Association of the plasma xanthine oxidoreductase activity with the metabolic parameters and vascular complications in patients with type 2 diabetes: a cross-sectional study in a hospital setting

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Original investigation

Keywords: xanthine oxidoreductase, xanthine, hypoxanthine, type 2 diabetes, diabetic vascular complications

Posted Date: May 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-26599/v1

Abstract

Background

Xanthine oxidoreductase (XOR) is a rate-limiting enzyme that catalyzes the oxidation of hypoxanthine to xanthine, and of xanthine to uric acid. XOR also enhances the production of reactive oxygen species (ROS) and causes endothelial dysfunction. To explore the association of XOR and its substrate with the vascular complications in patients with type 2 diabetes mellitus (hereafter simply, diabetes), we measured the plasma XOR activity and plasma levels of xanthine and hypoxanthine in patients with type 2 diabetes.

Materials and Methods

Plasma XOR activity and the plasma levels of xanthine and hypoxanthine were measured, and their associations with the clinical parameters and vascular complications were investigated in 94 Japanese inpatients with type 2 diabetes.

Results

Both the plasma XOR activity and plasma xanthine levels were correlated with the serum uric acid level. The plasma XOR activity was correlated with the plasma xanthine level, but not the plasma hypoxanthine level. The plasma XOR activity and plasma xanthine levels were positively correlated with the body mass index (BMI), serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum γ-GTP, fasting plasma insulin, and the homeostasis model of assessment of insulin resistance (HOMA-IR), and negatively correlated with the serum high density lipoprotein cholesterol levels (HDL-C). The plasma XOR activity also showed a positive correlation with the serum triglyceride (TG) levels. Plasma xanthine was positively correlated with the Fib4-index. Multivariate analyses identified AST, ALT, fasting plasma insulin and HOMA-IR as being independently associated with the plasma XOR activity. In addition, a negative correlation was observed between the plasma XOR activity and the duration of diabetes, and positive correlations were observed between the plasma XOR activity and the coefficient of variation of the R-R interval (CVR-R) and sensory nerve conduction velocity (SCV). Furthermore, the plasma XOR activity was found to be significantly decreased in patients with coronary artery disease.

Conclusions

The plasma XOR activity might be a surrogate marker for the development of vascular complications, as well as liver dysfunction and insulin resistance, in patients with type 2 diabetes

Background

Increasing evidence has been accumulated to show an association between oxidative stress and the pathogenesis of diabetes, as well as obesity, cardiovascular disease, heart failure, cancer, hypertension, atherosclerosis, and inflammatory disease [1–4]. Chronic hyperglycemia induces oxidative stress and enhances the production of reactive oxygen species (ROS), contributing to the development of insulin resistance, β-cell dysfunction, and vascular complications [2, 3, 5].

Oxidative stress is reported to be closely associated with vascular endothelial dysfunction in type 2 diabetes [6], and with the development of diabetic complications [7]. Xanthine oxidoreductase (XOR), which includes both xanthine oxidase (XO) and xanthine dehydrogenase (XDH), is a rate-limiting enzyme in purine catabolism and production of uric acid. XOR also plays a central role in the production of reactive oxygen species (ROS) and induces vascular endothelial dysfunction [3]. Increased XOR activity is reported in patients with coronary artery disease [8], obesity, and both type 1 and 2 diabetes [9, 10]. Recently, associations between XOR or XO activity and metabolic parameters were reported in patients with metabolic syndrome, renal dysfunction, and cardiovascular disease [11–17]. In addition, mice treated with an XOR inhibitor, allopurinol or febuxostat, showed improved insulin resistance, hypertension, atherosclerosis, and production of inflammatory cytokines by macrophages [18–20]. Furthermore, humans with chronic kidney disease (CKD) treated with XOR inhibitors were reported to show improvement of hypertension and renoprotective effects [21–27]. These findings implicate XOR activity in the pathogenesis of metabolic syndrome.

In purine catabolism, XOR oxidizes hypoxanthine to xanthine, and xanthine to uric acid [28]. Thus, hypoxanthine and xanthine are precursors of uric acid. Recently, an association between plasma hypoxanthine levels and obesity/smoking was reported, and differential regulation of hypoxanthine and xanthine was reported in a Japanese general population [29]. However, the association between the levels of these purines and the XOR activity in patients with type 2 diabetes remained obscure. Moreover, little is known about the associations of purine metabolism or XOR activity with the development of vascular complications in type 2 diabetes.

In this study, we investigated the plasma XOR activity, xanthine and hypoxanthine levels in patients with type 2 diabetes to clarify their associations with the metabolic parameters and vascular complications in type 2 diabetes.

Methods

Study Participants

We enrolled 94 Japanese patients with type 2 diabetes mellitus who were not receiving treatment for hyperuricemia. Patients who were pregnant, had severe renal dysfunction with an estimated glomerular filtration rate (eGFR) of < 30 ml/min/m^2, severe liver dysfunction, ketosis or infection, or any cancer were also excluded. All the patients were Japanese patients who were hospitalized at the Yokohama City University Hospital, Yokohama, Japan. This study was carried out from April 2017 to March 2019, with

the approval of the institutional ethics committee (approval number: B170900049, institutional ethical committee of Yokohama City University), and in accordance with the Declaration of Helsinki. All patients provided informed consent and signed informed consent forms.

Blood sampling and measurement of the plasma XOR activity, xanthine, and hypoxanthine levels

Blood samples were collected from the patients using PAX gene Blood DNA tubes (Becton, Dickinson and Company, Japan.) in the fasting condition early in the morning, and then centrifuged at 3000 rpm for 10 min at 4 °C within 8 h after blood collection to avoid the leak of hypoxanthine and xanthine from erythrocytes into the plasma [30]. The supernatant plasma samples were maintained at -80 °C until the assay. Plasma XOR activity was determined as previously described [31]. In brief, plasma sample was purified using a Sephadex G25 column, and 100 μL aliquots of the eluate was then mixed with [13C2, 15N2]-xanthine as the substrate and NAD + and [13C2, 15N2]-uric acid as the internal standard in Tris buffer (pH 8.5). Each of the mixtures was incubated at 37 °C for 90 min, quenched by methanol, and centrifuged at 2,000 × g for 15 min at 4 °C. The supernatants transferred to new tubes were evaporated, reconstituted with 150 μL distilled water, and filtered through an ultrafiltration membrane before undergoing a liquid chromatography-triple quadrupole mass spectrometry (LC/TQMS) analysis using the Nano Space SI-2 LC system (Shiseido, Ltd., Tokyo, Japan) and a TSQ-Quantum TQM spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an external systems interface. The amount of [13C2, 15N2]-UA produced was quantified using the calibration curve, with the XOR activity expressed as [13C2, 15N2]-uric acid in pmol/h/mL plasma. The lower limit of detection was 6.67 pmol/h/mL plasma, and intra- and inter-assay coefficients of variation were 6.5% and 9.1%, respectively. Plasma hypoxanthine and xanthine were also measured as previously reported [30, 31]. In brief, plasma samples were added into methanol containing [13C2, 15N2] xanthine and [13C3, 15N] hypoxanthine as internal standard and were centrifuged by 3,000 g at 4 °C for 15 min. The supernatant was diluted with distilled water, and concentrations of hypoxanthine and xanthine were measured using LC/TQMS (Nexera SCIEX QTRAP 4500, SHIMADZU, Japan).

