral fat accumulation and related metabolic disorders, including diabetes mellitus, hypertension, dyslipidemia, fatty liver and atherosclerotic cardiovascular diseases (CVDs)1,2. Xanthine oxidoreductase (XOR) is a rate-limiting enzyme that catalyzes the conversion of hypoxanthine and xanthine to xanthine and uric acid, respectively. XOR is also a pharmacological target of antihyperuricemic agents, such as allopurinol, febuxostat and topiroxostat. Furthermore, previous studies have shown that XOR is involved not only in the production of uric acid, but also in the pathogenesis of CVD3–5. XOR is initially synthesized as xanthine dehydrogenase, and its protein structure is converted into xanthine oxidase under pathophysiological conditions, such as tissue hypoxia6,7. Xanthine oxidase binds to negatively charged glycosaminoglycans at the apical surface of vascular endothelial cells8,9, and is well documented as a crucial source...
of reactive oxygen species through the production of $O_2^-$ and $H_2O_2$, which indicates a possible causal role of this enzyme in endothelial injury.$^{10,11}$

We previously reported that the tissue distribution of XOR gene expression differed between humans and rodents.$^{12}$ Although XOR messenger ribonucleic acid expression in murine adipose tissue is similar to that in the liver, its messenger ribonucleic acid expression in human tissues is limited in the liver, lungs and gut. Consequently, the secretion of hypoxanthine from human adipocytes was dominant over that of xanthine and uric acid, whereas greater amounts of uric acid were secreted from murine adipocytes than hypoxanthine and xanthine. The secretion of hypoxanthine from human adipocytes and uric acid from murine adipocytes was previously shown to increase under hypoxic conditions.$^{13}$

Recent technological advances have made it possible to quantitatively measure the XOR activity in human plasma, despite the presence of a much lower level of this enzyme than is present in mouse plasma.$^{13}$ Plasma XOR activity was recently shown to be associated with body mass index (BMI), homeostasis model assessment for insulin resistance (HOMA-IR), liver dysfunction and hyperuricemia in cross-sectional studies, on volunteers and participants subjected to annual health checkups.$^{14,15}$ Findings from these previous studies show a possible link between high plasma XOR activity and metabolic disorders, such as obesity and insulin resistance. However, limited information is currently available on plasma XOR activity in patients with type 2 diabetes mellitus, and also on its changes after short-term treatments of glycemic control. Here, we show a reduction in plasma XOR activity by glycemic control in admitted patients with type 2 diabetes mellitus, and clinical parameters associated with the change of plasma XOR activity.

**METHODS**

**Study participants**

The present study was carried out between December 2017 and March 2019, enrolling patients with type 2 diabetes mellitus aged between 20 and 75 years who were hospitalized in the Division of Endocrinology & Metabolism, Osaka University Hospital (Suita, Osaka, Japan) to improve glycemic control. The diagnosis of type 2 diabetes mellitus was based on the criteria of the World Health Organization National Diabetic Group 2006 and/or current treatment for diabetes. The exclusion criteria were: (i) patients for whom strict glycemic control was inappropriate; for example, because of frequent episodes of severe hypoglycemia and progressive diabetic retinopathy; (ii) patients who had advanced diabetic nephropathy (serum creatinine level $>2.0$ mg/dL); (iii) patients with acute infection, severe trauma or active cancer; (iv) patients in the pre- and postoperative periods; and (v) patients who were not considered to be eligible for the study based on the doctor’s assessment. A total of 31 patients were enrolled in the present study (MEDENGINE XOR study; XOR study based on medical-dental-engineering [MEDENGINE] cooperation). Of these, three patients taking XOR inhibitors for gout or asymptomatic hyperuricemia (two patients taking 10 mg/day and one taking 20 mg/day of febuxostat) were excluded from this study, and the remaining 28 patients were included in the analysis. During hospitalization, all the patients were treated with insulin, glucagon-like peptide-1 analogs and/or oral glucose-lowering drugs under dietary therapy to achieve the target levels of glycemic control. A total of 29 volunteers without diabetes aged between 20 and 75 years were also examined in the present study.

This study was approved by the Human Ethics Committee of Osaka University (no. 16374-6), and was carried out following the Declaration of Helsinki. Written informed consent was obtained from all of the study participants.

