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ORIGINAL ARTICLE

Independent links between plasma xanthine oxidoreductase activity and levels of adipokines

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Keywords
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ABSTRACT

Aims/Introduction: Xanthine oxidoreductase (XOR) is a rate-limiting enzyme that catalyzes uric acid formation in the purine metabolism, is involved in an increase in reactive oxygen species. Plasma XOR activity has been shown to be associated with obesity, smoking, liver dysfunction, hyperuricemia, dyslipidemia and insulin resistance.

Materials and Methods: The association between plasma XOR activity, measured by using liquid chromatography and mass spectrometry, and levels of adipokines, including adiponectin, fatty acid-binding protein 4 (FABP4) and fibroblast growth factor 21 (FGF21), was investigated in 282 participants (male/female: 126/156) of the Tanno-Sobetsu Study who were not taking medication.

Results: Women had lower plasma XOR activity than did men. Smoking habit was associated with increased activity. Plasma XOR activity was positively correlated with concentrations of FABP4 ($r = 0.192$, $P < 0.001$) and FGF21 ($r = 0.208$, $P < 0.001$), homeostasis model assessment of insulin resistance as an index of insulin resistance and uric acid, and was negatively correlated with adiponectin level ($r = -0.243$, $P = 0.001$). Multivariate regression analyses showed that levels of adiponectin, FABP4 and FGF21 were independent determinants of plasma XOR activity after adjusting age, sex, uric acid and homeostasis model assessment of insulin resistance. With additional adjustment of smoking habit, the level of FABP4, but not that of adiponectin or FGF21, remained as an independent predictor of plasma XOR activity.

Conclusions: Plasma XOR activity was independently associated with levels of adipokines in a general population of individuals not taking medication.

INTRODUCTION

Xanthine oxidoreductase (XOR) is a rate-limiting enzyme that catalyzes the formation of uric acid by the oxidative hydroxylation of hypoxanthine and xanthine in the purine metabolism[1]. XOR is transcribed and translated as xanthine dehydrogenase, which reduces oxidized nicotinamide adenine dinucleotide to the reduced form of nicotinamide adenine dinucleotide, and can be post-translationally converted to xanthine oxidase, which consumes oxygen to produce hydrogen peroxide and superoxide. Activation of XOR can increase reactive oxygen species and cause oxidative stress-induced injury in several tissues[2,3]. However, measurement of plasma XOR activity has been difficult because of very low activity in humans[4]. An accurate method for measuring plasma XOR activity in humans has recently been developed using liquid chromatography and triple quadrupole

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mass spectrometry[5]. Using this method, we and others have recently shown that plasma XOR activity is independently associated with obesity, smoking, liver dysfunction, hyperuricemia, dyslipidemia and insulin resistance, suggesting that plasma XOR activity might be a new metabolic biomarker[6,7].

Adipose tissue can secrete several hormones called adipokines, including adiponectin, fatty acid-binding protein 4 (FABP4) and fibroblast growth factor 21 (FGF21). Adiponectin, which is abundantly expressed in adipocytes, directly increases insulin sensitivity, and protects against initiation and progression of atherosclerosis[8]. FABP4, also known as adipocyte fatty acid-binding protein (A-FABP) or aP2, is expressed in adipocytes and macrophages, and is related to the development of insulin resistance and atherosclerosis[9–11]. FGF21 is expressed in several metabolic organs, including fat, liver and skeletal muscle, with profound effects and therapeutic relevance[12,13]. FGF21 derived from adipocytes, hepatocytes and myocytes has been reported to induce the browning of fat, activate the response to cold exposure in brown adipocytes,[14,15] and protect against diet-induced insulin resistance[16], atherosclerosis[17] and cardiac hypertrophy[18].

It has been shown that XOR is abundantly expressed in adipose tissue of mice and can promote the production of uric acid, which is related to obesity-induced insulin resistance[19]. However, little is known about the association between plasma XOR activity and adipokines. Several drugs have been reported to modulate levels of adiponectin[20,21], FABP4[22,23] and FGF21[24]. Therefore, we investigated the links between plasma XOR activity and adipokines, including adiponectin, FABP4 and FGF21, in the general population.