Statistical analysis

Statistical analyses were performed using SPSS statics 19 (IBM). Variables are presented as means ± standard deviation, medians (interquartile range) or number (%). Comparisons between two groups were carried out by Student's t-test and the Mann–Whitney U test for continuous variables, and by the chi-square test for categorical variables. Statistical calculations for significant differences were carried out using the Wilcoxon signed-rank test, Wilcoxon rank sum test and Spearman's rank correlation coefficients. Multivariate regression analyses were carried out to identify independent associations between the plasma XOR activity and variables, and the standardized regression coefficient (b) and percentage of variance for the selected independent predictors explained (R^2). Statistical significance was set at $P < 0.05$.

Results

Clinical characteristics of the participants

The clinical characteristics of the 94 participants (male; 59, female; 35) are shown in Table 1. The age was 64 ± 12 years, the BMI was 26.2 ± 7.1 kg/m^2, and the HbA1c was 8.8% (7.9–10.1). The duration of diabetes was 10 ± 11 years. The plasma XOR activity was 67.7 pmol/h/mL (31.1–184). The plasma concentrations of xanthine and hypoxanthine were 0.71 μM (0.60–0.86) and 3.6 μM (2.8–5.3), respectively. In the present study, we defined patients with cardiovascular disease (CVD) as subjects who had coronary artery disease, cerebral infarction, or peripheral artery disease. There were no significant differences in the age, plasma XOR activity, plasma xanthine level, plasma hypoxanthine level or serum uric acid level between the male and female patients (see **Additional file 1; Supplementary Fig. 1**).

Table 1
Clinical characteristics of the study patients.

Number (male/female)	**94 (59:35)**
Age (years)	64 ± 12
Duration of diabetes (years)	10 ± 11
BMI (kg/m^2)	26.2 ± 7.1
Waist circumference (cm)	97.3 ± 15.0
Disease	
hypertension	51 (54.3)
cardiovascular disease	19 (20.2)
Medications	
insulin	27 (28.7)
GLP-1 receptor agonist	7 (7.4)
metformin	32 (34.0)
DPP-4 inhibitor	49 (52.1)
SGLT2 inhibitor	16 (17.0)
Thiazolidine	9 (9.6)
Sulfonylurea	10 (10.6)
glinide	7 (7.4)
α-glucosidase inhibitor	12 (12.8)
HbA1c (%)	8.8 (7.9–10.1)
Glycated albumin (%)	22.0 (18.5–26.7)
Fasting blood glucose (mg/dL)	147 (129–185)
Fasting IRI (μIU/mL)	8.8 (4.3–13.9)

Data are presented as means ± standard deviation, medians (interquartile range) or number (%). BMI, body mass index; HbA1c, glycosylated hemoglobin; IRI, immunoreactive insulin; CPR, C-peptide immunoreactivity; HOMA-IR, homeostasis model assessment of insulin resistance; eGFR, estimated glomerular filtration rate; ACR, urinary albumin to creatinine ratio; UA, serum uric acid level; T-Chol, serum total cholesterol level; HDL-C, serum high-density lipoprotein cholesterol level; LDL-C, serum low density lipoprotein cholesterol level; TG, serum triglyceride level; AST, serum aspartate aminotransferase level; ALT, serum alanine aminotransferase level; γ-GTP, serum γ-glutamyl transpeptidase level; plasma XOR activity, plasma xanthine oxidoreductase activity level; CVR-R, coefficient of variation of the R-R interval; MCV, motor nerve conduction velocity (tibial nerve); SCV, sensory nerve conduction velocity (peroneal nerve)

Number (male/female)	**94 (59:35)**
Fasting CPR (ng/mL)	2.4 (1.4-3.0)
HOMA-IR	3.3 (1.7–5.1)
eGFR (mL/min/1.73 m^2)	75 (63–91)
ACR (mg/Cr)	10.8 (4.6–43.9)
UA (mg/dL)	5.4 (4.0-6.2)
T-Cho (mg/dL)	193 (169–214)
HDL-C (mg/dL)	49 (40–57)
LDL-C (mg/dL)	113 (97–136)
TG (mg/dL)	134 (95–185)
AST (IU/L)	21 (17–31)
ALT (IU/L)	21 (14–38)
γ-GTP (IU/L)	32 (20–48)
Fib-4 index	1.4 (0.9–1.9)
Urinary CPR (μg/day)	78 (46–110)
Plasma XOR activity (pmol/h/mL)	67.7 (31.1–184)
Xanthine (μM)	0.71 (0.60–0.86)
Hypoxanthine (μM)	3.6 (2.8–5.3)
CVR-R (%)	2.0 (1.3–2.7)
MCV (m/s)	42.2 (38.9–45.6)
SCV (m/s)	42.4 (37.2–47.6)
Diabetic retinopathy	25 (26.6)

Data are presented as means ± standard deviation, medians (interquartile range) or number (%). BMI, body mass index; HbA1c, glycosylated hemoglobin; IRI, immunoreactive insulin; CPR, C-peptide immunoreactivity; HOMA-IR, homeostasis model assessment of insulin resistance; eGFR, estimated glomerular filtration rate; ACR, urinary albumin to creatinine ratio; UA, serum uric acid level; T-Chol, serum total cholesterol level; HDL-C, serum high-density lipoprotein cholesterol level; LDL-C, serum low density lipoprotein cholesterol level; TG, serum triglyceride level; AST, serum aspartate aminotransferase level; ALT, serum alanine aminotransferase level; γ-GTP, serum γ-glutamyl transpeptidase level; plasma XOR activity, plasma xanthine oxidoreductase activity level; CVR-R, coefficient of variation of the R-R interval; MCV, motor nerve conduction velocity (tibial nerve); SCV, sensory nerve conduction velocity (peroneal nerve)

Correlations among the plasma XOR activity and the plasma xanthine, hypoxanthine and uric acid levels

First, we investigated the correlations among the plasma XOR activity, plasma xanthine, plasma hypoxanthine, and serum uric acid levels by simple regression analyses (Fig. 1). The plasma XOR activity was significantly and positively correlated with the plasma level of xanthine ($R = 0.56$, $P < 0.001$) and the serum uric acid level ($R = 0.42$, $P < 0.001$), but not with the plasma hypoxanthine level ($R = 0.04$, $P = 0.71$). In contrast, a positive correlation was observed between the plasma xanthine and hypoxanthine levels ($R = 0.35$, $P < 0.01$). Plasma xanthine level, but not the plasma hypoxanthine level was also correlated with the serum uric acid level ($R = 0.39$, $P < 0.001$).

