Xanthine Oxidoreductase Activity Is Correlated With Hepatic Steatosis

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Abstract

The enzyme xanthine oxidoreductase (XOR) catalyzes the formation of uric acid (UA) from hypoxanthine and xanthine, which in turn are products of purine metabolism starting from ribose-5-phosphate. Several studies suggested a relationship between hyperuricemia and hepatic steatosis; however, few previous studies have directly examined the relationship between XOR and hepatic steatosis. A total of 223 subjects with one or more cardiovascular risk factors were enrolled. Hepatic steatosis was calculated according to the liver-to-spleen (L/S) ratio on computed tomography and the hepatic steatosis index (HSI). We measured a plasma XOR activity assay using a newly established highly sensitive assay based on [$^{13}C_2$, $^{15}N_2$] xanthine and liquid chromatography/triple quadrupole mass spectrometry. XOR activity and the UA level were increased in subjects with L/S ratio <1.1 and HSI <36. Multivariate logistic regression analysis indicated that plasma XOR activity was associated with the risk of hepatic steatosis as assessed by the L/S ratio and HSI independently of insulin resistance, whereas UA levels were not associated with risk of hepatic steatosis. The results of this study indicated that plasma XOR activity is associated with hepatic steatosis independently of insulin resistance and serum UA levels.

Background

Nonalcoholic fatty liver disease (NAFLD) is defined as the presence of fatty accumulation in the liver on imaging or histology, excluding fatty liver secondary to alcohol, drugs, or genetic disorders [1, 2]. NAFLD is a major cause of liver disease, and its prevalence is reported to be increasing [1–3]. NAFLD has been reported to be a risk factor for not only hepatic diseases-related mortality but also cardiovascular disease [4–7].

Imaging techniques such as abdominal ultrasonography, computed tomography (CT), and magnetic resonance imaging are useful in the evaluation of hepatic steatosis. Abdominal ultrasound is the recommended screening test for NAFLD, but CT has also been used to evaluate hepatic steatosis. The CT value of the liver decreases with the degree of fat deposition, and by measuring the ratio of the CT values in the liver and to those in the spleen [the liver-to-spleen (L/S) ratio], it is possible to calculate the fat content of the liver [8–12]. In addition to imaging tests, the hepatic steatosis index (HSI) has been reported to be useful as screening indices for hepatic steatosis [13].

Metabolic syndrome and type 2 diabetes mellitus, which are associated with insulin resistance, are known to be risk factors for the development of NAFLD [14–16]. In addition, hyperuricemia has been reported to be a risk factor for metabolic syndrome and NAFLD [17, 18]. Xanthine oxidoreductase (XOR) is an enzyme regulating synthesis of uric acid (UA) and generation of reactive oxygen species [19, 20]. XOR activity is associated with insulin resistance [21], and it has been reported that XOR activity is elevated in metabolic syndrome and type 2 diabetes [22, 23]. In addition, previous studies demonstrated that XOR activity was significantly increased in a mouse model of NAFLD and that fatty liver induced by a high-fat diet was suppressed by administration of XOR inhibitors [24, 25].

It is assumed that XOR activity is associated with insulin resistance and hepatic steatosis in humans. However, in humans, XOR activity is extremely low compared with that in rodents; this makes accurate measurement difficult [26–28]. A novel human plasma XOR activity assay has been established using a combination of liquid chromatography (LC) and triple quadrupole mass spectrometry (TQMS) to detect [$^{13}C_2$, $^{15}N_2$] UA using [$^{13}C_2$, $^{15}N_2$] xanthine as a substrate [26–28].

The aim of this study was to clarify the relationship between XOR activity accurately evaluated by this novel method and hepatic steatosis assessed by the L/S ratio and HSI in humans.

Results

Characteristics of the study participants

Results are given as median (interquartile range), unless otherwise stated. The characteristics of the participants are presented in Table 1. There were 223 subjects, consisting of 142 females and 81 males. The age was 66 (53–73) years; the body mass index (BMI) was 23.1 (21.2–26.4) kg/m^2, the abdominal circumference (AC) assessed using CT was 83.9 (77.0–91.8) cm, subcutaneous fat area (SFA) was 162.7 (108.1–221.8) cm^2, and visceral fat area (VFA) was 86.1 (53.1–119.0) cm^2. XOR activity was 42.7 (25.3–78.6) pmol/h/mL; serum UA was 5.3 (4.5–6.2) mg/dL; the L/S ratio was 1.3 (1.2–1.5); and HSI was 32.6 (29.3–37.9).

