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Metabolism

Plasma Xanthine Oxidoreductase (XOR) Activity in Cardiovascular Disease Outpatients

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Background: The mechanisms of the increased plasma xanthine oxidoreductase (XOR) activity in outpatients with cardiovascular disease were unclear.

Methods and Results: A total of 372 outpatients were screened, and 301 outpatients with cardiovascular disease were prospectively analyzed. Blood samples were collected from patients who visited a daily cardiovascular outpatient clinic. Patients with diabetes mellitus (DM) were significantly more likely to be classified into the high-XOR group (≥100 pg/h/mL; 50%) than the low-XOR group (<100 pmol/h/mL; 28.7%). On multivariate logistic regression analysis, DM (OR, 2.683; 95% CI: 1.441–4.996) was independently associated with high plasma XOR activity in all cohorts. In the diabetic cardiovascular disease patients (n=100), median body mass index (BMI) in the high-XOR group (28.0 kg/m^2; IQR, 25.2–29.4 kg/m^2, n=32) was significantly higher than in the low-XOR group (23.6 kg/m^2; IQR, 21.2–25.7 kg/m^2, n=68), and BMI was independently associated with high plasma XOR activity (OR, 1.340; 95% CI: 1.149–1.540). Plasma hydrogen peroxide was significantly higher in DM patients with high plasma XOR activity and obesity (>22 kg/m^2) than in other patients.

Conclusions: DM with obesity is one of the mechanisms of XOR enhancement in cardiovascular disease. The increase of XOR is a possible pathway for the production of reactive oxygen species in obese cardiovascular disease patients with DM.

Key Words: Acute decompensated heart failure; Reactive oxygen species; Uric acid; XOR inhibitor

Xanthine oxidase (XO) and xanthine dehydrogenase (XDH), 2 interconvertible forms of xanthine oxidoreductase (XOR),[1,2] are the most important enzymes in the purine metabolic system. XOR activity is prominent in the liver and gut, which are associated with the mRNA level and the protein level of XOR, respectively. Therefore, the majority of plasma XOR in humans might be derived from the liver. Although XOR activity of fat tissue is increased in mammals, it is not so high in human fat tissue. XO and XDH both catalyze uric acid (UA) production, but their electron acceptors are different. XO and XDH both exist in human blood with different electron acceptors (oxygen, and NAD^+, respectively).[2] XDH converts NAD^+ to NADH, and XO produces hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) derived from oxygen. Through the production of UA in this metabolic system, a reaction catalyzed by XO, reactive oxygen species (ROS), such as H_2O_2 and O_2^-, are generated.[1] These byproducts will lead to cell damage. From this point of view, an excessive increase in XOR activity would not only lead to serum UA elevation, but would also induce increased oxidative stress.

A strategy for measuring XOR was recently established, and several reports have been published regarding the XOR levels in patients with chronic heart failure (HF)[3] and car-

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Table 1. Patient Characteristics

	Overall (n=301)	XOR activity		P-value
		<100 pmol/h/mL (n=237)	≥100 pmol/h/mL (n=64)	
General status and Vital signs				
Gender (male)	223 (74.0)	175 (73.9)	48 (75.0)	1.000
Age (years)	73 (67–78)	74 (67–80)	69 (60–74)	<0.001
SBP (mmHg)	126 (114–136)	124 (114–137)	128 (119–135)	0.361
Heart rate (beats/min)	73 (64–81)	71 (64–80)	75 (69–81)	0.215
Comorbidity				
Hypertension	200 (66.4)	158 (66.7)	42 (65.6)	0.882
Dyslipidemia	207 (68.8)	157 (66.2)	50 (78.1)	0.094
DM	100 (33.2)	68 (28.7)	32 (50.0)	0.002
Hyperuricemia	103 (34.2)	85 (35.9)	18 (28.1)	0.299
CKD	77 (25.6)	69 (29.1)	8 (12.5)	0.006
Etiology				
Heart failure	55 (18.3)	45 (19.0)	10 (15.6)	0.590
IHD	150 (49.8)	113 (47.7)	37 (57.8)	0.161
Arrhythmia	52 (17.3)	42 (17.7)	10 (15.6)	0.852
Vascular disease	19 (6.3)	16 (6.8)	3 (4.7)	0.773
PE	3 (1.0)	2 (0.8)	1 (1.6)	0.513
Valvular disease	11 (3.7)	10 (4.2)	1 (1.6)	0.468
CSA	8 (2.7)	7 (3.0)	1 (1.6)	1.000
Cardiomyopathy	3 (1.0)	2 (0.8)	1 (1.6)	0.513
Laboratory data				
BUN (mg/dL)	16.9 (14.2–20.5)	17.2 (14.3–21.1)	15.4 (13.8–19.0)	0.017
Creatinine (mg/dL)	0.91 (0.77–1.10)	0.94 (0.79–1.12)	0.87 (0.76–1.01)	0.027
eGFR (mL/min/1.73 m^2)	59.0 (49.0–70.4)	58.3 (46.7–69.3)	65.3 (54.8–77.9)	0.001
Total bilirubin (mg/dL)	0.7 (0.5–0.9)	0.7 (0.5–0.9)	0.7 (0.5–0.9)	0.740
Uric acid (mg/dL)	5.7 (4.8–6.6)	5.7 (4.8–6.5)	5.8 (4.9–6.7)	0.428
Hemoglobin (mg/dL)	13.7 (12.4–14.9)	13.6 (12.3–14.8)	14.1 (13.2–15.1)	0.014
CRP (mg/dL)	0.10 (0.05–0.21)	0.10 (0.05–0.20)	0.10 (0.05–0.31)	0.554
BNP (pg/mL)	52 (23–115)	62 (28–117)	33 (15–105)	0.018
XOR activity (pmol/h/mL)	40.0 (18.3–83.5)	29.8 (15.3–51.7)	165.5 (122.0–257.3)	<0.001
Medication				
XOR inhibitors	74 (24.6)	64 (27.0)	10 (15.6)	0.072
Febuxostat	62 (20.6)	52 (21.9)	10 (15.6)	
Allopurinol	9 (3.0)	9 (3.8)	0 (0.0)	
Topiroxostat	2 (0.7)	2 (0.8)	0 (0.0)	