Correlations between the plasma XOR activity and metabolic parameters

The correlations between the plasma XOR activity and the clinical parameters in the patients with type 2 diabetes were examined by simple regression analyses, and the results are shown in Fig. 2 **and** Table 2. There was a significant positive correlation between the plasma XOR activity and the body mass index (BMI) ($R = 0.50$, $P < 0.001$). In addition, the plasma XOR activity showed correlations with parameters of insulin resistance, such as fasting immunoreactive insulin (IRI) ($R = 0.54$, $P < 0.001$), homeostasis model assessment of the index of insulin resistance (HOMA-IR) ($R = 0.47$, $P < 0.001$) and urinary C-peptide excretion during the day ($R = 0.41$, $P = 0.01$). In addition, the plasma XOR activity showed significant positive correlations with the AST ($R = 0.81$, $P < 0.001$), ALT ($R = 0.88$, $P < 0.001$), γ-GTP ($R = 0.76$, $P < 0.001$), and TG ($R = 0.32$, $P < 0.01$), and negative correlations with the percent glycated albumin ($R = -0.27$, $P = 0.01$) and HDL-C ($R = -0.30$, $P < 0.05$); no significant correlation of the plasma XOR activity with the HbA1c ($R = 0.08$, $P = 0.43$) or the fasting blood glucose level ($R = 0.00$, $P = 0.983$) was observed in the patients with type 2 diabetes. The plasma XOR activity showed no significant correlations with the parameters of diabetic nephropathy, eGFR, severity of albuminuria, and serum cystatin C either in the patients with type 2 diabetes.

Table 2
Correlation analyses for plasma XOR activity.

	R	*P* value
Age	-0.146	0.161
BMI	0.502	0.000
Waist circumference	0.322	0.009
Duration conduction velocity	-0.295	0.006
HbA1c	0.083	0.427
Glycated albumin	-0.266	0.010
Fasting blood glucose	0.002	0.983
Fasting IRI	0.539	0.000
Fasting CPR	0.512	0.000
HOMA-IR	0.466	0.000
eGFR	0.005	0.964
Cystatin C	0.081	0.440
UA	0.417	0.000
LDL-C	0.156	0.134
HDL-C	-0.303	0.003
TG	0.318	0.002
AST	0.808	0.000
ALT	0.881	0.000
γ-GTP	0.759	0.000
Fib4-index	0.310	0.054
ACR	0.037	0.723
Urinary CPR	0.406	0.010

XOR, xanthine oxidoreductase; BMI, body mass index; HbA1c, glycosylated hemoglobin; IRI, immunoreactive insulin; CPR, C-peptide immunoreactivity; HOMA-IR, homeostasis model assessment of insulin resistance; eGFR, estimated glomerular filtration rate; UA, serum uric acid level; T-Chol, serum total cholesterol level; HDL-C, serum high-density lipoprotein cholesterol level; LDL-C, serum low density lipoprotein cholesterol level; TG, serum triglyceride level; AST, serum aspartate aminotransferase level; ALT, serum alanine aminotransferase level; γ-GTP, serum γ-glutamyl transpeptidase level; ACR, urinary albumin to creatinine ratio

Correlations between the plasma xanthine concentrations and metabolic parameters

We also analyzed the correlations between the plasma xanthine levels and the clinical parameters in patients with type 2 diabetes by simple regression analyses (Fig. 3 **and** Table 3**)**. The plasma concentration of xanthine showed significant positive correlations with the BMI ($R = 0.39$, $P < 0.001$), waist circumference ($R = 0.42$, $P = 0.001$), fasting IRI ($R = 0.35$, $P = 0.001$), fasting C-peptide ($R = 0.32$, $P < 0.01$), HOMA-IR ($R = 0.27$, $P < 0.01$), AST ($R = 0.62$, $P < 0.001$), ALT ($R = 0.56$, $P < 0.001$), γ-GTP ($R = 0.54$, $P < 0.001$), and Fib4-index ($R = 0.43$, $P < 0.01$), and a negative correlation with the HDL-C ($R = -0.21$, $P < 0.05$) in patients with type 2 diabetes.

Table 3
Correlation analyses for plasma xanthine.

	R	*P* value
Age	0.057	0.586
BMI	0.386	0.000
Waist circumference	0.419	0.001
Duration	-0.128	0.239
HbA1c	-0.003	0.975
Glycated albumin	-0.165	0.111
Fasting blood glucose	-0.075	0.472
Fasting IRI	0.347	0.001
Fasting CPR	0.320	0.002
HOMA-IR	0.271	0.009
eGFR	-0.122	0.241
Cystatin C	0.191	0.065
UA	0.392	0.000
LDL-C	0.147	0.157
HDL-C	-0.207	0.045
TG	0.178	0.086
AST	0.620	0.000
ALT	0.562	0.000
γ-GTP	0.535	0.000
Fib4-index	0.427	0.007
ACR	0.124	0.234
Urinary CPR	0.133	0.421

BMI, body mass index; HbA1c, glycosylated hemoglobin; IRI, immunoreactive insulin; CPR, C-peptide immunoreactivity; HOMA-IR, homeostasis model assessment of insulin resistance; eGFR, estimated glomerular filtration rate; UA, serum uric acid level; T-Chol, serum total cholesterol level; HDL-C, serum high-density lipoprotein cholesterol level; LDL-C, serum low density lipoprotein cholesterol level; TG, serum triglyceride level; AST, serum aspartate aminotransferase level; ALT, serum alanine aminotransferase level; γ-GTP, serum γ-glutamyl transpeptidase level; ACR, urinary albumin to creatinine ratio

Correlations between the plasma concentrations of hypoxanthine and metabolic parameters

We further investigated the correlations between the plasma hypoxanthine levels and clinical parameters in patients with type 2 diabetes (Fig. 4 **and** Table 4**)**. The plasma concentration of hypoxanthine was negatively correlated with the HDL-C ($R = -0.23$, $P < 0.05$), but showed no significant correlation with any other parameter.

Table 4
Correlation analyses for plasma hypoxanthine.