**Measurements of clinical parameters and collection of blood samples**

Bodyweight and waist circumference (WC) at the level of the umbilicus were measured in the standing position. Systolic and diastolic blood pressure was measured in the sitting position using a standard mercury sphygmomanometer. Blood samples were collected in the morning after overnight fasting to measure plasma XOR activity, plasma glucose, C-peptide, glycated hemoglobin (HbA1c; National Glycohemoglobin Standardization Program), glycoalbumin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), $\gamma$-glutamyl transpeptidase ($\gamma$-GTP), lactate dehydrogenase, creatinine, uric acid, high-sensitivity C-reactive protein (hs-CRP), acetoacetate and 3-hydroxybutyrate. Plasma adiponectin concentrations were determined by an enzyme-linked immunosorbent assay (human adiponectin enzyme-linked immunosorbent assay kit; Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan). The estimated glomerular filtration rate was calculated using the formula proposed by the Japanese Society of Nephrology.$^{16}$ These clinical parameters of the inpatients with type 2 diabetes mellitus were examined at admission and after 2 weeks of treatment during hospitalization. HOMA-IR was calculated as (fasting insulin [mU/mL] $\times$ fasting plasma glucose [mg/dL]) $\div$ 405.

The maximum intima-media thickness of the common carotid artery was measured using B-mode ultrasonography.$^{17}$ For assessing the arterial stiffness, the brachial-ankle pulse wave velocity was measured by an automatic form analyzer (BP-203RPE II; Colin, Komaki, Japan).

**Measurements of plasma XOR activity**

Plasma XOR activity was quantified using triple quadrupole mass spectrometry and liquid chromatography by detecting the production of $^{15}$C$_2^{15}$N$_2$-uric acid from $^{13}$C$_2^{15}$N$_2$-xanthine as a substrate as reported previously.$^{13}$ Triple quadrupole mass spectrometry and liquid chromatography comprised an LC system (Shimadzu, Kyoto, Japan) and a QTRAP 4500 Mass Spectrometry System (SCIEX, Framingham, MA, USA) equipped with an ESI interface. Calibration standard samples of $^{13}$C$_2$-
RESULTS
Baseline characteristics of patients with type 2 diabetes mellitus
The baseline characteristics of patients with type 2 diabetes mellitus (10 men/18 women) enrolled in the present study are shown in Table 1. Briefly, the mean age, BMI and HbA1c were 63.4 years, 26.8 kg/m² and 9.2%, respectively. The median duration of diabetes was 15 years (IQR 8–20.8 years), and there was no newly diagnosed patient. Complications of hypertension, dyslipidemia and CVD were observed in 68, 82 and 25% of the participants, respectively. The median value of plasma XOR activity was 83.1 pmol/h/mL (IQR 31.6–254 pmol/h/mL), with a wide range of 14.4–1150 pmol/h/mL.

Clinical parameters correlated with plasma XOR activity at admission
We examined the relationships between plasma XOR activity and each of the clinical parameters at the time of admission. As shown in Table S1, in the univariate analysis, plasma XOR activity positively correlated with bodyweight, BMI, WC, C-peptide, liver enzymes (AST, ALT and γ-GTP), estimated glomerular filtration rate and hs-CRP, and negatively with age. No significant correlation was found between the XOR activity and serum uric acid levels. Next, a stepwise multiple regression was carried out to determine the parameters that were independently related to the baseline plasma XOR activity. To avoid the problem of multicollinearity, BMI and AST were selected into the model instead of bodyweight, WC and ALT. As a result, serum AST was identified as the only significant factor associated with plasma XOR activity (Table S2a). When ALT was added into the model instead of AST, it was also the only independent factor for the baseline plasma XOR activity (Table S2b). Among 16 out of 28 patients who were not treated with long-acting insulin, HOMA-IR positively correlated with plasma XOR activity (R = 0.59, P = 0.02). However, this association was no longer statistically significant after adjustment for AST (P = 0.06) or ALT (P = 0.38).

Relationships between changes in plasma XOR activity and those in other clinical parameters after 2 weeks of treatment during hospitalization
As shown in Table 2, 2 weeks of treatment during hospitalization resulted in an average weight loss of 1.3 kg (P < 0.0001), which was associated with significant improvements in multiple clinical parameters, including fasting plasma glucose, HbA1c, TG, γ-GTP and hs-CRP. During the same period, plasma XOR activity was also modestly, but significantly, decreased (P = 0.041). A tendency of decrease in serum AST and ALT was also observed.