METHODS

Study Participants

In a population-based cohort, the Tanno-Sobetsu Study, a total of 627 Japanese participants (male/female: 292/335) were recruited from residents of Sobetsu Town in 2016. This population was the same as that in our previous study for investigating plasma XOR activity[7]. Participants treated with any medications were excluded for the elimination of drug effects on plasma XOR activity and adipokines, and participants not taking any medication (n = 282, male/female: 126/156) were enrolled. Medical checkups, including measurement of blood pressure and calculation of body mass index (BMI), and collection of blood samples were carried out as previously described[7]. This study was approved with the ethics committee of Sapporo Medical University, and was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all of the study participants.

Measurements

Concentrations of adiponectin, FABP4 and FGF21 were measured using enzyme-linked immunosorbent assays kits for adiponectin (R&D Systems, Minneapolis, MN, USA), FABP4 (Biovendor, Modrice, Czech Republic) and FGF21 (R&D Systems), respectively. Variables of liver function, renal function, glucose and lipid metabolism were measured as previously described[7]. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as insulin (μU/mL) × glucose (mg/dL) / 405.

Plasma XOR Activity

Plasma XOR activity was measured by using a combination of liquid chromatography and triple quadrupole mass spectrometry to detect [$^{13}C_2$, $^{15}N_2$]-uric acid using [$^{13}C_2$, $^{15}N_2$]-xanthine as a substrate, as previously reported[5,7]. Inter- and intra-assay coefficients of variation were 9.1 and 6.5%, respectively, and the lower limit of detection was 6.67 pmol/h/mL plasma[5].

Statistical Analysis

Variables are presented as the mean ± standard deviation for normal distributions, or medians (interquartile ranges) for skewed variables. The normality of each variable was tested by the Shapiro–Wilk W-test. Comparison between two groups for parametric and non-parametric parameters was carried out by the Student's t-test and the Mann–Whitney U-test, respectively. The χ^2-test was carried out for intergroup differences in percentages of parameters. A Pearson's correlation analysis was carried out for the correlation between two variables. Non-normally distributed variables were logarithmically transformed for regression analyses. Multivariate regression analyses were carried out to identify independent links between plasma XOR activity and adipokines, including adiponectin, FABP4 and FGF21, after adjustment of age, sex, smoking habit and levels of uric acid, and HOMA-IR by several models, showing the standardized regression coefficient (β) and the percentage of variance for the selected independent predictors explained (R^2). Statistical significance was determined as a P-value <0.05. JMP 9 software for Macintosh (SAS Institute, Cary, NC, USA) was used for statistical analyses.

RESULTS

Basal Characteristics of the Studied Participants

Basal characteristics of the 282 recruited participants not taking any medications (male/female: 126/156) are shown in Table 1. The numbers of participants with smoking and drinking habits were 68 (24.1%) and 118 (41.8%), respectively. Hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg), diabetes mellitus (a combination of hemoglobin A1c ≥6.5% and fasting glucose ≥126 mg/dL), dyslipidemia (low-density lipoprotein cholesterol ≥140 mg/dL, high-density lipoprotein [HDL] cholesterol <40 mg/dL or triglycerides ≥150 mg/dL) and hyperuricemia (uric acid >7 mg/dL) were found in 76, 3, 128 and 25 participants, respectively. Women had significantly lower adiposity, including BMI and waist circumference; significantly lower frequencies of current smoking and drinking habits; and lower levels of blood pressure, liver enzymes and parameters of renal function, and glucose and lipid metabolism except for cholesterols than did men. Levels