	R	P value
Age	-0.108	0.301
BMI	0.141	0.176
Waist circumference	0.232	0.063
Duration	-0.144	0.182
HbA1c	0.178	0.086
Glycated albumin	-0.022	0.836
Fasting blood glucose	-0.013	0.898
Fasting IRI	0.089	0.400
Fasting CPR	0.057	0.589
HOMA-IR	0.052	0.623
eGFR	0.104	0.318
Cystatin C	-0.082	0.434
UA	0.046	0.662
LDL-C	0.075	0.471
HDL-C	-0.234	0.023
TG	0.190	0.067
AST	0.085	0.605
ALT	0.053	0.746
γ-GTP	0.107	0.517
Fib4-index	0.007	0.967
ACR	-0.143	0.170
Urinary CPR	0.101	0.543

BMI, body mass index; HbA1c, glycosylated hemoglobin; IRI, immunoreactive insulin; CPR, C-peptide immunoreactivity; HOMA-IR, homeostasis model assessment of insulin resistance; eGFR, estimated glomerular filtration rate; UA, serum uric acid level; T-Chol, serum total cholesterol level; HDL-C, serum high-density lipoprotein cholesterol level; LDL-C, serum low density lipoprotein cholesterol level; TG, serum triglyceride level; AST, serum aspartate aminotransferase level; ALT, serum alanine aminotransferase level; γ-GTP, serum γ-glutamyl transpeptidase level; ACR, urinary albumin to creatinine ratio

Multiple regression analyses to factors independently associated with the plasma XOR activity

Next, we performed stepwise multiple regression analyses to investigate the factors showing independent associations with the plasma XOR activity in patients with type 2 diabetes. In the multiple regression analysis model, the variables of age, gender, HbA1c, serum uric acid, BMI, fasting IRI, HOMA-IR, AST, ALT, and TG were entered as explanatory variables, and the plasma XOR activity was entered as the dependent variable (Table 5). The ALT ($\beta = 0.465$, $P < 0.001$), AST ($\beta = 0.365$, $P = 0.003$) and TG ($\beta = 0.161$, $P = 0.01$) were found to be significantly and independently associated with the plasma XOR activity (Model 1). No association was observed between the serum uric acid ($\beta = 0.034$, $P = 0.807$) and the plasma XOR activity in the current study. When ALT and AST were excluded, fasting IRI ($\beta = 0.775$, $P < 0.001$) and HOMA-IR ($\beta = -0.43$, $P = 0.019$) showed significant independent associations with the plasma XOR activity (Model 3).

Table 5
Multivariate regression analysis to identify factors independently associated with the plasma XOR activity.

	Model 1		Model 2		Model 3	
	Adjusted R^2		Adjusted R^2		Adjusted R^2	
	R^2 = 0.664		R^2 = 0.642		R^2 = 0.200	
	β	*p*	*β*	*p*	*β*	*p*
Age	0.076	0.235	0.039	0.568	0.086	0.877
Gender	-0.073	0.262	-0.079	0.211	-0.155	0.104
BMI	-0.017	0.373	0.036	0.604	0.015	0.904
HbA1c	-0.055	0.622	-0.037	0.559	0.012	0.909
UA	0.034	0.807	0.1	0.123	0.099	0.342
Fasting IRI	0.024	0.743	0.096	0.165	0.775	< 0.001
HOMA-IR	-0.019	0.785	0.047	0.468	-0.43	0.019
TG	0.161	0.01	-	-	0.037	0.72
AST	0.365	0.003	0.325	0.011	-	-
ALT	0.465	< 0.001	0.509	< 0.001	-	-

Multiple regression analysis to determine factors independently associated with the plasma xanthine oxidase activity. Plasma xanthine oxidase activity was included as a dependent variable, and age, gender, body mass index, HbA1c, uric acid, fasting IRI and HOMA-IR were used as common explanatory variables.

We also included TG, AST and ALT as explanatory variables in model 1, AST and ALT in model 2, and TG in model 3.

Associations between plasma XOR activity, diabetic complications, and cardiovascular disease

In this study, no significant correlations were observed between the plasma XOR activity and parameters of diabetic nephropathy, such as the eGFR or severity of albuminuria. We also focused on the association between the plasma XOR activity and parameters of diabetic neuropathy, such as the coefficient of variation of the R-R interval (CVR-R), tibial motor nerve conduction velocity (MCV) and peroneal sensory nerve conduction velocity (SCV). While the CVR-R ($R = 0.21$, $P < 0.05$) and SCV ($R = 0.27$, $P < 0.01$) showed a weak, but significant, correlation with the plasma XOR activity (Fig. 5), the plasma XOR activity did not differ significantly among patients who showed normal, blunted or absent Achilles tendon reflex. No

significant difference in the plasma XOR activity was observed between patients with normal and abnormal vibration perception threshold either. In addition, plasma XOR activity were also similar between patients with and without diabetic retinopathy. We also compared the plasma XOR activity between patients with cardiovascular disease (CVD) and patients without CVD. Notably, we found significantly decreased plasma XOR activity in patients with CVD (33.3 pmol/h/mL (28.6–78.8)) as compared to patients without CVD (74.5 pmol/h/mL (37.8–204)).

Discussion

In the current study, we demonstrated associations among the plasma XOR activity, xanthine, and hypoxanthine levels in patients with type 2 diabetes. Previous studies have shown an association between the plasma XOR activity and the severity of cardiovascular disease [8, 14, 17].

In this study, a significant correlation was observed between the plasma XOR activity and plasma xanthine level, but not the plasma hypoxanthine level. In contrast, the plasma xanthine levels were correlated with the plasma hypoxanthine levels. Since hypoxanthine is a precursor of xanthine, this association between hypoxanthine and xanthine might be easy to conceive. Significant associations between the plasma levels of hypoxanthine and xanthine, and between the plasma xanthine and XOR activity were also reported by another study conducted in the general population [29]. The plasma XOR activity and xanthine levels, but not the plasma hypoxanthine level, were correlated with the BMI, unlike the results of a previous study [29]. Hypoxanthine is a known marker of hypoxia [32, 33] and a recent study revealed that the secretion of hypoxanthine from the adipose tissue increased in response to local hypoxia [34]. It is possible that the production of hypoxanthine in the adipose tissue and other tissues differs from that under chronic hyperglycemia conditions in type 2 diabetes.

It is well known that the liver is the main source of XOR, and that hepatic damage caused by an infection and a variety of toxic agents is associated with elevation of the serum XOR activity [35]. Furthermore, many clinical trials have shown correlation between the degree of liver dysfunction and the plasma XOR activity [13, 36]. In fact, the plasma XOR activity level was strongly correlated with the serum transaminase and γ-GTP levels in the present study. The plasma xanthine, but not plasma hypoxanthine, level was also correlated with the serum transaminase and γ-GTP levels. The plasma XOR activity also tended to show a weak correlation with the Fib4-index, a marker of hepatic fibrosis [37], although it did not reach statistical significance. However, interestingly, on the other hand, the plasma xanthine concentration showed a significant positive correlation with the Fib4-index. It is unknown whether plasma XOR activity is associated with liver fibrosis. Recently, the contribution of MiR-218-XOR-ROS pathway in the development of non-alcoholic steatohepatitis (NASH) was reported *in vitro* and in animal model [38]. These results indicate that both the plasma xanthine and plasma XOR activity are possibly associated with severity of liver fibrosis, as well as the degree of liver dysfunction.