We then assessed the relationship between changes in each clinical parameter and those in plasma XOR activity 2 weeks after admission. As shown in Table 3, in the univariate analysis, changes in the log plasma XOR activity positively correlated...
with those in low-density lipoprotein cholesterol, log-AST, log-ALT and log-adiponectin, whereas no correlation was found between the changes in log plasma XOR activity and those in BMI, glycemic parameters (fasting blood glucose, HbA1c and glycocalcium) and plasma purine metabolites (uric acid, log hypoxanthine and log xanthine). The stepwise multiple regression analysis showed that the changes in log-AST (ΔLog-AST) was the sole factor correlating changes in the log plasma XOR activity (ΔLog-XOR), when ΔLog-AST instead of ΔLog-ALT was entered into the model because of its higher correlation coefficient with ΔLog-XOR (Table 4). As shown in Figure 1a, ΔLog-AST was strongly and positively correlated with ΔLog-XOR (R = 0.82, P < 0.0001).

Table 1 | Baseline clinical characteristics of patients with type 2 diabetes

| Clinical parameters | n (males/females) | Age (years) | BW (kg) | BMI (kg/m²) | WC (cm) | sBP (mmHg) | dBP (mmHg) | FPG (mg/dL) | C-peptide (ng/mL) | HbA1c (%) | HbA1c (mmol/mol) | GA (%) | T-cho (mg/dL) | LDL-cho (mg/dL) | HDL-cho (mg/dL) | TG (mg/dL) | AST (IU/L) | ALT (IU/L) | γ-GTP (IU/L) | LDH (IU/L) | Creatinine (mg/dL) | eGFR (mL/min/1.73 m²) | UA (mg/dL) | Xan (μmol/L) | HX (μmol/L) | XOR activity (pmol/h/mL) | hs-CRP (mg/dL) | Adiponectin (μg/mL) | AcAc (μmol/L) | 3-OHBA (μmol/L) | CCA max IMT (mm) | baPWV (m/s) | Duration of diabetes (years) | Glucose-lowering agents |
|---------------------|-------------------|-------------|---------|-------------|---------|------------|------------|------------|-----------------|-----------|------------------|--------|-----------------|-----------------|-----------------|---------|----------|----------|----------------|----------------|-------------------|----------------|-------------|----------------|----------------|----------------|------------------|------------------|
|                     | 28 (10/18)        | 63.4 ± 11   | 67.8 ± 14.1 | 26.8 ± 5.3 | 989 ± 10.5 | 131.8 ± 18.4 | 766.6 ± 16.9 | 147.9 ± 47.4 | 1.62 ± 0.99 | 9.2 ± 2.1 | 77 ± 23 | 23.7 ± 5.9 | 1998 ± 62.5 | 120.3 ± 50.1 | 534 ± 12.3 | 125 (75.3–232.3) | 22 (17–27) | 21.5 (15.5–39.3) | 265 (183–45.8) | 1895 (1560–2088) | 0.75 ± 0.23 | 720 ± 21.2 | 5.6 ± 1.2 | 0.63 (0.45–0.83) | 1.01 (0.55–1.48) | 83.1 (31.6–254) | 0.05 (0.04–0.11) | 6.6 (4.1–8.9) | 63.5 (36.5–97.8) | 93.5 (45.3–195.8) | 1.9 ± 1.0 | 1753 ± 280 | 15 (8–20.8) | (SU/BG/TZD/DPP-4i/Gl-GL/SGLT-2i/RA/Insulin) 8/12/19/9/10/6/14 Smoking habit (current/passive) |
| Complication of CVD | 23 (82%) | 19 (68%) |
| Complication of dyslipidemia | 7 (25%) |

Data are presented as the mean ± standard deviation, median (interquartile range) or the number of participants (%). α-GI, α-glucosidase inhibitor; γ-GTP, γ-glutamyl transpeptidase; 3-OHBA, 3-hydroxybutyrate; AcAc, acetacetate; ALT, alanine transaminase; AST, aspartate transaminase; BG, biguanide; BMI, body mass index; BW, bodyweight; CCA, common carotid artery; CVD, cardiovascular disease; dBP, diastolic blood pressure; DPP-4i, dipeptidyl peptidase-4 inhibitor; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GA, glycoalbumin; GLP-1 RA, glucagon-like peptide-1 receptor agonist; HDL-cho, high-density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive protein; HX, hypoxanthine; IMT, intima-media thickness; LDH, lactate dehydrogenase; LDL-cho, low-density lipoprotein cholesterol; PWV, brachial-ankle pulse wave velocity; sBP, systolic blood pressure; SGLT-2i, sodium–glucose cotransporter 2 inhibitor; SU, sulfonlurea; T-cho, total cholesterol; TG, triglyceride; TZZ, thiazolidinedione; UA, uric acid; WC, waist circumference; Xan, xanthine; XOR, xanthine oxidoreductase.