Table 1
Participant characteristics

N (Female : Male)	**223 (142 : 81)**
Age (years)	66 (53–73)
BMI (kg/m^2)	23.1 (21.2–26.4)
Abdominal circumference (cm)	83.9 (77.0–90.8)
Subcutaneous fat area (cm^2)	162.7 (108.1–221.8)
Visceral fat area (cm^2)	86.1 (53.1–119.0)
HbA1c (%)	5.8 (5.6–6.2)
HOMA–R	1.4 (0.9–2.2)
T-Chol (mg/dL)	194.0 (174.0–215.0)
TG (mg/dL)	108.0 (77.0–154.5)
HDL-Chol (mg/dL)	57.0 (48.5–70.0)
XOR activity (pmol/h/mL)	42.7 (25.3–78.6)
UA (mg/dL)	5.3 (4.5–6.2)
L/S ratio	1.3 (1.2–1.5)
HSI	32.6 (29.3–37.9)
AST (U/L)	20.0 (16.0–25.0)
ALT (U/L)	18.0 (13.0–26.0)
γ-GTP	21.0 (15.8–32.0)
Hypertension	171 (76.7%)
Diabetes	51 (22.9%)
Dyslipidemia	145 (65.0%)

The results are presented as median (interquartile range). BMI, body mass index; HbA1c, hemoglobin A1c; HOMA-R, homeostasis model assessment ratio; T-Chol, total-cholesterol; TG, triglycerides; HDL-Chol, high-density lipoprotein-cholesterol; XOR, xanthine oxidoreductase; UA, uric acid; L/S, liver-to-spleen; HSI, hepatic steatosis index; AST, aspartate transaminase; ALT, alanine transaminase: γ-GTP, γ-glutamyl transpeptidas.

Differences in patient background categorized by XOR activity and UA levels

Participants were divided into quartiles based on XOR activity (Table 2). BMI, AC, SFA, and VFA tended to increase with increasing XOR activity: quartile 1 with the lowest and quartile 4 with the highest XOR activity. HbA1c, homeostasis model assessment ratio (HOMA-R), aspartate transaminase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γ-GTP) also tended to increase with increasing XOR activity. The proportion of the L/S ratio < 1.1 and HSI > 36.0 for each XOR activity is illustrated in Fig. 1. The results indicated that the proportion of patients with L/S ratio < 1.1 and HSI > 36.0 tended to increase with increasing XOR activity.

Table 2
Differences in clinical parameters among quadrants of xanthine oxidoreductase (XOR) activities

XOR Variables	Quartile 1 (N = 56)	Quartile 2 (N = 55)	Quartile 3 (N = 56)	Quartile 4 (N = 56)	P for trend
Female : Male	44 : 12	42 : 13	26 : 30	30 : 26	< 0.001
Age (years)	65 (52–75)	69 (65–74)	62 (50–71)	62 (53–71)	0.080
BMI (kg/m^2)	21.5 (20.3–23.3)	23.0 (21.5–24.9)	24.0 (21.8–27.1)	26.0 (22.9–29.4)	< 0.001
AC (cm)	77.4 (71.9–85.3)	83.1 (78.5–87.5)	84.1 (76.9–92.4)	88.9 (84.2–99.2)	< 0.001
SFA (cm^2)	122.2 (82.8–174.3)	165.6 (126.8–203.9)	160.2 (105.1–232.1)	193.7 (138.6–254.7)	< 0.001
VFA (cm^2)	54.4 (27.6–87.3)	81.1 (53.1–108.4)	82.1 (58.3–113.3)	119.8 (90.8–156.0)	< 0.001
HbA1c (%)	5.6 (5.4–5.9)	5.8 (5.5–6.2)	5.7 (5.6–6.2)	6.1 (5.8–6.9)	< 0.001
HOMA–R	1.1 (0.7–1.6)	1.2 (0.9–1.7)	1.7 (1.1–2.1)	2.1 (1.4–2.8)	< 0.001
T-Chol (mg/dL)	190.0 (174.8–209.0)	191.0 (177.0–214.0)	198.5 (174.8–215.0)	194.5 (169.5–217.0)	0.383
TG (mg/dL)	81.5 (63.8–126.3)	108.0 (75.0–130.0)	109.0 (85.0–159.5)	130.5 (98.5–199.3)	< 0.001
HDL-Chol (mg/dL)	66.0 (55.0–76.5)	63.0 (52.5–75.0)	53.5 (48.0–64.5)	50.0 (43.8–58.0)	< 0.001
XOR activity (pmol/h/mL)	18.0 (14.4–21.0)	31.4 (27.5–34.6)	59.1 (49.3–70.0)	114.5 (90.5–179.0)	< 0.001
UA (mg/dL)	4.8 (4.0–5.8)	5.0 (4.4–5.6)	5.4 (4.7–6.1)	5.8 (5.0–6.5)	< 0.001
L/S ratio	1.4 (1.3–1.5)	1.4 (1.3–1.5)	1.3 (1.2–1.5)	1.2 (1.0–1.3)	< 0.001
HSI	29.9 (26.9–33.7)	31.6 (29.0–34.1)	33.3 (29.5–37.5)	38.0 (32.7–41.2)	< 0.001
AST (U/L)	16.0 (14.0–21.3)	18.0 (16.0–21.0)	21.0 (19.0–26.0)	25.0 (21.8–29.3)	< 0.001
ALT (U/L)	12.0 (9.0–16.0)	15.0 (12.0–19.0)	19.0 (17.0–29.3)	28.5 (21.0–38.3)	< 0.001
γ-GTP (U/L)	16.0 (11.3–21.5)	19.0 (15.0–24.3)	24.0 (19.0–34.0)	29.0 (21.5–44.5)	< 0.001
Hypertension	40	41	46	44	0.252