Data given as n (%) or median (IQR). BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CKD, chronic kidney disease; CRP, C-reactive protein; CSA, coronary spastic angina; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; IHD, ischemic heart disease; PE, pulmonary thromboembolism; SBP, systolic blood pressure; XOR, xanthine oxidoreductase.

diac/renal disease,[4,5] and in normal volunteers and general populations.[6,7] Insulin resistance, subclinical inflammation, and metabolic syndrome are independently associated with high XOR activity in young normal humans and general populations, but this has not been examined in outpatients with cardiovascular disease. Thus, we hypothesized that increased XOR activity would occur in outpatients with cardiovascular disease with severe cardiac disease or cardiac risk factors (i.e., hypertension, diabetes mellitus [DM], dyslipidemia, hyperuricemia), and that it would lead to increased ROS. In the present study, we therefore investigated the factors independently associated with extremely high XOR activity in outpatients with cardiovascular disease.

Methods

Subjects

A total of 372 patients who attended the cardiovascular outpatient clinic of Nippon Medical School Chiba Hokusoh Hospital, Hasegawa Hospital, and Toho Kamagaya Hospital between December 2016 and November 2018 were enrolled. Outpatients included patients with cardiovascular disease (i.e., ischemic heart disease, compensated HF, arrhythmia, vascular disease, a history of pulmonary artery embolism [PE], valvular disease, coronary spasm angina [CSA] and cardiomyopathy) and patients without pre-existing cardiovascular conditions (i.e., only hypertension, dyslipidemia, or DM). Seventy-one patients without pre-existing cardiovascular disease were excluded from the present study. Finally, 301 outpatients with cardiovascular disease were

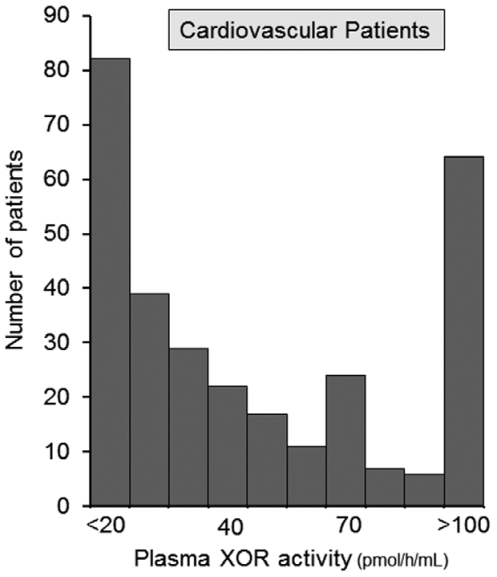

Figure 1. Distribution of plasma xanthine oxidoreductase (XOR) activity. Plasma XOR activity was <20 pmol/h/mL in 81 patients (26.9%) and ≥100 pmol/h/mL in 64 (21.3%).

prospectively analyzed.