A previous study showed the existence of a relation between the plasma XOR activity and the severity of dyslipidemia in the general population [36]. In this study also, we found a significant positive correlation

between the serum triglyceride levels and plasma XOR activity, and a negative correlation between the serum HDL-C levels and plasma XOR activity. Moreover, there were also significant correlations between the plasma xanthine and hypoxanthine and serum HDL-C levels. The plasma XOR activity was correlated with multiple parameters indicative of insulin resistance, such as the fasting IRI, fasting C-peptide, HOMA-IR, and urinary C-peptide levels during the day in the current study. Thus, the plasma XOR activity may be a marker of the severity of metabolic syndrome.

The plasma XOR activity showed no correlation with the fasting glucose level or percent HbA1c in the patients with type 2 diabetes. Previous studies in which patients with type 1 diabetes or subjects from the general population were enrolled, a positive correlation was observed between the plasma XOR activity and the percent HbA1c [15, 39]. The association between glycemic control and plasma XOR activity remains controversial. In this study, the percent glycated albumin showed a weak, but significant negative correlation with the plasma XOR activity. In a previous study of hemodialysis patients with type 2 diabetes, the percent glycated albumin showed a positive and independent association with the plasma XOR activity [16]. Our discrepant results could be explained by the fact that the patients in the aforementioned study were on maintenance hemodialysis and the backgrounds of the patients were different from those in our study: 1) the enrolled individuals in our study were hospitalized because of poor glycemic control, and 2) we excluded patients with severe renal dysfunction (eGFR < 30 mL/min/1.73 m^2) 3) hypoalbuminemia in patients with hemodialysis. Since the percent glycated albumin is a precursor of advanced glycation end products and enhances oxidative stress, it is possible that this result reflected oxidative stress, at least in part.

The results of our multiple regression analysis identified the serum levels of ALT, AST, and triglyceride as independent indicators of the plasma XOR activity in patients with type 2 diabetes. A previous study also showed associations between the serum levels of liver enzymes and the plasma XO activity level in patients with type 2 diabetes or metabolic syndrome [13]. When the serum levels of transaminases were excluded from the possible determinants, fasting IRI and HOMA-IR were found to be independent predictors of the plasma XOR activity. These findings suggest that both liver dysfunction and insulin resistance are associated with the elevation of the plasma XOR activity in patients with type 2 diabetes.

We also found a weak, but significant negative correlation between the plasma XOR activity and the duration of diabetes, indicating that the plasma XOR activity could decrease with the development of diabetes. This might be consistent with the finding that the CVR-R and sensory nerve conduction velocity, a parameter of diabetic neuropathy, showed a positive correlation with the plasma XOR activity. However, since there was no significant correlation between the plasma XOR activity and the motor nerve conduction velocity or CVR-R, and patients with impaired vibration perception threshold or blunted Achilles tendon reflex did not show decreased plasma XOR activity, further investigation is needed to elucidate the association between plasma XOR activity and diabetic neuropathy.

In the present study, no association was observed between the plasma XOR activity and the presence of diabetic nephropathy or diabetic retinopathy. Since we excluded patients with progressive nephropathy

from the study and the mean duration of diabetes was 10 years in the patients enrolled in this study, further studies would be needed to clarify these relationships.

However, notably, patients with CVD showed significantly reduced plasma XOR activity as compared to patients without CVD. Our results seem to be inconsistent with those of previous studies which showed elevated plasma XO activity in patients with coronary artery disease [8] or coronary artery spasm [40]. Multiple factors, such as chronic hyperglycemia, increased oxidative stress, and other pathogenetic factors in patients with type 2 diabetes could explain this discrepant result. No significant correlation was observed between the plasma XOR activity and the brachial-ankle pulse wave velocity (baPWV) or ankle-brachial index (ABI) (data not shown). To the best of our knowledge, there have been no previous reports about the relationship between the plasma XOR activity and diabetic vascular complications, these results give a new insight into the pathogenesis of diabetes and associated diseases. Furthermore, our findings might be of practical value if/when XOR inhibitors are used in patients with the expectation of protective effects against oxidative stress; a renoprotective effect of XOR inhibitors has already been reported in patients with CKD.

Study Limitations

There were some limitations of our current study. First, the number of patients in this study was relatively small, and the patients were all Japanese subjects. Second, most of the subjects had poor glycemic control, and further analysis is needed to determine the influence of the grade of glycemic control. Third, the patients were receiving drugs for diabetes, as shown in Table 1; the drugs, including insulin, GLP-1 receptor analogs, metformin, SGLT2 inhibitors and thiazolidinedione, could also influence the serum insulin levels, insulin resistance index, and other parameters. Finally, since this study was a clinical observational, cross-sectional study, we could not determine the causal relations of the plasma XOR activity or purine levels with the other parameters in patients with type 2 diabetes.

Conclusions

We demonstrated the significance of the plasma XOR activity and the levels of its substrates, xanthine and hypoxanthine, in patients with type 2 diabetes. Associations have been reported previously between the plasma XOR activity and liver dysfunction/ severity of insulin resistance. In this study, we revealed, for the first time, the association between the plasma xanthine level and these parameters in patients with diabetes mellitus. In addition, the plasma XOR activity was negatively correlated with the duration of diabetes, and the existence of a possible association between the plasma XOR activity and the risk of development of diabetic vascular complications was revealed. Further investigations are needed to determine whether the plasma XOR activity and plasma levels of the related purines could be useful biomarkers of the development of diabetes or its vascular complications.

Abbreviations

ACR: urinary albumin to creatinine ratio; ALT: serum alanine aminotransferase level; AST: serum aspartate aminotransferase level; BMI: body mass index; CPR: C-peptide immunoreactivity; CVD: cardiovascular disease; eGFR: estimated glomerular filtration rate; HbA1c: glycosylated hemoglobin; HDL-C: serum high-density lipoprotein cholesterol level; HOMA-IR: homeostasis model assessment of insulin resistance; IRI: immunoreactive insulin; LDL-C: serum low density lipoprotein cholesterol level; T-Chol: serum total cholesterol level; TG: serum triglyceride level; XOR: xanthine oxidoreductase; γ-GTP: serum γ-glutamyl transpeptidase level;

Declarations

Acknowledgements

We acknowledge the entire staff of the Department of Endocrinology and Metabolism, Yokohama City University, Graduate School of Medicine, Yokohama, Japan. We also thank all participants.

Authors' contributions

T.O., J.S., and Y.Te. designed the study. T.O.M.K.,Y.T.,and J.S. collected the data. T.O., T.N., and T.M analyzed the data. T.O., J.S., and Y.Te wrote the manuscript. All authors gave final approval of the version to be published.