Table 2 | Changes in clinical parameters from admission to 2 weeks after treatment for diabetes

| Clinical parameters | Adm | Post | P-value |
|---------------------|-----|------|---------|
| BW (kg)             | 67.8 ± 14.1 | 66.5 ± 13.7 | <0.0001 |
| BMI (kg/m²)         | 26.8 ± 5.3  | 26.3 ± 5.1  | <0.0001 |
| sBP (mmHg)          | 131 ± 18   | 124 ± 12   | 0.085   |
| dBP (mmHg)          | 76 ± 17    | 75 ± 12    | 0.817   |
| FPG (mg/dL)         | 147.9 ± 47.4 | 109 ± 19 | <0.0001 |
| C-peptide (ng/mL)   | 1.62 ± 0.99 | 1.41 ± 0.80 | 0.146   |
| HbA1c (%)           | 9.2 ± 2.1  | 8.5 ± 1.7  | <0.0001 |
| HbA1c (mmol/mol)    | 77 ± 23    | 69 ± 18    | <0.0001 |
| GA (%)              | 23.7 ± 5.9 | 19.4 ± 3.5 | <0.0001 |
| T-cho (mg/dL)       | 1998 ± 62.5 | 1581 ± 303 | 0.0001  |
| LDL-cho (mg/dL)     | 120.3 ± 50.1 | 88.3 ± 28.8 | <0.0001 |
| HDL-cho (mg/dL)     | 534 ± 12.3 | 49.6 ± 10.9 | 0.003   |
| Log-TG (mg/dL)      | 4.85 ± 0.61 | 4.58 ± 0.41 | 0.001   |
| Log-AST (IU/L)      | 3.16 ± 0.40 | 3.07 ± 0.31 | 0.092   |
| Log-ALT (IU/L)      | 3.15 ± 0.61 | 3.04 ± 0.53 | 0.085   |
| Log-γ-GTP (IU/L)    | 3.42 ± 0.65 | 3.19 ± 0.65 | <0.0001 |
| Log-LDH (IU/L)      | 5.20 ± 0.21 | 5.13 ± 0.20 | 0.006   |
| Creatinine (mg/dL)  | 0.75 ± 0.23 | 0.79 ± 0.24 | 0.042   |
| eGFR (mL/min/1.73 m²) | 720 ± 21.2 | 681 ± 18.6 | 0.018   |
| UA (mg/dL)          | 5.6 ± 1.2  | 5.4 ± 1.0  | 0.161   |
| Log-Xan (μmol/L)    | −0.50 ± 0.35 | −0.48 ± 0.59 | 0.894   |
| Log-HX (μmol/L)     | −0.008 ± 0.69 | −0.058 ± 0.59 | 0.745   |
| Log-XOR (pmol/mL)   | 45.2 ± 1.2  | 43.0 ± 1.0  | 0.041   |
| Log-hs-CRP (mg/dL)  | −2.50 ± 1.06 | −2.85 ± 0.71 | 0.047   |
| Log-adiponectin (μg/mL) | 1.76 ± 0.67 | 1.68 ± 0.66 | 0.037   |
| Log-AcAc (μmol/L)   | 4.12 ± 0.67 | 4.07 ± 0.70 | 0.713   |
| Log-3-OHBA (μmol/L) | 4.60 ± 0.86 | 4.71 ± 0.82 | 0.487   |

Data are presented as the means ± standard deviation. γ-GTP, γ-glutamyl transpeptidase; 3-OHBA, 3-hydroxybutyrate; AcAc, acetacetate; Adm, admission; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; BW, bodyweight; dBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GA, glycoalbumin; HDL-cho, high-density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive protein; HX, hypoxanthine; IMT, intima-media thickness; LDH, lactate dehydrogenase; LDL-cho, low-density lipoprotein cholesterol; Post, 2 weeks after treatment during hospitalization; sBP, systolic blood pressure; T-cho, total cholesterol; TG, triglyceride; UA, uric acid; Xan, xanthine; XOR, xanthine oxidoreductase.
Table 3 | Correlations between changes in plasma xanthine oxidoreductase activity and those in other clinical parameters during hospitalization