XOR Measurement and Comparisons

Blood samples were collected during the daily outpatient clinic appointment. The blood samples were centrifuged at 4°C, ≤5 min after collection, and were immediately frozen at −80°C; they were stored at this temperature until use. Plasma XOR activity assay was performed at Sanwa Kagaku Kenkyusho, Japan.

To remove small molecules, including hypoxanthine, xanthine, and UA, 100 μL of each plasma sample was purified using a Sephadex G25 column. The eluate was then mixed with 16 μmol/L [$^{13}C_2$, $^{15}N_2$]-xanthine as the substrate and 16 μmol/L NAD^+ and 1 μmol/L [$^{13}C_2$, $^{15}N_2$]-UA as the internal standard in Tris buffer (pH 8.5). Each of the mixtures, the total volume of which was 250 μL for each, was incubated for 90 min at 37°C. Subsequently, the mixtures were mixed with 500 μL 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 liquid chromatography-triple quadrupole mass spectrometry (LC/TQMS) using a Nano Space SI-2 LC system (Shiseido, Tokyo, Japan) and a TSQ-Quantum Discovery MAX Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an electrospray ionization interface. The amount of [$^{13}C_2$, $^{15}N_2$]-UA produced was quantified using the calibration curve, with the XOR activity expressed as [$^{13}C_2$, $^{15}N_2$]-UA in pmol/h/mL plasma. The lower and upper limits for the detection of XOR activity were 6.67 pmol/h/mL and 6.670 pmol/h/mL, respectively. The inter-detection assay coefficients of variation of pooled human plasma activity were 6.5% and 9.1%, respectively.[8] The XOR activity was reported with the addition of NAD^+; thus, it was impossible to measure the actual XO activity. The standard units for reporting XO and XOR are U/mL plasma (1U=1 μmole UA formed/min) and pmol/h/mL of plasma (600 pmol/h/mL plasma, which equals 10 μU/mL plasma), respectively.

Outpatients were divided into the high-XOR group (≥100 pmol/h/mL, n=64) and the low-XOR group (<100 pmol/h/mL, n=237). Because the normal range of XOR activity has not been established as yet, the present cut-off (100 pmol/h/mL) was defined using the mean determined in normal volunteers by Murase et al (89.1± 55.1 pmol/h/mL).[8] We compared patient characteristics (gender, age), vital signs (systolic blood pressure [SBP], heart rate [HR]), risk factors for atherosclerosis and comorbidities (DM, hypertension, dyslipidemia, hyperuricemia, and chronic kidney disease [CKD]), laboratory data (blood urea nitrogen,[9] creatinine, total bilirubin, UA, hemoglobin [Hb], brain natriuretic peptide [BNP], and C-reactive protein [CRP]), medication (XOR inhibitor), and etiology (HF [chronic or compensated], ischemic heart disease [after percutaneous coronary intervention or coronary artery bypass graft due to acute coronary syndrome, ischemic cardiomyopathy, stable angina pectoris and silent myocardial ischemia], arrhythmia [chronic or paroxysmal arterial fibrillation, history of ventricular arrhythmia, after pacemaker implantation due to bradycardia arrhythmia], vascular disease [aortic dissection or aortic aneurysm or peripheral arterial disease], PE, valvular disease, CSA and cardiomyopathy [dilated cardiomyopathy or hypertrophic cardiomyopathy]). Some patients had multiple etiologies. In such cases, we selected the most important and serious disease for the statistical analysis. Multivariate logistic regression analysis was performed to identify the factors significantly associated with increased XOR activity.

We also compared the following factors in a subgroup analysis of patients with DM: body mass index (BMI), laboratory data (blood glucose [BG], Hb A1c [HbA1c], total cholesterol, triglyceride, low-density lipoprotein cholesterol [LDL-C] and high-density lipoprotein cholesterol [HDL-C]), and medication use (diabetic and cardiovascular medica-

Table 2. Factors Associated With XOR ≥100 pmol/h/min

Influencing factor	Univariate			Multivariate		
	OR	95% CI	P-value	OR	95% CI	P-value
Age (per 1.0-year increase)	0.959	0.936–0.982	0.001	0.971	0.945–0.997	0.031
DM	2.485	1.412–4.373	0.002	2.683	1.441–4.996	0.002
CKD	0.348	0.158–0.680	0.009	0.360	0.156–0.831	0.017
BUN (per 0.1-mg/dL increase)	0.945	0.898–0.994	0.029			
Hemoglobin (per 1.0-mg/dL increase)	1.203	1.030–1.404	0.020			
BNP (per 1.0-pg/mL increase)	0.999	0.996–1.001	0.355			

Abbreviations as in Table 1.