XOR Variables	Quartile 1 (N = 56)	Quartile 2 (N = 55)	Quartile 3 (N = 56)	Quartile 4 (N = 56)	P for trend
Diabetes	8	11	13	19	0.013
Dyslipidemia	29	37	34	45	0.005

Based on the plasma XOR activity, the subjects were divided into quadrants. Clinical parameters and the proportion of comorbidities between quartiles were examined using Jonckheere–Terpstra test or Cochran–Armitage test.

BMI, body mass index; AC, abdominal circumference; SFA, subcutaneous fat area; visceral fat area, VFA; HbA1c, hemoglobin A1c; HOMA-R, homeostasis model assessment ratio; T-Chol, total-cholesterol; TG, triglycerides; HDL-Chol, high-density lipoprotein-cholesterol; UA, uric acid; L/S, liver-to-spleen; HSI, hepatic steatosis index; AST, aspartate transaminase; ALT, alanine transaminase: γ-GTP, γ-glutamyl transpeptidas.

Participants were divided into quartiles based on serum UA levels (Table 3). Similar to XOR activity, BMI, AC, SFA, VFA, AST, ALT, and γ-GTP tended to increase with increasing UA levels. HOMA-R, but not HbA1c, tended to increase with increasing UA levels. The proportion of the L/S ratio < 1.1 and HSI > 36.0 for each serum UA level is presented in Fig. 1. The results indicated that the proportion of the L/S ratio < 1.1 and HSI > 36.0 tended to increase with increasing serum UA level.

Table 3
Differences in clinical parameters among quadrants of serum uric acid (UA) levels

UA Variables	Quartile 1 (N = 54)	Quartile 2 (N = 57)	Quartile 3 (N = 56)	Quartile 4 (N = 56)	P for trend
Female : Male	45 : 9	41 : 16	32 : 24	24 : 32	< 0.001
Age (years)	70 (57–75)	66 (60–71)	64 (51–70)	65 (51–71)	0.030
BMI (kg/m^2)	21.9 (19.5–24.3)	22.8 (20.6–25.0)	24.3 (21.7–28.7)	25.4 (22.5–28.9)	< 0.001
AC (cm)	81.2 (71.4–86.3)	80.8 (74.5–86.6)	86.3 (80.5–92.8)	88.0 (81.2–98.4)	< 0.001
SFA (cm^2)	146.2 (94.7–207.8)	142.8 (102.4–186.5)	187.6 (130.2–226.6)	173.6 (122.1–282.0)	< 0.001
VFA (cm^2)	60.4 (33.7–85.3)	79.5 (45.1–100.7)	87.6 (59.4–124.6)	110.0 (85.4–145.4)	< 0.001
HbA1c (%)	5.7 (5.5–6.3)	5.8 (5.6–6.1)	5.8 (5.6–6.4)	5.8 (5.6–6.1)	0.469
HOMA-R	1.1 (0.9–1.6)	1.1 (0.8–1.7)	1.7 (1.1–2.8)	1.9 (1.3–2.6)	< 0.001
T-Chol (mg/dL)	188.5 (168.5–208.0)	194.0 (181.0–214.0)	197.5 (174.8 -214.5)	194.5(174.3 -217.3)	0.201
TG (mg/dL)	94.5 (73.8–123.8)	97.0 (66.0–142.0)	111.5 (83.0–172.8)	129.0 (101.8–176.8)	< 0.001
HDL−Chol (mg/dL)	64.5 (55.0–75.8)	58.0 (51.0–71.0)	57.0 (48.0–66.5)	51.5 (42.0–61.3)	< 0.001
XOR activity (pmol/h/mL)	27.6 (19.2–45.9)	39.6 (25.6–71.6)	54.6 (30.9–92.6)	59.0 (26.8–93.8)	< 0.001
UA (mg/dL)	3.9 (3.3–4.2)	4.9 (4.7–5.1)	5.6 (5.4–6.0)	6.6 (6.5–7.1)	< 0.001
L/S ratio	1.4 (1.3–1.5)	1.4 (1.2–1.5)	1.3 (1.2–1.4)	1.3 (1.1–1.4)	< 0.001
HSI	31.2 (28.3–34.5)	31.3 (28.4–34.8)	35.7 (30.0–39.6)	34.7 (31.6–39.8)	< 0.001
AST (U/L)	19.0 (15.0–24.0)	20.0 (16.0–22.0)	21.5 (17.8–26.0)	22.0 (17.0–26.3)	0.020
ALT (U/L)	15.0 (11.0–21.0)	16.0 (12.0–22.0)	21.0 (15.5–34.0)	21.0 (14.8–33.0)	0.001
γ-GTP (U/L)	18.0 (12.0–25.0)	20.0 (14.8–27.3)	22.0 (17.0–30.5)	27.5 (20.0–40.8)	< 0.001
Hypertension	38	37	50	46	0.018