| Clinical parameters | Univariate | P-value |
|---------------------|------------|---------|
| ΔBW                 | 0.26       | 0.180   |
| ΔBMI                | 0.29       | 0.140   |
| ΔsBP                | −0.06      | 0.750   |
| ΔdBP                | −0.10      | 0.600   |
| ΔFGF                | 0.08       | 0.690   |
| ΔC-peptide          | −0.02      | 0.921   |
| ΔHbA1c              | 0.05       | 0.810   |
| ΔGA                 | 0.07       | 0.720   |
| ΔT-cho              | −0.45      | 0.017   |
| ΔLDL-cho            | −0.47      | 0.011   |
| ΔHDL-cho            | −0.10      | 0.630   |
| ΔLog-TG             | 0.15       | 0.460   |
| ΔLog-AST            | 0.82       | <0.0001 |
| ΔLog-ALT            | 0.72       | <0.0001 |
| ΔLog-γ-GTP          | −0.12      | 0.540   |
| ΔLog-LDH            | 0.17       | 0.380   |
| ΔCreatinine         | −0.03      | 0.880   |
| ΔeGFR               | 0.13       | 0.520   |
| ΔUA                 | 0.23       | 0.240   |
| ΔLog-Xan            | 0.11       | 0.570   |
| ΔLog-HX             | −0.18      | 0.370   |
| ΔLog-hs-CRP         | −0.01      | 0.950   |
| ΔLog-adiponectin    | 0.54       | 0.003   |
| ΔLog-AcAc           | 0.01       | 0.970   |
| ΔLog-3-OHBα         | −0.02      | 0.930   |

γ-GTP, γ-glutamyl transpeptidase; 3-OHBα, 3-hydroxybutyrate; AcAc, acetacetate; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; BW, body weight; dBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GA, glycoalbumin; HDL-cho, high-density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive protein; HX, hypoxanthine; LDL, lactate dehydrogenase; LDL-cho, low-density lipoprotein cholesterol; sBP, systolic blood pressure; T-cho, total cholesterol; TG, triglyceride; UA, uric acid; Xan, xanthine; XOR, xanthine oxidoreductase.

Because plasma XOR activity levels at admission varied widely among the patients, we divided the patients into two groups according to their baseline plasma XOR activity, higher or lower than the median value, and examined changes in plasma XOR and serum AST separately for each group. As shown in Figure 1b, plasma XOR activity was markedly decreased in patients with high baseline XOR activity, and was associated with significant reductions in serum AST. On the contrary, no significant changes were observed in these two parameters in the low XOR group (Figure 1b).

Plasma XOR activity in control participants without diabetes

Finally, 29 healthy volunteers (20 men/9 women) were examined to investigate whether such associations between plasma XOR activity and liver enzymes also emerge for individuals without diabetes. Mean age, BMI and HbA1c were 42.4 years, 22.6 kg/m² and 5.4%, respectively (Table S3). The median plasma XOR activity was 35.6 pmol/h/mL (IQR 24.4–69.1 pmol/h/mL), ranging from 8.67 to 173 pmol/h/mL. The plasma XOR activity levels were higher in men than in women, and positively correlated with bodyweight, BMI, WC, HOMA-IR, liver enzymes (AST, ALT and γ-GTP) and hs-CRP (Table S4). Plasma purine metabolites, including uric acid and xanthine, also positively correlated with the plasma XOR activity in these participants. However, the stepwise multiple regression analysis showed that just three parameters – namely, sex, serum ALT and γ-GTP levels – were significant independent predictors of the plasma XOR activity, when ALT instead of AST was incorporated into the model because of its higher correlation coefficient with plasma XOR (Table S5). Among these, ALT had the strongest correlation with the XOR activity (standard β = 0.664, P < 0.0001).

Table 4 | Multiple stepwise regression analysis of changes in clinical parameters associated with those in plasma XOR activity during hospitalization

| Clinical parameters | Multivariate | β  | SE  | Std β | P-value |
|---------------------|--------------|----|-----|-------|---------|
| ΔDL-cho             | −0.0009      | 0.002 | −0.050 | 0.716  |
| ΔLog-AST            | 2.059        | 0.282 | 0.820 | <0.0001 |
| ΔLog-adiponectin    | −0.005       | 0.047 | −0.002 | 0.991  |
| Adjusted R²         |              |     | 0.659 |       |         |

Parameters shown in boldface were statistically significant. AST, aspartate transaminase; LDL-cho, low-density lipoprotein cholesterol.

DISCUSSION

The main results of the present study of Japanese inpatients with type 2 diabetes mellitus are as follows: (i) cross-sectionally, the serum levels of liver transaminases (AST and ALT) were identified as significant factors correlating with the baseline plasma XOR activity; (ii) after 2 weeks of treatment during hospitalization, plasma XOR activity significantly decreased, particularly in patients with initially high plasma XOR; and (iii) changes in plasma XOR activity were strongly and independently associated with changes in serum AST.

