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

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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 of 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 CVD[3–5]. XOR is initially synthesized as xanthine dehydrogenase, and its protein structure is converted into xanthine oxidase under pathophysiological conditions, such as tissue hypoxia[6,7]. Xanthine oxidase binds to negatively charged glycosaminoglycans at the apical surface of vascular endothelial cells[8,9], and is well documented as a crucial source

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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[12].

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), γ-glutamyl transpeptidase (γ-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 [μU/mL] × fasting plasma glucose [mg/dL] / 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 [$^{13}C_2$, $^{15}N_2$]-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 [$^{13}C_2$,

$^{15}N_2$]-uric acid were also measured, and production levels were calculated from the calibration curve. Similar to the previous study, XOR activities were expressed as pmol/h/mL. In this method, the lower detection limit was 6.67 pmol/h/mL, and coefficients of variation of the intra- and interassay were 6.5% and 9.1%, respectively.

Measurements of hypoxanthine and xanthine

Hypoxanthine and xanthine concentrations in plasma were measured as reported previously[18]. In brief, plasma samples were obtained using blood collection tubes coated with ethylenediaminetetraacetic acid-2K and placed in a cube cooler within 1 min of collection. Within 30 min of collection, the samples were centrifuged at 800 *g* at 4°C for 10 min. The plasma samples were added to methanol containing [$^{13}C_2$, $^{15}N_2$]-xanthine and [$^{13}C_3$, ^{15}N]-hypoxanthine as internal standards, and then centrifuged at 3,000 *g* at 4°C for 15 min. The supernatant (40 μL) was diluted with distilled water (160 μL), and concentrations of both the compounds were determined by triple quadrupole mass spectrometry and liquid chromatography (Nexera, SCIEX QTRAP 4500, SHIMADZU, Kyoto, Japan).

Definitions

Hypertension was defined as blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or the use of antihypertensive agents. Dyslipidemia was defined as low-density lipoprotein cholesterol ≥140 mg/dL, TG ≥150 mg/dL and/or high-density lipoprotein cholesterol ≤ 40 mg/day, or the use of antihyperlipidemic agents. CVD was defined as a history of cerebrovascular disease, coronary artery disease and/or peripheral artery disease, which was determined by an ankle-brachial pressure index <0.9.

Statistical analysis

All values are expressed as the mean ± standard deviation or the median (interquartile range [IQR]). Non-normally distributed variables were log-transformed before the analysis. Relationships between two continuous variables were analyzed using scatter plots and Pearson's correlation coefficient. Changes in all the clinical parameters from baseline to 2 weeks after admission were subjected to the paired *t*-test. The relationships between the changes in plasma XOR activity and those in other clinical parameters were calculated using Pearson's correlation coefficient. In all cases, *P*-values <0.05 were considered to be significant. To identify parameters that independently related to the baseline plasma XOR activity or changes in plasma XOR activity during hospitalization, those with *P*-values <0.05 in the simple regression analysis were entered into a forward-backward stepwise multiple regression analysis as independent variables. All analyses were carried out with the JMP Statistical Discovery Software 14.0 (SAS Institute, Cary, NC, USA).

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/m^2 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

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

Clinical parameters	
n (males/females)	28 (10/18)
Age (years)	63.4 ± 11
BW (kg)	67.8 ± 14.1
BMI (kg/m^2)	26.8 ± 5.3
WC (cm)	98.9 ± 10.5
sBP (mmHg)	131.8 ± 18.4
dBP (mmHg)	76.6 ± 16.9
FPG (mg/dL)	147.9 ± 47.4
C-peptide (ng/mL)	1.62 ± 0.99
HbA1c (%)	9.2 ± 2.1
HbA1c (mmol/mol)	77 ± 23
GA (%)	23.7 ± 5.9
T-cho (mg/dL)	199.8 ± 62.5
LDL-cho (mg/dL)	120.3 ± 50.1
HDL-cho (mg/dL)	53.4 ± 12.3
TG (mg/dL)	125 (75.3–232.3)
AST (IU/L)	22 (17–27)
ALT (IU/L)	21.5 (15.5–39.3)
γ-GTP (IU/L)	26.5 (18.3–45.8)
LDH (IU/L)	189.5 (156.0–208.8)
Creatinine (mg/dL)	0.75 ± 0.23
eGFR (mL/min/1.73 m^2)	72.0 ± 21.2
UA (mg/dL)	5.6 ± 1.2
Xan (μmol/L)	0.63 (0.45–0.83)
HX (μmol/L)	1.01 (0.55–1.48)
XOR activity (pmol/h/mL)	83.1 (31.6–254)
hs-CRP (mg/dL)	0.05 (0.04–0.11)
Adiponectin (μg/mL)	6.6 (4.1–8.9)
AcAc (μmol/L)	63.5 (36.5–97.8)
3-OHBA (μmol/L)	93.5 (45.3–195.8)
CCA max IMT (mm)	1.9 ± 1.0
baPWV (m/s)	1753 ± 280
Duration of diabetes (years)	15 (8–20.8)
Glucose-lowering agents (SU/BG/TZD/DPP-4i/α-GI/SGLT-2i/GLP-1 RA/insulin)	8/12/1/9/2/6/6/14
Smoking habit (current/past/non)	2/12/14
Complication of hypertension	19 (68%)
Complication of dyslipidemia	23 (82%)
Complication of CVD	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, acetoacetate; 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, sulfonylurea; T-cho, total cholesterol; TG, triglyceride; TZD, thiazolidine; 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/m^2)	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)	199.8 ± 62.5	158.1 ± 30.3	<0.0001
LDL-cho (mg/dL)	120.3 ± 50.1	88.3 ± 28.8	<0.0001
HDL-cho (mg/dL)	53.4 ± 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 m^2)	72.0 ± 21.2	68.1 ± 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/h/mL)	4.5 ± 1.2	4.3 ± 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, acetoacetate; 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; 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.