UA Variables	Quartile 1 (N = 54)	Quartile 2 (N = 57)	Quartile 3 (N = 56)	Quartile 4 (N = 56)	P for trend
Diabetes	13	11	14	13	0.892
Dyslipidemia	35	33	40	37	0.536

Based on the serum UA levels, the subjects were divided into quadrants. Jonckheere–Terpstra test or Cochran–Armitage test were conducted to assess trends in individual clinical parameters among quadrants.

BMI, body mass index; AC, abdominal circumference; SFA, subcutaneous fat area; visceral fat area, VFA; HbA1c, hemoglobin A1c; HOMA-R, homeostasis model assessment ratio; T-Chol, total-cholesterol; TG, triglycerides; HDL-Chol, high-density lipoprotein-cholesterol; XOR, xanthine oxidoreductase; L/S, liver-to-spleen; HSI, hepatic steatosis index; AST, aspartate transaminase; ALT, alanine transaminase.

Association of the UA levels and XOR activity with hepatic steatosis

An ordinal logistic regression analysis with the L/S ratio as the objective variable and UA levels as the explanatory variable indicated that UA levels had the crude odds ratio (OR) of 1.454 [95% confidence interval (CI): 1.159 − 1.824, P < 0.001] (Table 4). The similar analysis showed that the crude OR for XOR activity per 10 pmol/h/mL of 1.080 (95% CI: 1.039–1.123, P < 0.001). Thus, both UA and XOR activity were associated with a lower L/S ratio. Next, since hepatic steatosis, hyperuricemia, and XOR activity were all associated with insulin resistance, we performed a logistic regression analysis with UA, XOR activity, and HOMA-R as explanatory variables in model 1. The results indicated that XOR activity and HOMA-R were associated with a lower L/S ratio, with OR for XOR activity per 10 pmol/h/mL of 1.052 (95% CI: 1.013–1.093, P = 0.009) and OR for HOMA-R of 1.593 (95% CI: 1.230–2.062, P < 0.001), whereas UA was no longer associated with a lower L/S ratio, with OR of 1.228 (95% CI: 0.960–1.570, P = 0.102). Furthermore, since NAFLD is known to be associated with obesity, hypertension, dyslipidemia, and diabetes mellitus [1, 3, 14], we performed a logistic regression analysis adjusted for age, gender, and presence of hypertension, dyslipidemia, and diabetes mellitus in model 3. The results indicated that XOR activity was associated with a lower L/S ratio with OR for XOR activity per 10 pmol/h/mL of 1.047 (95% CI: 1.009–1.086, P = 0.016), whereas UA was not associated with a lower L/S ratio with OR of 1.047 (95% CI: 0.800–1.370, P = 0.737).

Table 4
Logistic regression analyses of the factors associated with liver-to-spleen (L/S) ratio

	L/S ratio							
			Model 1 (n = 216)		Model 2 (n = 216)		Model 3 (n = 216)	
	Crude OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
UA	1.454 (1.159–1.824)	< 0.001	1.228 (0.960–1.570)	0.102	1.128 (0.875–1.454)	0.354	1.047 (0.800–1.370)	0.737
XOR activity per 10 pmol/h/mL	1.080 (1.039–1.123)	< 0.001	1.052 (1.013–1.093)	0.009	1.047 (1.009–1.086)	0.016	1.047 (1.009–1.086)	0.016
HOMA-R	1.836 (1.437–2.347)	< 0.001	1.593 (1.230–2.062)	< 0.001	1.411 (1.070–1.860)	0.015	1.407 (1.050–1.884)	0.022
BMI	1.190 (1.117–1.268)	< 0.001			1.102 (1.022–1.188)	0.012	1.087 (1.006–1.174)	0.034

In model 1, an ordinal logistic regression analysis was performed with L/S ratio as the objective variable and serum uric acid (UA) levels, plasma xanthine oxidoreductase (XOR) activity, and homeostasis model assessment ratio (HOMA-R) as explanatory variables. In Model 2, body mass index (BMI) was added as an explanatory variable. In Model 3, logistic regression analysis was performed adjusting for age, gender, and presence of hypertension, dyslipidemia, and diabetes mellitus.