To our knowledge, this is the first study to assess plasma XOR activity cross-sectionally and across treatments for patients with type 2 diabetes mellitus. The baseline plasma XOR activity was correlated with age, BMI, C-peptide, hs-CRP and serum liver enzymes (AST, ALT and γ-GTP) in a simple regression analysis. Similar findings were recently reported in a cross-sectional study on 60 Japanese patients with type 2 diabetes mellitus and metabolic syndrome, in which plasma xanthine oxidase activity correlated with BMI, HOMA-IR and liver transaminases. In contrast, a subsequent stepwise multivariate analysis in the present study showed that the only independent
predictors of plasma XOR in patients with type 2 diabetes mellitus were serum AST and ALT, with similar results being obtained for control participants without diabetes (Tables S2, S5). Additionally, a 2-week treatment for diabetes was associated with a significant decrease in plasma XOR activity, particularly in patients with high baseline XOR levels, and this change was only and strongly associated with that in AST (Tables 3,4). Not only HbA1c, but also glycoalbumin, which reflects short-term glycemic control (during the last 2–3 weeks), improved during hospitalization. However, there was no significant correlation between the changes in these glycemic parameters and those in plasma XOR, suggesting that changes in glycemic control within a short period, at least 2 weeks in the present study, were not associated with the decrease in XOR activity. Taken together, caloric restriction and improved glycemic control under hospitalized conditions might have ameliorated liver function, which was associated with decreases in plasma XOR activity.

To date, few studies have evaluated changes in the XOR activity. It was recently reported that, in a population-based cohort, an annual change in plasma XOR activity was independently associated with a change in bodyweight, as well as that in liver enzymes. In the present study, changes in bodyweight and BMI did not correlate with those in plasma XOR activity. A possible explanation for this difference might be that the absolute change in bodyweight in the present study (the mean value of −1.3 kg) was small and might have little association with plasma XOR activity. We previously reported XOR expression and uric acid secretion in murine adipose tissue, both of which were elevated in obese mice. In contrast, in a subsequent study, we found that the expression levels of the XOR messenger ribonucleic acid were far lower in the human adipose tissue than in the liver and small intestine. Consequently, XOR activity in human adipose tissue was markedly lower than that in murine adipose tissue, resulting in the secretion of more hypoxanthine from human adipose tissue than in the liver and small intestine. Consequently, XOR activity in human adipose tissue was markedly lower than that in murine adipose tissue, resulting in the secretion of more hypoxanthine from human adipose tissue than xanthine and uric acid. In addition, a recent study showed that a significant rise in plasma XOR activity in high-fat-induced obesity was almost canceled by hepatocyte-specific ablation of XOR in mice, suggesting a limited contribution of adipose tissue to the circulating XOR levels in mice as well.

Previous findings and the present results collectively show that a large proportion of the XOR protein in the human bloodstream is derived from the continuous leakage of hepatic XOR, but not from adipocytes. Furthermore, plasma XOR activity might be elevated in the background of liver diseases, such as non-alcoholic fatty liver disease (NAFLD), often associated with obesity and diabetes. A number of clinical investigations have shown that NAFLD increases the risk of CVD, independent of established cardiovascular risk factors. Although the underlying mechanism for this association is still not fully understood, insulin resistance, dietary intake, altered...
lipid metabolism, the gut microbiome and the pro-inflammatory state in NAFLD patients are considered to play important roles in the pathogenesis of atherosclerosis. In addition to these, an excess XOR release into the systemic circulation due to chronic hepatic injury might participate in the cross-talk between NAFLD and CVD progression. Further clinical and experimental studies are required to clarify whether increased plasma XOR activity per se could be involved in atherosclerosis associated with type 2 diabetes mellitus.

Plasma XOR activity in patients with type 2 diabetes mellitus (median value of 83.1 pmol/h/mL) was relatively high compared with that previously reported in the general population (median value of 36 pmol/h/mL), in which XOR activity levels were assessed by the same method. We also found that the value in individuals with diabetes was significantly higher than that in our group without diabetes (median value of 35.6 pmol/h/mL; \( P = 0.002 \)), and the statistical significance between the two groups persisted even after adjustment for sex, age, BMI, TG, AST, ALT and Hba1c (\( P = 0.004 \)), suggesting that pathogenesis of diabetes per se might be associated with increased plasma XOR activity. However, because of clear differences in baseline characteristics of the type 2 diabetes mellitus patients and the healthy volunteers (Tables 1, S3), further large-scale case-control studies are required to confirm this preliminary finding. Previous studies in rodents have shown that inflammatory mediators including bacterial lipopolysaccharide and interleukin-1β upregulated XOR expression in liver and lung tissues. As such, in addition to leakage from damaged hepatocytes, the pro-inflammatory state in patients with diabetes might relate to increased plasma XOR activity.