Table 3. DM Patient Characteristics

	XOR activity		P-value
	<100 pmol/h/mL (n=68)	≥100 pmol/h/mL (n=32)	
General status and vital signs			
Gender (male)	57 (83.8)	28 (87.5)	0.769
Age (years)	73 (67–78)	67 (60–73)	0.002
SBP (mmHg)	128 (116–139)	129 (123–137)	0.673
Heart rate (beats/min)	72 (61–80)	76 (70–80)	0.225
Comorbidities			
Hypertension	52 (76.4)	23 (71.9)	0.628
Dyslipidemia	54 (79.4)	25 (79.1)	1.000
Hyperuricemia	27 (39.7)	10 (31.3)	0.507
CKD	27 (39.7)	5 (15.6)	0.021
Diagnosis			
Heart failure	18 (26.4)	4 (12.5)	0.130
IHD	36 (52.9)	20 (62.5)	0.396
Arrhythmia	10 (14.7)	5 (15.6)	1.000
Vascular disease	3 (4.4)	1 (3.1)	1.000
PE	0 (0.0)	0 (0.0)	–
Valvular disease	0 (0.0)	0 (0.0)	–
CSA	1 (1.5)	1 (3.1)	0.540
Cardiomyopathy	0 (0.0)	1 (3.1)	0.320
Laboratory data			
BUN (mg/dL)	18.3 (15.7–22.7)	15.8 (14.0–18.5)	0.008
Creatinine (mg/dL)	1.00 (0.83–1.26)	0.87 (0.74–1.05)	0.005
eGFR (mL/min/1.73 m²)	52.5 (41.1–66.7)	68.3 (55.2–79.0)	<0.001
Total bilirubin (mg/dL)	0.7 (0.5–0.9)	0.7 (0.5–0.9)	0.865
Uric acid (mg/dL)	5.8 (4.8–6.5)	5.7 (4.9–6.3)	0.946
Hemoglobin (mg/dL)	14.0 (12.6–15.0)	14.6 (13.3–15.2)	0.137
CRP (mg/dL)	0.10 (0.05–0.20)	0.11 (0.05–0.39)	0.388
BNP (pg/mL)	66 (21–98)	23 (14–86)	0.043
XOR activity (pmol/h/mL)	34.6 (15.0–57.8)	180.0 (155.0–292.5)	<0.001
Medication			
XOR inhibitors	20 (29.4)	5 (15.6)	0.215
Febuxostat	16 (23.5)	5 (15.6)	
Allopurinol	3 (4.4)	0 (0.0)	
Topiroxostat	1 (1.5)	0 (0.0)	

Data given as n (%) or median (IQR). Abbreviations as in Table 1.

tions). Finally, to evaluate ROS production, we further measured hydrogen peroxide in patients with DM.

Hydrogen peroxide in plasma (blood) was measured on fluorescence intensity (Ex, 560 nm; Em, 590 nm) using amplex red reagent.

Statistical Analysis

All data were statistically analyzed using SPSS 22.0 J (SPSS Japan Institute, Tokyo, Japan). All numerical data are expressed as median (IQR), depending on normality. Normality was assessed using the Shapiro-Wilk W-test. The Mann-Whitney U-test was used for comparisons between 2 groups (high-XOR group vs. low-XOR group). Comparisons of proportions were performed using chi-squared test. $P<0.05$ was considered to indicate statistical significance.

All clinically relevant factors affecting increased XOR activity, including age (per 1.0-year increase), DM, CKD, serum BUN (per 0.1-mg/dL increase), Hb level (per 1.0-mg/dL increase), and serum BNP level (per 1.0-pg/mL increase) were selected for inclusion in the multivariate logistic regression analysis of the whole study population. In the subgroup analysis of patients with DM, BMI (per 1.0-kg/m² increase), HbA1c (per 1.0% increase), triglyceride (per 10-mg/dL increase) and biguanides use were selected for inclusion in the multivariate logistic regression model. Multivariate logistic regression analysis was performed using backward stepwise selection.

Ethical Considerations

The Research Ethics Committee of Nippon Medical School Chiba Hokusoh Hospital, the responsible committee on ethical standards in human experimentation, approved the study protocol. The procedures were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all of the participants before commencing the study.