The results of HSI as a dependent factor are presented in Table 5. Similarly to the L/S ratio, XOR activity was associated with increased HSI with OR for XOR activity per 10 pmol/h/mL of 1.138 (95% CI: 1.064–1.217, P < 0.001). On the contrary, UA value was not significantly associated with increased HSI, with OR of 1.360 (95% CI: 1.000–1.850, P = 0.050).

Table 5
Logistic regression analyses of the factors associated with hepatic steatosis index (HSI)

	HSI			
			Model 1 (n = 216)	
	Crude OR (95% CI)	P	OR (95% CI)	P
UA	1.620 (1.250–2.100)	< 0.001	1.360 (1.000–1.850)	0.050
XOR activity per 10 pmol/h/mL	1.198 (1.116–1.287)	< 0.001	1.138 (1.064–1.217)	< 0.001
HOMA-R	2.226 (1.623–3.051)	< 0.001	1.747 (1.243–2.454)	0.001

A logistic regression analysis was performed for HSI, and HSI > 36.0 as high values. In Model 1, serum uric acid (UA) levels, plasma xanthine oxidoreductase (XOR) activity, and homeostasis model assessment ratio (HOMA-R) were used as explanator

Discussion

In this study, we investigated the relationship between XOR activity measured accurately using LC/TQMS and indices of hepatic steatosis such as the L/S ratio and HSI in humans. We demonstrate that plasma XOR activity was significant risk factors of hepatic steatosis as assessed by the L/S ratio and HSI, independent of insulin resistance index and serum UA levels.

The prevalence of NAFLD has been reported to be increasing worldwide [1–4]. Insulin resistance is important as a major risk factor for NAFLD [1, 14]. In this study, we demonstrated that hepatic steatosis is related to HOMA-R. Insulin resistance promotes the transfer of free fatty acids from adipose tissue to the liver [29–31]. Furthermore, insulin resistance stimulates sterol receptor-binding protein 1c (SREBP-1c), a transcription factor that controls the synthesis of fatty acids and triglycerides in the liver, leading to hepatic steatosis [32–36]. Thus, insulin resistance is involved in the development of NAFLD through various mechanisms. Furthermore, NAFLD is also known to exacerbate insulin resistance through direct and indirect mechanisms [32–37].

Although it is known that there is a correlation between insulin resistance and XOR activity [21], the results of this study indicate that plasma XOR activity is associated with hepatic steatosis independently of insulin resistance. It has been reported that XOR activity is increased in NAFLD model mice and that

administration of XOR activity inhibitors improves high-fat-diet-induced hepatic steatosis [24, 25]. Various mechanisms can be considered through which XOR activity promotes the development of hepatic steatosis. The nucleotide-binding oligomerization domain-like receptor family, pyrin domain-containing 3 (NLRP3), forms the NLRP3 inflammasome in response to free fatty acids and stress. NLRP3 is involved in the pathogenesis of NAFLD by inducing the production of Interleukin (IL)-1β and IL-18 [38]. XOR activity and intrahepatic UA have been reported to activate NLRP3 [24]. In addition, c-Jun N-terminal kinase (JNK) is a protein kinase that is activated in response to oxidative stress. XOR activity induces oxidative stress and activates JNK, and the activated JNK has been reported to inhibit insulin signaling and induce hepatic steatosis [39, 40].

UA itself has been reported to induce hepatic lipid accumulation by inducing mitochondrial oxidative stress and insulin resistance [41, 42]. In fact, it has been reported that hyperuricemia is associated with the development and progression of NAFLD [17, 18, 43]. Contrarily, our study demonstrated that plasma XOR activity, rather than serum UA levels, was associated with hepatic steatosis.

There is a study in which the XOR inhibitors, febuxostat, and allopurinol were administered to mice models of NAFLD. In that study, the authors reported that febuxostat significantly reduced hepatic XOR activity and significantly improved insulin resistance and lipid peroxidation in the liver, even though blood UA levels were similarly reduced in both febuxostat and allopurinol [39]. This result, similarly to that in our study, suggests that XOR activity plays a more important role in hepatic steatosis than UA levels. It has been reported that XOR activity is more related to vascular endothelial dysfunction than blood UA level [44, 45]. Because XOR activity is involved in both hyperuricemia and the development of NAFLD, its management is potentially important in clinical settings [39, 46].