The present study has several limitations. Although patients taking antihyperuricemic drugs were excluded from the present study, some glucose-lowering agents, such as selective sodium-glucose cotransporter 2 inhibitors, might affect bodyweight, serum liver enzymes and plasma uric acid levels. The number of patients was relatively small to carry out a multivariate analysis and to identify clinical parameters that had weaker associations with plasma XOR activity. Although no study participant had hepatitis B or C, examinations other than blood tests were not undertaken to assess the etiology and severity of liver dysfunction. Therefore, it currently remains unclear whether the pathological progression of liver damage affects plasma XOR activity, independent of the serum liver transaminase levels.

In conclusion, the present results showed a close relationship between plasma XOR activity and circulating levels of liver transaminases in patients with type 2 diabetes mellitus, cross-sectionally and across hospitalized treatment, suggesting that plasma XOR reflects liver damage in patients with diabetes.

ACKNOWLEDGMENTS

We thank Miho Minami, Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, for her excellent technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research (C) no. 18K16229 (to YF), no. 19K09023 (to HN), no. 19K08980 (to NM) and no. 19K08978 (to SK); Grants-in-Aid for Scientific Research (B) no. 18H02863 (to IS); a grant from the ‘Creation of Innovative Technology for Medical Applications Based on the Global Analyses and Regulation of Disease-Related Metabolites Project’ of the Japan Agency for Medical Research and Development (to IS), Japan Foundation for Applied Enzymology (to YF), MSD Life Science Foundation (to YF), Japan Heart Foundation & Astellas Grant for Research on Atherosclerosis Update (to YF), and Gout and Uric Acid Foundation of Japan (to HN). The funding agencies had no role in study design, data collection, analysis, decision to publish or preparation of the manuscript.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Yamashita S, Matsuzawa Y, Tokunaga K, et al. Studies on the impaired metabolism of uric acid in obese subjects: marked reduction of renal urate excretion and its improvement by a low-calorie diet. Int J Obes 1986; 10: 255–264.
2. Neeland IJ, Ross R, Després JP, et al. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. Lancet Diabetes Endocrinol 2019; 7: 715–725.
3. Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol 2004; 555: 589–606.
4. Battelli MG, Bolognesi A, Polito L. Pathophysiology of circulating xanthine oxidoreductase: new emerging roles for a multi-tasking enzyme. Biochim Biophys Acta 2014; 1842: 1502–1517.
5. Kelley EE. A new paradigm for XOR-catalyzed reactive species generation in the endothelium. Pharmacol Rep 2015; 67: 669–674.
6. Engerson TD, Mckelvey TG, Rhine DB, et al. Conversion of xanthine dehydrogenase to oxidase in ischemic rat tissues. J Clin Invest 1987; 79: 1564–1570.
7. Brass CA, Narciso J, Gollan JL. Enhanced activity of the free radical producing enzyme xanthine oxidase in hypoxic rat liver. Regulation and pathophysiologic significance. J Clin Invest 1991; 87: 424–431.
8. Houston M, Estvez A, Chumley P, et al. Binding of xanthine oxidase to vascular endothelium. Kinetic characterization and oxidative impairment of nitric oxide-dependent signaling. J Biol Chem 1999; 274: 4985–4994.
9. Adachi T, Fukushima T, Usami Y, et al. Binding of human xanthine oxidase to sulfated glycosaminoglycans on the endothelial-cell surface. Biochem J 1993; 289: 523–527.
10. Meneshian A, Bulkeley GB. The physiology of endothelial xanthine oxidase: from urate catabolism to reperfusion injury to inflammatory signal transduction. Microcirculation 2002; 9: 161–175.
11. Battelli MG, Polito L, Bolognesi A. Xanthine oxidoreductase in atherosclerosis pathogenesis: not only oxidative stress. *Atherosclerosis* 2014; 237: 562–567.

12. Nagao H, Nishizawa H, Tanaka Y, et al. Hypoxanthine secretion from human adipose tissue and its increase in hypoxia. *Obesity* 2018; 26: 1168–1178.

13. Murase T, Nampei M, Oka M, et al. A highly sensitive assay of human plasma xanthine oxidoreductase activity using stable isotope-labeled xanthine and LC/TQMS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2016; 1039: 51–58.