Funding

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the institutional ethics committee at each participating hospital and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent prior to participation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1.

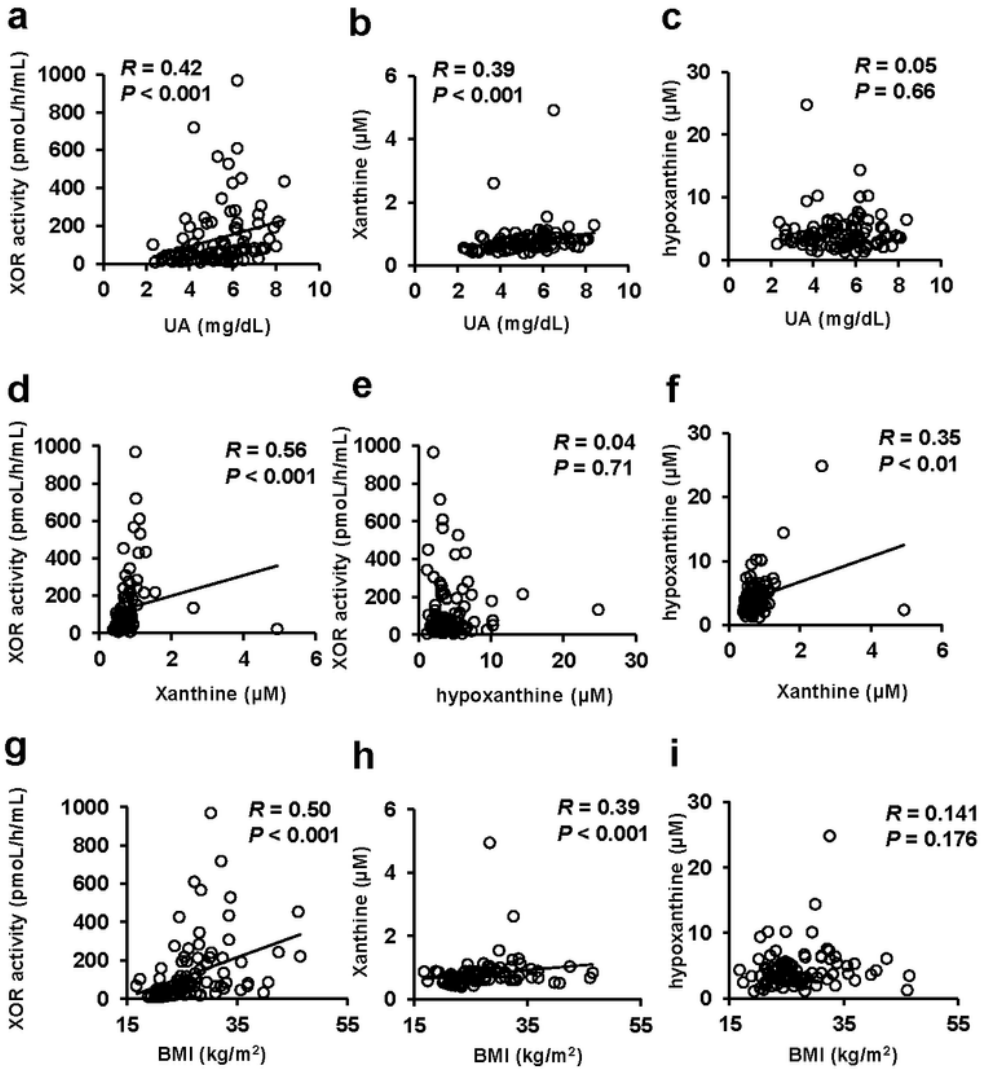

Figure 1

Correlations among the plasma XOR activity and plasma levels of xanthine and hypoxanthine. (a) Correlation between the plasma XOR activity (pmol/h/mL) and serum uric acid level. (b) Correlation between the plasma xanthine concentration (μM) and serum uric acid level (mg/dL). (c) Correlation between the plasma hypoxanthine concentration (μM) and serum uric acid level (mg/dL). (d) Correlation between the plasma XOR activity (pmol/h/mL) and plasma xanthine concentration (μM). (e) Correlation

between the plasma XOR activity (pmol/h/mL) and plasma hypoxanthine concentration (μM). (f) Correlation between the plasma xanthine concentration (μM) and plasma hypoxanthine concentration (μM). (g) Correlation between the plasma XOR activity (pmol/h/mL) and BMI (kg/m2). (h) Correlation between the plasma xanthine concentration (μM) and BMI (kg/m2). (i) Correlation between the plasma hypoxanthine concentration (μM) and BMI (kg/m2).