In this study, we used the L/S ratio to evaluate hepatic steatosis, which has been reported to indicate moderate or severe fatty liver with a sensitivity of 93% [9]. In the study of Westerners, L/S ratio < 0.9 is considered to indicate the presence of moderate to severe fatty liver [9]. In Japan, a study using liver transplant donors reported that the L/S ratio > 1.1 was sufficient to rule out moderate or severe fatty liver [12]. Furthermore, when liver biopsies were performed to histologically evaluate the liver and compared with the L/S ratio, it was reported that the L/S ratio cutoff value for detecting clinically problematic fatty liver was over 1.1, and the L/S ratio assuming absence of hepatic steatosis was over 1.296 [11]. However, it has been reported that it is difficult to adequately assess mild fatty liver using the L/S ratio [9]. Therefore, cases of mild fatty liver may be included in cases with L/S ratio ≥ 1.1, and further investigation is considered necessary in the future.

In a case-control study conducted in Korea, it was reported that HSI was useful in predicting NAFLD and that approximately 90% of cases could be properly diagnosed [13]. In this study, we used NAFLD fibrosis score (NFS) and the FIB-4 index to evaluate the progression of hepatic fibrosis [47, 48], but only 5 cases with L/S ratio < 1.1 had high NFS, and only 4 patients had a high FIB-4 index, and the significant relationship was not observed between hepatic fibrosis and XOR activity. In this study, liver biopsy was not performed because of its high invasiveness. However, a prospective study including liver biopsy is

necessary to examine the relationship more accurately between NAFLD onset and progression and XOR activity.

The limitation of this study is that it was a single-center, cross-sectional analysis. In this study, we used the L/S ratio and HSI as indices of hepatic steatosis and NFS and the FIB-4 index as indices of hepatic fibrosis progression, but liver biopsy is necessary for diagnosis of fatty liver, and further studies are required. Furthermore, a prospective study is required to further clarify the relationship between XOR and hepatic steatosis.

In conclusion, the results of this study indicated that plasma XOR activity is associated with hepatic steatosis independently of insulin resistance and serum UA levels. Thus, XOR activity may be potentially involved in hepatic steatosis in humans.

Methods

Study design and participants.

This cross-sectional analysis was conducted as part of the Hyogo Sleep Cardio-Autonomic Atherosclerosis (HSCAA) study [49-51]. In summary, the HSCAA study is a single-center cohort study which aims to investigate the interrelationships among sleep disorders, autonomic neuropathy, metabolic diseases, and atherosclerotic diseases [49-51]. The HSCAA study included patients aged 20 years and older with one or more cardiovascular risk factors (obesity, smoking, presence of cardiovascular event history, hypertension, dyslipidemia, diabetes mellitus, chronic kidney disease) and being treated at the Hyogo College of Medicine Hospital.

Since we started XOR measurements from 2018 for the subjects who were registered or followed in the HSCAA study, this cross-sectional study included 310 patients, from January 2018 to July 2021, who consented abdominal CT examinations. In the end, 223 patients were analyzed in the present study after excluding 87 with alcoholic habits (>30 g/day for males and >20 g/day for females), autoimmune hepatitis, viral hepatitis, and under treatment with XOR inhibitors.

The HSCAA study has been approved by the Ethics Committee of Hyogo College of Medicine Hospital (Approval No. 2351). Written informed consent was obtained from all subjects and the study was conducted in full accordance with the Declaration of Helsinki. The present study protocol was approved by the Ethics Committee of Hyogo College of Medicine Hospital (Approval No. 3601) and performed with an opt-out option, as explained in instructions posted on the website of the hospital. All methods in our study were performed in accordance with the relevant guidelines and regulations.

Visceral fat area and subcutaneous fat area

CT was performed using SIEMENS SOMATOM Definition AS+ or SOMATOM Definition H (Siemens Healthcare GmbH, Erlangen, Germany) with 10 mm slices. We evaluated the visceral fat area (VFA),

subcutaneous fat area (SFA), and waist circumference using Ziostation 2 (AMIN Ltd., Tokyo, Japan). The abdominal circumference (AC) was measured at the umbilical height.

Hepatic steatosis and liver fibrosis

The L/S ratio and HSI were used to evaluate hepatic steatosis. Hepatic and splenic attenuation values were measured on non-contrast-CT scans using four circular region-of-interest (ROI) cursors in the liver and two in the spleen. In the liver, four ROIs were located in each of the right lobe and the left lobe of the liver. All measurements were manually obtained in regions of uniform parenchymal attenuation, with care being taken to avoid vessels, artifacts, and other areas that might have spuriously increased or decreased measurements. Calculation of the L/S ratio was as follow: L/S ratio = (Average attenuation value of the liver) / (Average attenuation value of the spleen) [9-12]. HSI was calculated from ALT, AST, BMI, gender, and the presence of diabetes mellitus [13].