14. Washio KW, Kusunoki Y, Murase T, et al. Xanthine oxidoreductase activity is correlated with insulin resistance and subclinical inflammation in young humans. *Metabolism* 2017; 70: 51–56.

15. Furuhashi M, Matsumoto M, Tanaka M, et al. Plasma xanthine oxidoreductase activity as a novel biomarker of metabolic disorders in a general population. *Circ J* 2018; 82: 1892–1899.

16. Japanese Society of Nephrology. Evidence-based practice guideline for the treatment of CKD. *Clin Exp Nephrol* 2009; 13: 537–566.

17. Katakami N, Matsuhisa M, Kaneto H, et al. Serum endogenous secretory RAGE level is an independent risk factor for the progression of carotid atherosclerosis in type 1 diabetes. *Atherosclerosis* 2009; 204: 288–292.

18. Nakamura T, Murase T, Satoh E, et al. Establishment of the process in blood sampling and sample handling as a biomarker of hypoxia-inducible diseases; plasma hypoxanthine and xanthine measurement. *J Mol Biomark Diagn* 2018; 9: 404.

19. Sunagawa S, Shirakura T, Hokama N, et al. Activity of xanthine oxidase in plasma correlates with indices of insulin resistance and liver dysfunction in patients with type 2 diabetes mellitus and metabolic syndrome: a pilot exploratory study. *J Diabetes Investig* 2019; 10: 94–103.

20. Furuhashi M, Koyama M, Matsumoto M, et al. Annual change in plasma xanthine oxidoreductase activity is associated with changes in liver enzymes and body weight. *Endocr J* 2019; 66: 777–786.

21. Tsushima Y, Nishizawa H, Tochino Y, et al. Uric acid secretion from adipose tissue and its increase in obesity. *J Biol Chem* 2013; 288: 27138–27149.

22. Harmon DB, Mandler WK, Sipula UJ, et al. Hepatocyte-specific ablation or whole-body inhibition of xanthine oxidoreductase in mice corrects obesity-induced systemic hyperuricemia without improving metabolic abnormalities. *Diabetes* 2019; 68: 1221–1229.

23. Yki-Järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol* 2014; 2: 901–910.

24. Oni ET, Agatston AS, Blaha MJ, et al. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver; should we care? *Atherosclerosis* 2013; 230: 258–267.

25. Ampuero J, Gallego-Durán R, Romero-Gómez M. Association of NAFLD with subclinical atherosclerosis and coronary-artery disease: meta-analysis. *Rev Esp Enferm Dig* 2015; 107: 10–16.

26. Wu S, Wu F, Ding Y, et al. Association of non-alcoholic fatty liver disease with major adverse cardiovascular events: a systematic review and meta-analysis. *Sci Rep* 2016; 6: 33386.

27. Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015; 62: 547–564.

28. Adams LA, Anstee QM, Tilg H, et al. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut* 2017; 66: 1138–1153.

29. Lim S, Taskinen MR, Borén J. Crosstalk between nonalcoholic fatty liver disease and cardiometabolic syndrome. *Obes Rev* 2019; 20: 599–611.

30. Kurosaki M, Li Calzi M, Scanziani E, et al. Tissue- and cell-specific expression of mouse xanthine oxidoreductase gene in vivo: regulation by bacterial lipopolysaccharide. *Biochem J* 1995; 306: 225–234.

31. Hassoun PM, Yu FS, Cote CG, et al. Upregulation of xanthine oxidase by lipopolysaccharide, interleukin-1, and hypoxia. Role in acute lung injury. *Am J Respir Crit Care Med* 1998; 158: 299–305.

32. Sattar N, Taskinen MR, Borén J. Crosstalk between nonalcoholic fatty liver disease and cardiometabolic syndrome: a comprehensive review. *Diabetologia* 2018; 61: 2153–2163.

33. Zhao Y, Xu L, Tian D, et al. Effects of sodium-glucose cotransporter 2 (SGLT2) inhibitors on serum uric acid level: a meta-analysis of randomized controlled trials. *Diabetes Obes Metab* 2018; 20: 458–462.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1** | Correlations between plasma xanthine oxidoreductase activity and clinical parameters in patients with type 2 diabetes at admission.

**Table S2** | Multiple stepwise regression analysis of clinical parameters associated with the baseline plasma xanthine oxidoreductase activity.
Table S3 | Clinical characteristics in control participants without diabetes.

Table S4 | Correlations between the plasma xanthine oxidoreductase activity and clinical parameters in control participants without diabetes.

Table S5 | Multiple stepwise regression analysis of clinical parameters associated with the plasma xanthine oxidoreductase activity in control participants without diabetes.