Table 4. Patient Characteristics Associated With DM

	XOR activity		P-value
	<100 pmol/h/mL (n=68)	≥100 pmol/h/mL (n=32)	
General status			
BMI (kg/m^2)	23.6 (21.2–25.7)	28.0 (25.2–29.4)	<0.001
Laboratory data			
BG (mg/dL)	142 (115–179)	138 (122–179)	0.535
HbA1c (%)	6.7 (6.4–7.1)	7.0 (6.7–7.3)	0.040
Total cholesterol (mg/dL)	157 (143–179)	158 (149–181)	0.668
Triglyceride (mg/dL)	122 (89–177)	161 (122–217)	0.031
LDL-C (mg/dL)	83 (66–99)	86 (77–102)	0.231
HDL-C (mg/dL)	46 (37–59)	45 (38–54)	0.773
Diabetic medication			
Insulin	10 (14.7)	3 (9.4)	0.541
Sulfonylureas	6 (8.8)	3 (9.4)	1.000
Biguanides	21 (30.9)	17 (53.1)	0.046
α-Glucosidase inhibitor	15 (22.1)	6 (18.8)	0.797
Thiazolidinediones	3 (4.4)	0 (0.0)	0.549
Glinides	12 (17.6)	4 (12.5)	0.575
DPP-4 inhibitor	51 (75.0)	25 (78.1)	0.806
SGLT-2 inhibitor	6 (8.8)	3 (9.4)	1.000
Cardiovascular medication			
Diuretics	34 (50.0)	9 (28.1)	0.052
ACEI/ARB	45 (66.2)	27 (84.4)	0.093
β-blocker	49 (72.1)	20 (62.5)	0.361
Spironolactone	8 (11.8)	6 (18.8)	0.367
Statin	53 (77.9)	28 (87.5)	0.291

Data given as n (%) or median (IQR). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BG, blood glucose; BMI, body mass index; DPP-4, dipeptidyl peptidase-4; DM, diabetes mellitus; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SGLT-2, sodium glucose cotransporter 2; XOR, xanthine oxidoreductase.

Table 5. Factors Associated With XOR ≥100 pmol/h/min in DM Patients

Influencing factor	Univariate			Multivariate		
	OR	95% CI	P-value	OR	95% CI	P-value
BMI (per 1.0-kg/m^2 increase)	1.328	1.152–1.531	<0.001	1.330	1.149–1.540	<0.001
HbA1c (per 1.0% increase)	0.974	0.872–1.088	0.638			
Triglyceride (per 10-mg/dL increase)	1.008	0.978–1.040	0.593			
Biguanides	2.537	1.069–6.019	0.035	2.360	0.882–6.311	0.087

Abbreviations as in Table 4.

Results

Patient Characteristics

The outpatient cohort consisted of 223 male patients (74.0%) and 78 female patients (26.0%; median age, 73 years). A total of 200 patients (66.4%) had hypertension, 207 (68.8%) had dyslipidemia, 100 (33.2%) had DM, 103 (34.2%) had hyperuricemia and 77 (25.6%) had CKD. The main outpatient diseases were as follows: HF (n=55; 18.3%), ischemic heart disease (n=150, 49.8%), arrhythmia (n=52, 17.3%) and others (n=44; 14.6%; vascular disease, PE, valvular disease, CSA and cardiomyopathy; **Table 1**).

Plasma XOR Activity

The distribution of XOR activity in patients with cardiovascular disease is shown in **Figure 1**. Plasma XOR activity was <20 pmol/h/mL in 81 patients (26.9%) and ≥100 pmol/h/mL in 64 (21.3%).

In the high-XOR group, the patients were significantly younger (P<0.001), the incidence of DM was significantly higher (P=0.002) and the incidence of CKD was significantly lower (P=0.006) compared with the low-XOR group. Furthermore, in the high-XOR group serum BUN, creatinine and BNP were significantly lower (P=0.017, P=0.027 and P=0.018, respectively), while serum Hb was significantly higher (P=0.014) compared with the low-XOR group (**Table 1**).

On multivariate logistic regression modeling, age (OR, 0.971; 95% CI: 0.945–0.997; P=0.031), DM (OR, 2.683; 95% CI: 1.441–4.996; P=0.002) and CKD (OR, 0.360; 95% CI: 0.156-0.831, P=0.017) were independently associated with high plasma XOR activity (≥100 pmol/h/mL) in

outpatients with cardiovascular disease (**Table 2**). This suggests that DM was positively correlated with extremely high XOR activity. We therefore performed a subgroup analysis of DM outpatients.