In addition, the NAFLD fibrosis score (NFS) and the Fibrosis-4 (Fib-4) index were calculated to predict the progression of liver fibrosis in patients with L/S ratio <1.1 [47, 48]. NFS was calculated from age, BMI, AST, ALT, the presence of glucose intolerance, platelet count, and albumin [47]. It has been reported that by applying the high cutoff score (NFS >0.676), the presence of advanced fibrosis could be diagnosed with high accuracy [47]. The FIB-4 index was calculated from age, AST, ALT, and platelet count. It has been reported that its cutoff value <1.45 can exclude hepatic fibrosis, and its cutoff value >3.25 can predict hepatic fibrosis [52].

Plasma XOR activity measurement

The assay protocol of XOR activity in humans was reported previously [26–28]. In brief, 100 μL of plasma samples (purified by Sephadex G25 resin) were mixed with a Tris buffer (pH 8.5) containing [$^{13}C_2$,$^{15}N_2$] xanthine as a substrate, NAD+, and [$^{13}C_3$,$^{15}N_3$] UA as an internal standard. The mixtures were incubated at 37°C for 90 min. Subsequently, the mixtures were mixed with 500 μL of methanol and centrifuged at 2,000 × g for 15 min at 4°C. The supernatants were transferred to new tubes and dried using a centrifugal evaporator. The residues were reconstituted with 150 μL of distilled water, filtered through an ultrafiltration membrane, and measured using LC/TQMS. LC/TQMS comprised a Nano Space SI-2 LC system (Shiseido Co., Ltd., Tokyo, Japan) and a TSQ Triple Quadrupole LC-MS system (ThermoFisher Scientific GmbH, Bremen, Germany) equipped with an ESI interface. Calibration standard samples of [$^{13}C_2$,$^{15}N_2$] UA were also measured, and the amounts of production were quantitated from the calibration curve. XOR activities were expressed in pmol/mL/h [26-28].

Other parameters

At the same time as that for the CT scan, blood samples were taken for AST, ALT, UA, fasting blood glucose, immunoreactive insulin, total cholesterol (T-Chol), high-density lipoprotein cholesterol (HDL-Chol), and TG. Serum UA levels were measured using the uricase/peroxidase technique with an

autoanalyzer using UA (Pureauto S UA Sekisui Medical, Ltd., Tokyo, Japan). Height, weight, and blood pressure were also measured.

Type 2 diabetes was diagnosed based on results showing fasting plasma glucose ≥126 mg/dL, causal plasma glucose ≥200 mg/dL, or 2-h plasma glucose ≥200 mg/dl during a 75-g oral glucose tolerance test, or previous therapy for diabetes [53]. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or taking treatment for hypertension. We defined dyslipidemia as the presence of LDL-C ≥140 mg/dL, HDL-C ≤40 mg/dL, TG level ≥150 mg/dL, or taking treatment for dyslipidemia.

Statistical analysis

The results were presented as median (interquartile range), unless otherwise stated. We used the Jonckheere-Terpstra test to compare the trend of data between three or more groups. The Cochran–Armitage test was used for the trend of the ratio between three or more groups.

Hepatic steatosis was graded as follows: with hepatic steatosis (L/S ratio <1.1) [11, 12], without hepatic steatosis (L/S ratio >1.296), and intermediate (L/S ratio = 1.1–1.296) [11]. In model 1, an ordinal logistic regression analysis was performed with L/S ratio as the objective variable and serum UA levels, plasma XOR activity, and HOMA-R as explanatory variables. In Model 2, BMI was added as an explanatory variable. In Model 3, we used an ordinal logistic regression analysis, and the L/S ratio was used as the objective variable; UA, XOR activity, and the HOMA-R were used as the explanatory variables, adjusted for age, gender, and components for Japanese diagnostic criteria of metabolic syndrome (AC, blood pressure, plasma glucose, HDL, and TG). Furthermore, HSI >36.0 were defined as high values [13], and logistic regression analyses was performed with HSI as the objective variables and UA, XOR, and HOMA-R as explanatory variables.

Statistical analyses were conducted using the BellCurve software version 2.15 (Social Survey Research Information Co., Ltd., Tokyo, Japan), with $P < 0.05$ indicating statistical significance.

Declarations

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Author Contributions

C.Y, Y.K, T.T, and H.K were engaged in the preparation of the study protocol, the analysis of data, laboratory tests, and the preparation of this article. T.M and T.N were engaged in laboratory test. K.O, M.O, A.Mo, A.Mi, M.K-H, K.K-H, M.K, K.K, and T.S were engaged in the data collection. All authors listed have contributed to the work and approved the final version.