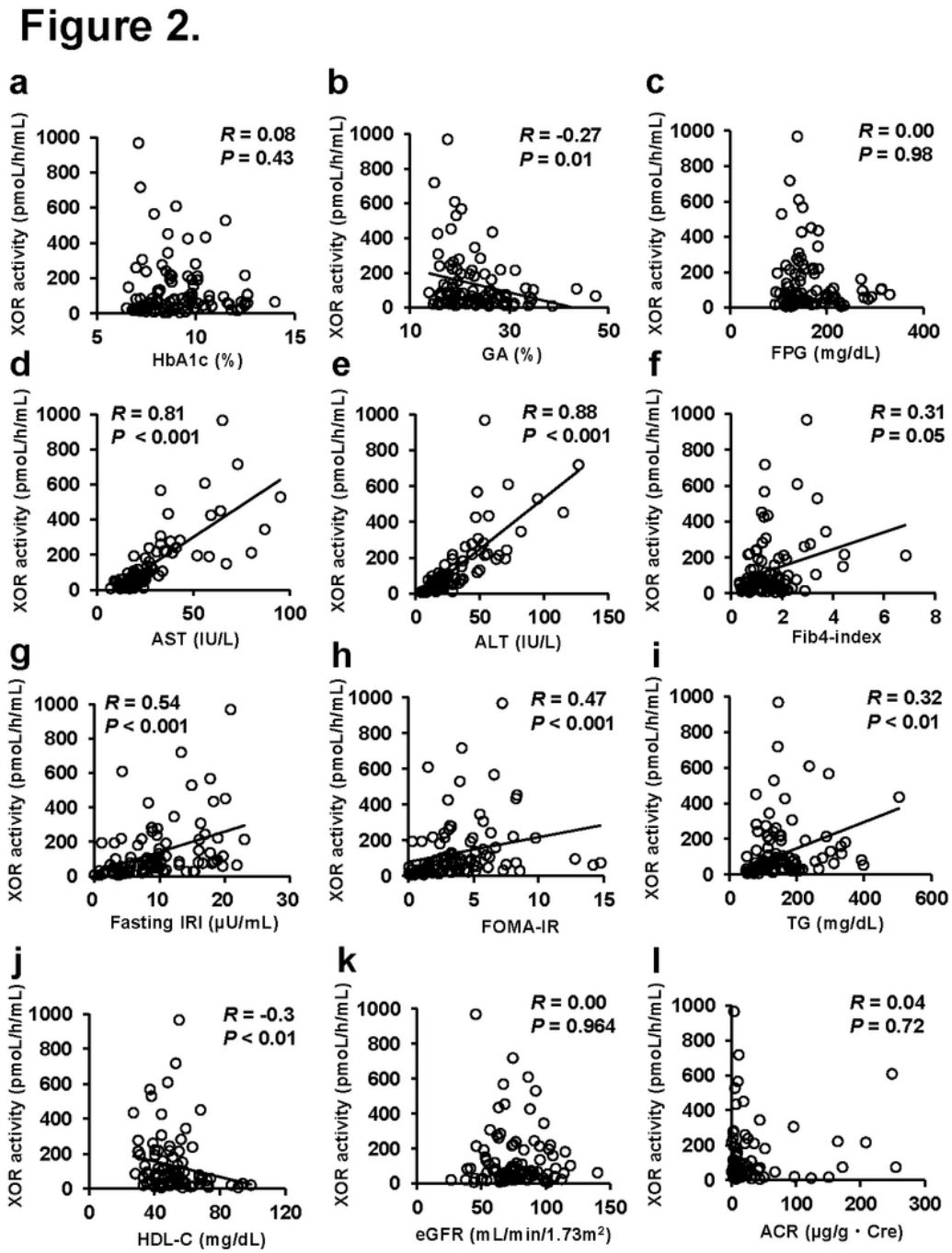

Figure 2

Correlations between the plasma XOR activity level and clinical parameters. Correlations between the plasma XOR activity (pmol/h/mL) and (a) HbA1c (%), (b) glycated albumin (%), (c) fasting plasma glucose level (mg/dL), serum levels of (d) AST (IU/L) and (e) ALT (IU/L), (f) Fib4-index, (g) fasting serum IRI (μU/mL), (h) HOMA-IR, (i) serum TG level (mg/dL), (j) HDL-C (mg/dL), (k) eGFR (ml/min/1.73m2), and urinary albumin to creatinine ratio (ACR) (mg/gCr).

Figure 3.

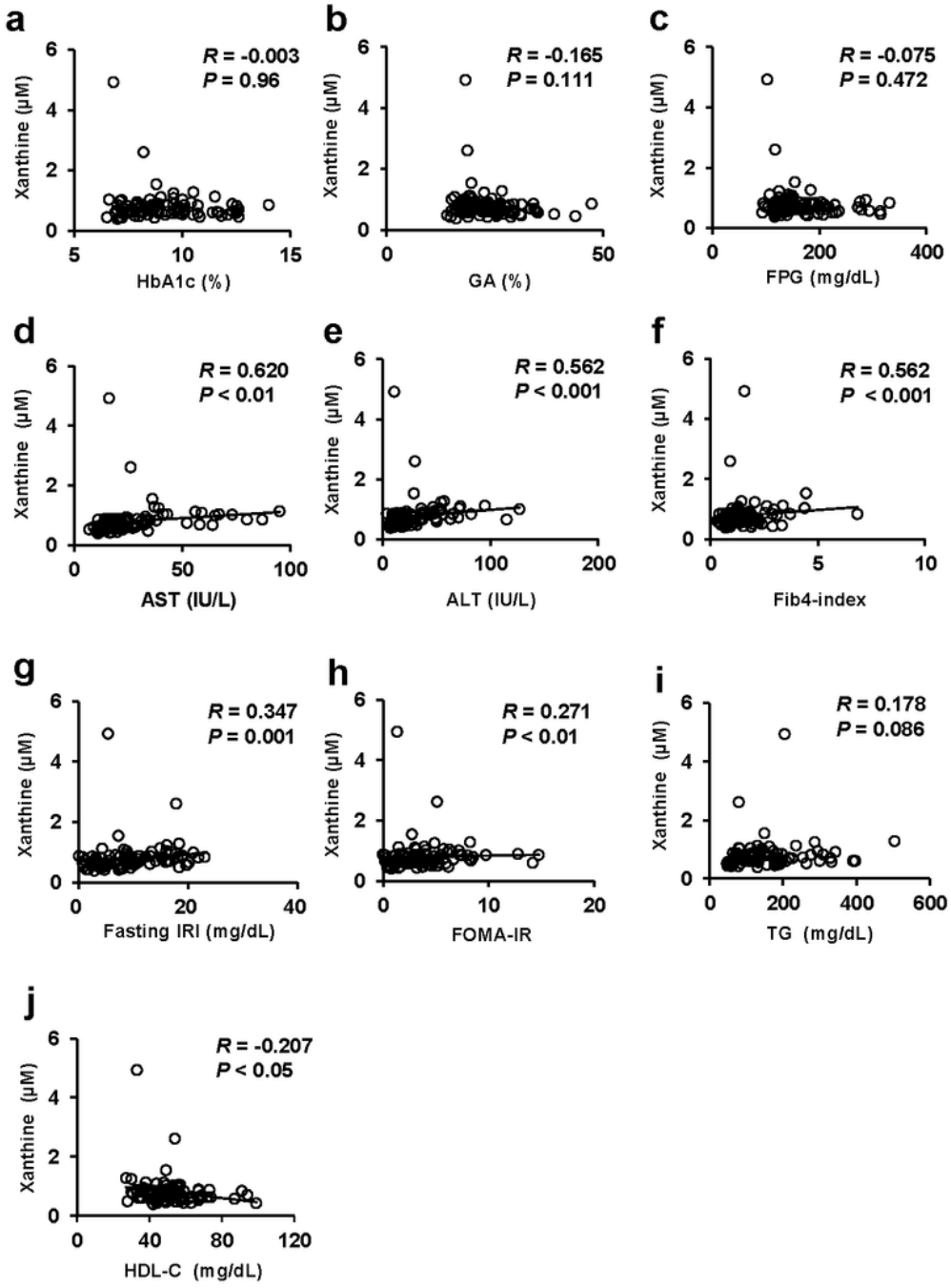

Figure 3

Correlations between the plasma xanthine level and clinical parameters. Correlations between the plasma concentration of xanthine (μM) and (a) HbA1c (%), (b) glycated albumin (%), (c) fasting plasma glucose level (mg/dL), serum levels of (d) AST (IU/L) and (e) ALT (IU/L), (f) Fib4-index, (g) fasting serum IRI (μU/mL), (h) HOMA-IR, (i) serum TG level (mg/dL), (j) HDL-C (mg/dL), (k) eGFR (ml/min/1.73m2), and urinary albumin to creatinine ratio (ACR) (mg/gCr).

Figure 4.