As noted in **Table 3**, the results of the diabetic cohort were similar to those of the overall patient cohort. In the high-XOR group, the patients were significantly younger (P=0.002) and the incidence of CKD was significantly lower (P=0.021) compared with the low-XOR group. Furthermore, serum BUN, creatinine and BNP were significantly lower (P=0.008, P=0.005 and P=0.043, respectively) compared with the low-XOR group (**Table 3**). **Table 4** lists the characteristics associated with DM. In the high-XOR group, BMI and serum HbA1c and triglycerides were significantly higher (P<0.001, P=0.040 and P=0.031, respectively) than in the low-XOR group. Furthermore, biguanides were given significantly more frequently (P=0.046) in the high-XOR group (**Table 4**). On multivariate logistic regression modeling, only BMI (OR, 1.339; 95% CI: 1.149–1.540; P<0.001).was independently associated with high plasma XOR activity (≥100 pg/h/mL) in outpatients with cardiovascular disease and DM (**Table 5**). We defined obesity as BMI >22 kg/m^2.[10] Indeed, hydrogen peroxide in plasma was significantly higher in DM patients with high plasma XOR activity (≥100 pg/h/mL) and obesity (BMI >22 kg/m^2; n=70) than in other patients (n=30; median, 2,967 vs. 2,725 relative fluorescence unit (RFU); **Figure 2**).

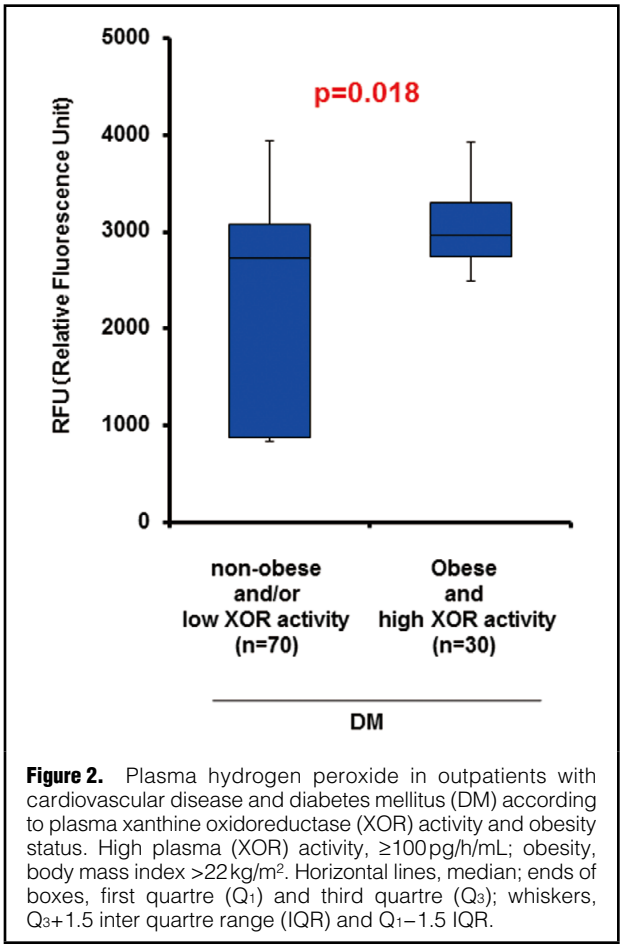

Figure 2. Plasma hydrogen peroxide in outpatients with cardiovascular disease and diabetes mellitus (DM) according to plasma xanthine oxidoreductase (XOR) activity and obesity status. High plasma (XOR) activity, ≥100 pg/h/mL; obesity, body mass index >22 kg/m^2. Horizontal lines, median; ends of boxes, first quartre (Q_1) and third quartre (Q_3); whiskers, Q_3+1.5 inter quartre range (IQR) and Q_1−1.5 IQR.

Discussion

In present study, DM had the strongest association with extremely high XOR activity in outpatients with cardiovascular disease. In other words, patients with diabetic cardiovascular disease would have extremely increased XOR activity. ROS are generated through the production of UA, which is catalyzed by XO.[1] Although the protein kinase C (PKC) pathway, polyol pathway and advanced glycation end products (AGE) pathway in hyperglycemic status[11–13] have traditionally been discussed as mechanisms of increased ROS production, this XOR-associated mechanism might be another pathway via which ROS are produced in diabetic cardiovascular disease patients. Furthermore, obesity was identified as a factor associated with increased XOR activity in diabetic cardiovascular disease patients. Based on this finding, we discuss the mechanisms underlying the increased XOR activity in outpatients with cardiovascular disease with DM (**Figure 3**).