Competing interests

While Takayo Murase and Takashi Nakamura are affiliated with Sanwa Kagaku Kenkyusho Co. Ltd., their involvment does not alter our adherence regarding sharing of data and materials. The other authors have no conflicts of interest to declare.

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Figures

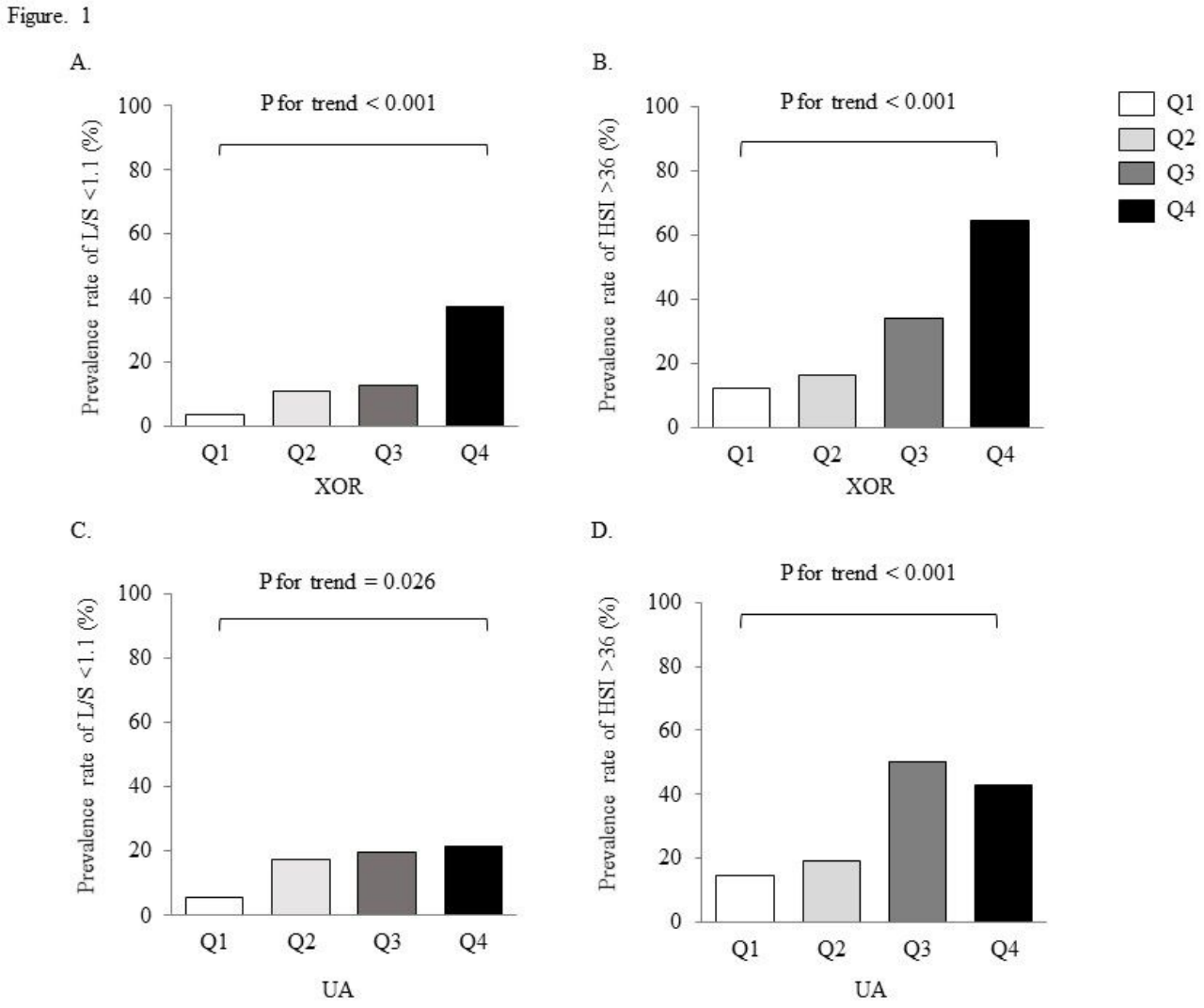

Figure 1

Comparisons of indices of hepatic steatosis among quadrants of XOR activity or UA levels A. Xanthine oxidoreductase (XOR) activity and prevalence rate of liver-to-spleen (L/S) ratio <1.1, B. Uric acid (UA) levels and prevalence rate of L/S ratio <1.1, C. XOR and prevalence rate of hepatic steatosis index (HSI) >36.0, D. UA and prevalence rate of HSI >36.0. The proportion of hepatic steatosis between quartiles was examined using the Cochran–Armitage test. Abbreviations: Q, quadrant