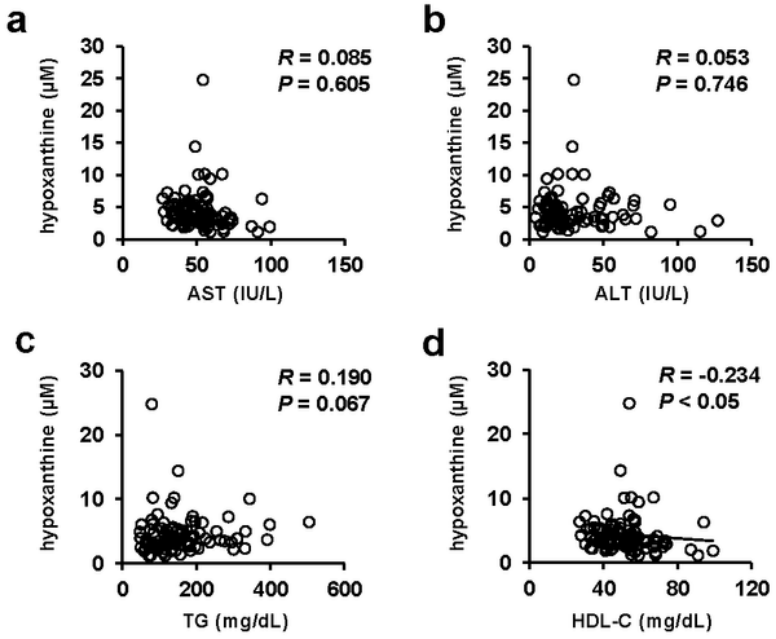

Figure 4

Correlations between the plasma hypoxanthine level and clinical parameters. Correlations between the plasma concentration of plasma xanthine (μM) and serum levels of (a) AST (IU/L) and (b) ALT (IU/L), (c) TG (mg/dL), and (d) HDL-C (mg/dL).

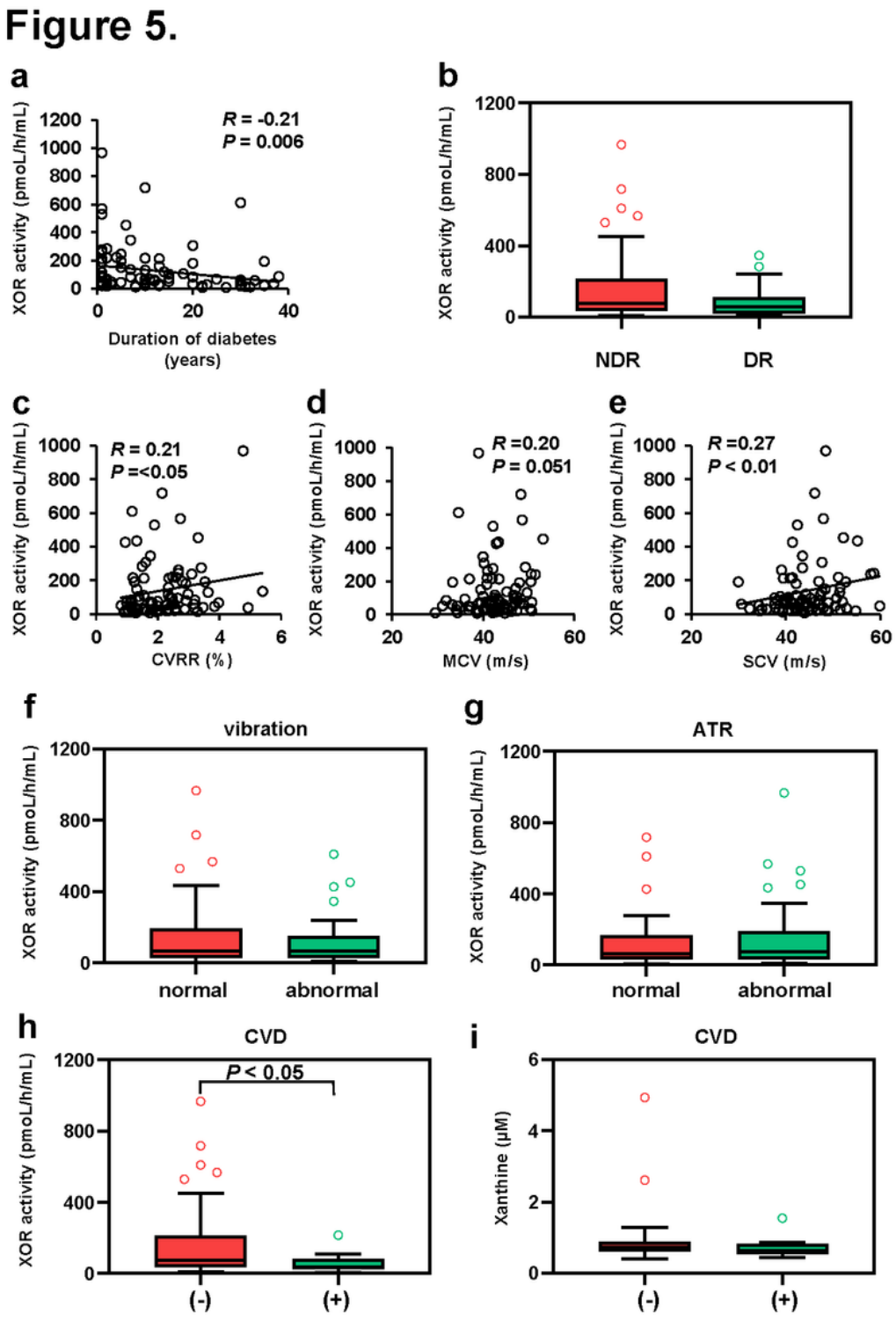

Figure 5

Correlations between plasma XOR activity and diabetic complications. (a) Correlation between plasma XOR activity (pmol/h/mL) and the duration of diabetes. (b) Plasma XOR activity (pmol/h/mL) in patients

with (DR) and without (NDR) diabetic retinopathy. (c) Correlation between plasma XOR activity (pmol/h/mL) and coefficient of variation of the R-R interval (CVR-R) (%). (d) Correlation between plasma XOR activity (pmol/h/mL) and tibial nerve motor conduction velocity (MCV) (m/s). (e) Correlation between plasma XOR activity (pmol/h/mL) and peroneal nerve sensory conduction velocity (SCV) (m/s). (f) Plasma XOR activity (pmol/h/mL) in patients with normal and abnormal vibration sense. (g) Plasma XOR activity (pmol/h/mL) in patients with normal and blunted Achilles tendon reflex. (h) Plasma XOR activity (pmol/h/mL) in patients with or without cardiovascular disease. (i) Plasma xanthine concentrations (μM) in patients with and without cardiovascular disease.

Supplementary Files

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- Additionalfile1.pdf