Mechanisms of Increased XOR Activity in DM Patients

An association between type 1 or type 2 DM (especially hyperglycemia) and XOR activity has been suggested in several reports. Plasma XO in experimental type 1 DM mice was reported to be increased 3-fold at 2 weeks after the onset of DM,[14] and also XOR activity has been reported to be increased in type 2 DM mice.[15] In Asian type 2 DM patients, including Indian, Chinese and Malaysian patients, serum XO activity was higher than in age-matched healthy control subjects.[16] And in a type 1 DM experimental model, the level of XO and the XO/XDH ratio were increased in plasma and liver tissue.[17] Insulin resistance, HbA1c and fasting glucose were also noted as factors that were independently associated with high XOR in the general population.[7]

A relationship between glycemic control and XOR activity was also reported in patients with DM. A strong positive association between HbA1c and XO was also identified in type 2 DM patients.[16] Thus, poorer glycemic control would increase the activation of XO. This was also reported in hemodialysis patients.[4] The clinical implications of plasma XO activity in Japanese patients with type 2 DM have recently been reported, and plasma XO activity was found to be correlated with indices of insulin resistance and liver dysfunction.[18] Obesity is strongly associated with insulin resistance, therefore the present results might support this conclusion. It has also been suggested that fatty liver is a significant predictor of higher plasma XOR activity.[19] Obesity is also sometimes associated with fatty liver. Although we had no liver echo data in present study, high BMI was independently associated with increased XOR activity, and this might reflect the existence of fatty liver in the present subjects.

As noted here, there is already a great deal of knowledge regarding XOR in patients with DM. There are some reasonable hypotheses as to why DM was associated with XOR activity in cardiovascular disease patients with DM. First, hyperglycemia itself is known to activate endothelial XO. Kuppusamy et al indicated that blood XO is activated under high glucose concentrations.[16] Second, an association with liver damage has been suggested. Some patients with DM were reported to have been exposed to liver damage. Sunagawa et al reported that the plasma XO activity in patients with type 2 DM was correlated with liver trans-

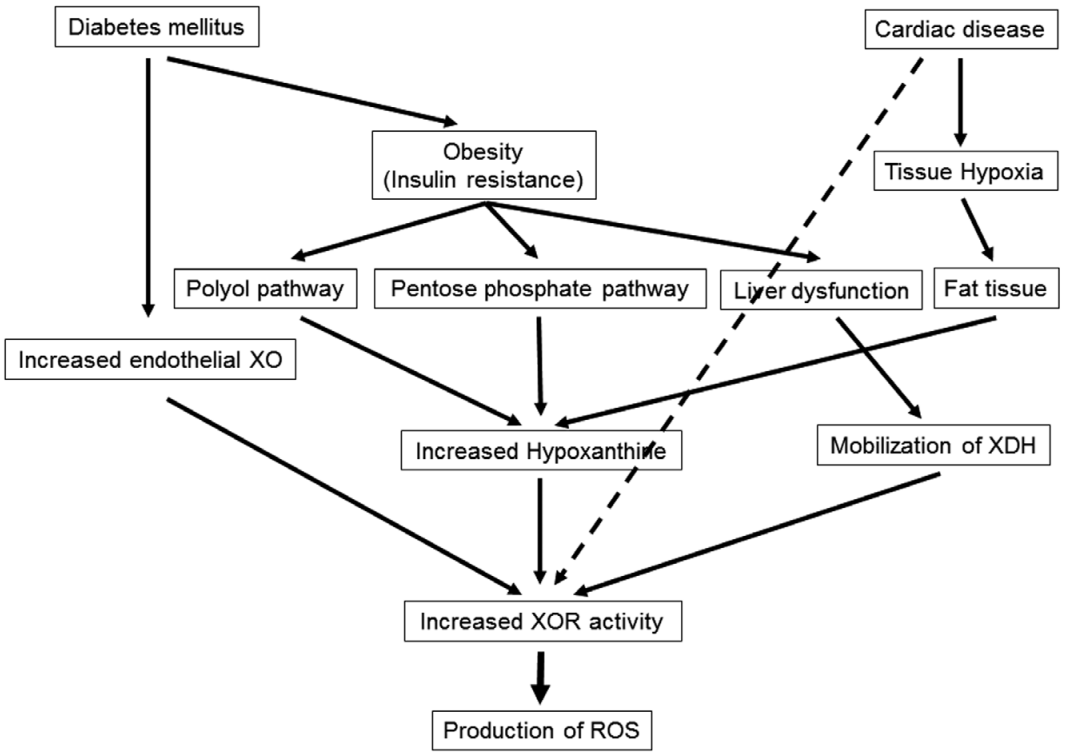

Figure 3. Schematic diagram of the mechanisms of increased xanthine oxidoreductase (XOR) activity and reactive oxygen species (ROS) production in diabetic cardiovascular patients with obesity. XDH, xanthine dehydrogenase; XO, xanthine oxidase.