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 glycoalbumin) 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 3 | Correlations between changes in plasma xanthine oxidoreductase activity and those in other clinical parameters during hospitalization

Clinical parameters	Univariate	
	R	*P*-value
ΔBW	0.26	0.180
ΔBMI	0.29	0.140
ΔsBP	−0.06	0.750
ΔdBP	−0.10	0.600
ΔFPG	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-OHBA	−0.02	0.930

γ-GTP, γ-glutamyl transpeptidase; 3-OHBA, 3-hydroxybutyrate; AcAc, acetoacetate; 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; LDH, 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/m^2 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 $\beta = 0.664$, $P < 0.0001$).

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
ΔLDL-cho	−0.0009	0.002	−0.050	0.716
ΔLog-AST	**2.059**	**0.282**	**0.820**	**<0.0001**
ΔLog-adiponectin	−0.005	0.487	−0.002	0.991
Adjusted R^2		**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[19]. In contrast, a subsequent stepwise multivariate analysis in the present study showed that the only independent

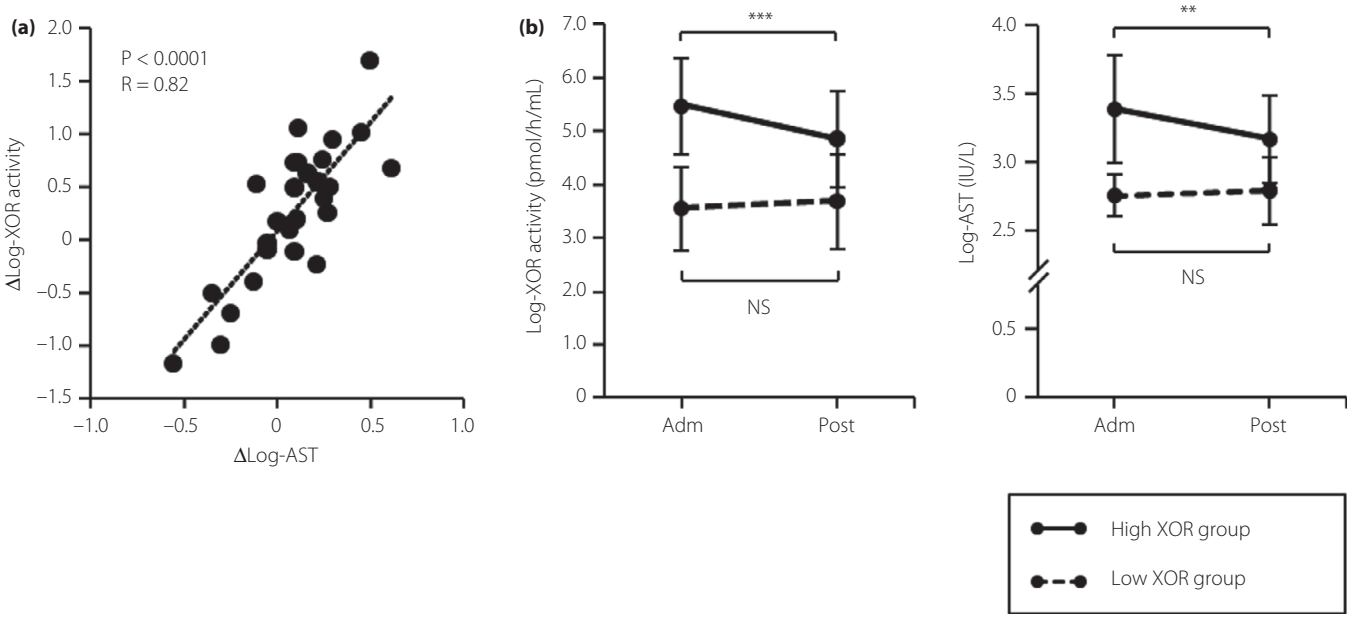

Figure 1 | Relationship between changes in serum aspartate aminotransferase (AST) with those in plasma xanthine oxidoreductase (XOR) activity during 2 weeks of hospitalization. (a) Pearson's correlation coefficient was used to examine the relationship between changes in serum AST and those in plasma XOR activity ($P < 0.0001$, $R = 0.82$). (b) Patients were divided into two groups according to their baseline plasma XOR activity levels, higher or lower than the median value (83.1 pmol/h/mL). Changes in plasma XOR activity and serum AST from admission (Adm) to 2 weeks after treatment during hospitalization (Post) are shown for each group. Data are the mean ± standard deviation. $^{**}P < 0.01$, $^{***}P < 0.001$. NS, not significant.

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[20]. 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[21]. 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 xanthine and uric acid[12]. 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[22].

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[23]. A number of clinical investigations have shown that NAFLD increases the risk of CVD, independent of established cardiovascular risk factors[24–26]. 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[27–29]. 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)[15], 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[30,31]. 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[32,33]. 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.

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DISCLOSURE

The authors declare no conflict of interest.

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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.