aminase (aspartate aminotransferase, alanine aminotransferase) activity.[18] Leakage of XDH into the circulation and subsequent conversion of XDH to XO might be exacerbated by liver damage. Third, the glycolytic pathway is disordered and the polyol pathway is accelerated in a hyperglycemic state.[11] Fructose is increased by the polyol pathway; as a result, a large amount of adenosine triphosphate (ATP) is consumed. When the level of ATP is insufficient, the disease state cannot be maintained and stored ATP is broken down. Hypoxanthine is also produced after the increase of adenosine diphosphate (ADP), adenylic acid and inosine acid, with the latter accelerating this purine metabolism pathway and activating XOR. Finally, fat synthesis is accelerated in the liver of obese diabetic patients. As a result, nicotinamide-adenine dinucleotide phosphate (NADPH) is consumed, and the pentose phosphate pathway is accelerated to compensate for the loss of NADPH. Hypoxanthine is also produced by the acceleration of this pathway, with the latter accelerating this purine metabolism pathway, activating XOR. Further studies are therefore required to investigate the association between XOR activity and DM.

Cardiovascular disease itself was also indicated as an activator of XOR. We have already reported extremely high plasma XOR activity in patients with acute HF (AHF).[20] Lactate is associated with extremely high XOR. Tissue hypoxia may be involved in the mobilization of XDH into the circulation and the subsequent conversion of XDH to XO in AHF. Some recent reports have discussed the association between high XOR activity and cardiac disease (i.e., left ventricular hypertrophy, low left ventricular ejection fraction, increased BNP, compensated HF, CAS and ischemic heart disease).[3,5,21–23] Although we could not show a direct relationship between cardiac disease and XOR activity in the present study, cardiovascular disease itself would increase XOR activity. Furthermore, according to a recent study, human fat tissues are a potential source of hypoxanthine under hypoxic conditions.[24] Hypoxic conditions induced by cardiovascular disease might be another pathway for increasing XOR activity.

In addition, the relationship between XOR activity and endothelial function in patients with DM has been investigated recently.[25] Endothelial dysfunction is well established as a response to cardiovascular risk factors and precedes the development of atherosclerosis. The role of XOR activity in the modulation of endothelial and vascular function should be discussed as a mechanism of increased XOR activity in diabetic outpatients with cardiovascular disease.

Inactivation of XOR as a Treatment Strategy

In the present study, we identified the factors that were independently associated with extremely high XOR activity in outpatients with cardiovascular disease. Obesity with DM in cardiovascular disease patients was strongly associated with extremely high XOR. Diabetic cardiovascular disease patients might produce ROS as a result of increased XOR activity; thus, the present study supports the possibility that XOR inhibitor treatment (i.e., allopurinol, febuxostat and topiroxostat) to reduce ROS, as was recently reported in an experimental model and clinical study,[17,26–29] may

lead to better outcomes in outpatients with cardiovascular disease and DM. Furthermore, as weight loss leads to reduced XO activity,[30] it might induce the reduction of ROS in these patients and lead to better outcomes with regard to the cardiovascular disease and DM.

Study Limitations

This study had several limitations. First, because it was a single-center study, some patient-related biases might have been included. Second, the study cohort included patients who were treated with XOR inhibitors at the time of sampling. Sephadex G25 was used to remove small molecules such as xanthine and hypoxanthine, which are competitive inhibitors of stable isotope-labeled [$^{13}C_2$, $^{15}N_2$] xanthine in the XOR activity assay, and to remove the interfering drug molecules from plasma samples. The subjects, however, included patients who had been treated with medication that decreased UA, including allopurinol (n=9), febuxostat (n=62), and topiroxostat (n=2). If any of these drugs remained in the samples, the XOR activity may have been underestimated. Furthermore, the time after XOR inhibitor treatment is an important additional consideration. The percentage of these drugs that remain after exclusion with Sephadex G25 has not been reported. Further studies are required to investigate this issue. Third, the reasons for the association between XOR activity and young age, low BNP and low CKD are not clear, and some of the findings are controversial. Further study with a large population is needed to explain these findings. Fourth, the patients enrolled in the present study were heterogeneous. Several patients had various coexisting cardiovascular diseases. Outpatients with cardiovascular disease have multiple comorbidities and it is very difficult to identify a single etiology in such cases. Furthermore, because the patients were not all enrolled consecutively, patient selection might have been biased. Finally, most of the cited studies have been conducted in the Asian population. We have few reports from Western countries regarding XOR activity. This may be due to a difference in the view of hyperuricemia between Western and Asian countries. Ideally, global citations involving many ethnicities would be required. This issue might be one of the limitations of the present study.

Conclusions

DM was positively associated with high plasma XOR activity in outpatients with cardiovascular disease. High BMI was also associated with high plasma XOR activity in outpatients with cardiovascular disease and DM. The activation of XOR by obesity is a possible pathway for the production of ROS in diabetic cardiovascular disease patients.

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Disclosures

The authors declare no conflicts of interest.

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