Table 1 | Characteristics of the participants not taking medication

	Total (n = 282)	Male (n = 126)	Female (n = 156)	P
Age (years)	56 ± 16	55 ± 17	57 ± 16	0.425
Body mass index (kg/m^2)	22.8 ± 3.8	23.7 ± 3.6	22.0 ± 3.8	<0.001
Waist circumference (cm)	83.2 ± 11.4	85.7 ± 10.9	81.3 ± 11.4	0.001
Systolic blood pressure (mmHg)	127 ± 20	131 ± 17	124 ± 21	0.009
Diastolic blood pressure (mmHg)	75 ± 11	76 ± 10	73 ± 11	0.031
Pulse rate (b.p.m.)	70 ± 11	69 ± 12	71 ± 11	0.108
Smoking habit	68 (24.1)	40 (31.7)	28 (17.9)	0.007
Alcohol drinking habit	118 (41.8)	68 (54.0)	50 (32.1)	<0.001
Disease				
Hypertension	76 (27.0)	35 (27.8)	41 (26.3)	0.789
Diabetes mellitus	3 (1.1)	3 (2.4)	0 (0)	0.088
Dyslipidemia	128 (45.3)	53 (42.1)	75 (48.1)	0.337
Hyperuricemia	25 (8.9)	22 (17.5)	3 (1.9)	<0.001
Biochemical data				
AST (IU/L)	22 (19–26)	22 (20–27)	21 (18–25)	0.008
ALT (IU/L)	18 (14–24)	21 (16–29)	16 (13–20)	<0.001
γGTP (IU/L)	21 (15–32)	26 (20–39)	17 (14–27)	<0.001
Blood urea nitrogen (mg/dL)	15 ± 4	15 ± 4	15 ± 4	0.091
Creatinine (mg/dL)	0.8 (0.7–0.9)	0.9 (0.8–0.9)	0.7 (0.6–0.8)	<0.001
eGFR (mL/min/1.73 m^2)	73 ± 15	76 ± 16	71 ± 14	0.004
Uric acid (mg/dL)	5.2 ± 1.3	6.0 ± 1.1	4.6 ± 1.0	<0.001
Total cholesterol (mg/dL)	213 ± 38	201 ± 35	223 ± 38	<0.001
LDL cholesterol (mg/dL)	125 ± 34	118 ± 31	132 ± 35	0.001
HDL cholesterol (mg/dL)	63 ± 17	56 ± 15	70 ± 16	<0.001
Triglycerides (mg/dL)	83 (60–116)	92 (65–148)	76 (54–107)	0.001
Fasting glucose (mg/dL)	89 (85–95)	92 (86–98)	89 (83–93)	0.001
Insulin (μU/mL)	8.4 (3.9–17.6)	9.6 (4.4–20.0)	7.3 (3.4–14.3)	0.036
HOMA-IR	1.80 (0.88–4.03)	2.23 (1.05–4.49)	1.55 (0.84–3.32)	0.015
HbA1c (%)	5.4 (5.1–5.6)	5.4 (5.2–5.6)	5.3 (5.1–5.6)	0.015
Adiponectin (μg/mL)	7.1 (4.7–10.6)	5.4 (3.7–8.0)	9.0 (6.2–12.5)	<0.001
FABP4 (ng/mL)	10.1 (6.2–16.7)	8.7 (5.6–15.5)	11.2 (6.9–17.1)	0.038
FGF21 (pg/mL)	96 (58–149)	105 (69–158)	91 (53–140)	0.020
XOR (pmol/h/mL plasma)	32 (19–58)	44 (22–82)	26 (18–45)	<0.001

Variables are expressed as number (%), mean ± standard deviation or median (interquartile range). γGTP, γ-glutamyl transpeptidase; ALT, alanine transaminase; AST, aspartate transaminase; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; FGF21, fibroblast growth factor 21; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin resistance; XOR, xanthine oxidoreductase.

of plasma XOR activity and FGF21 were significantly higher in men than in women. Levels of adiponectin and FABP4 were lower in men than in women.

Correlations of Plasma XOR Activity with Clinical Variables

Plasma XOR activity was significantly lower in participants without a smoking habit than in those with a smoking habit (Figure 1a). No significant difference was found between plasma XOR activities in participants with and those without an alcohol drinking habit. As shown in Table 2, plasma XOR activity was positively correlated with adiposity, diastolic blood pressure, and levels of liver enzymes, estimated glomerular filtration rate (eGFR), triglycerides, fasting glucose, insulin, hemoglobin A1c, uric acid (Figure 1b), HOMA-IR (Figure 1c), FABP4 (Figure 1d) and FGF21 (Figure 1e), and was negatively correlated with concentrations of adiponectin (Figure 1f) and HDL cholesterol. Similar correlations of parameters were found when sex was separately analyzed (Table 2).

Correlations of Levels of Adipokines with Clinical Variables

As shown in Table S1, adiponectin concentration was positively correlated with age and HDL cholesterol level, and was negatively correlated with adiposity and levels of alanine aminotransferase (ALT), γ-glutamyl transpeptidase, uric acid, eGFR, triglycerides, parameters of glucose metabolism and plasma XOR activity. Similar correlations of adiponectin level with age, adiposity, and levels of eGFR, HDL cholesterol and triglycerides were found when sex was separately analyzed. Adiponectin level was not significantly correlated with levels of other adipokines, FABP4 and FGF21.

As shown in Table S2, FABP4 concentration was positively correlated with age, adiposity, systolic blood pressure and levels

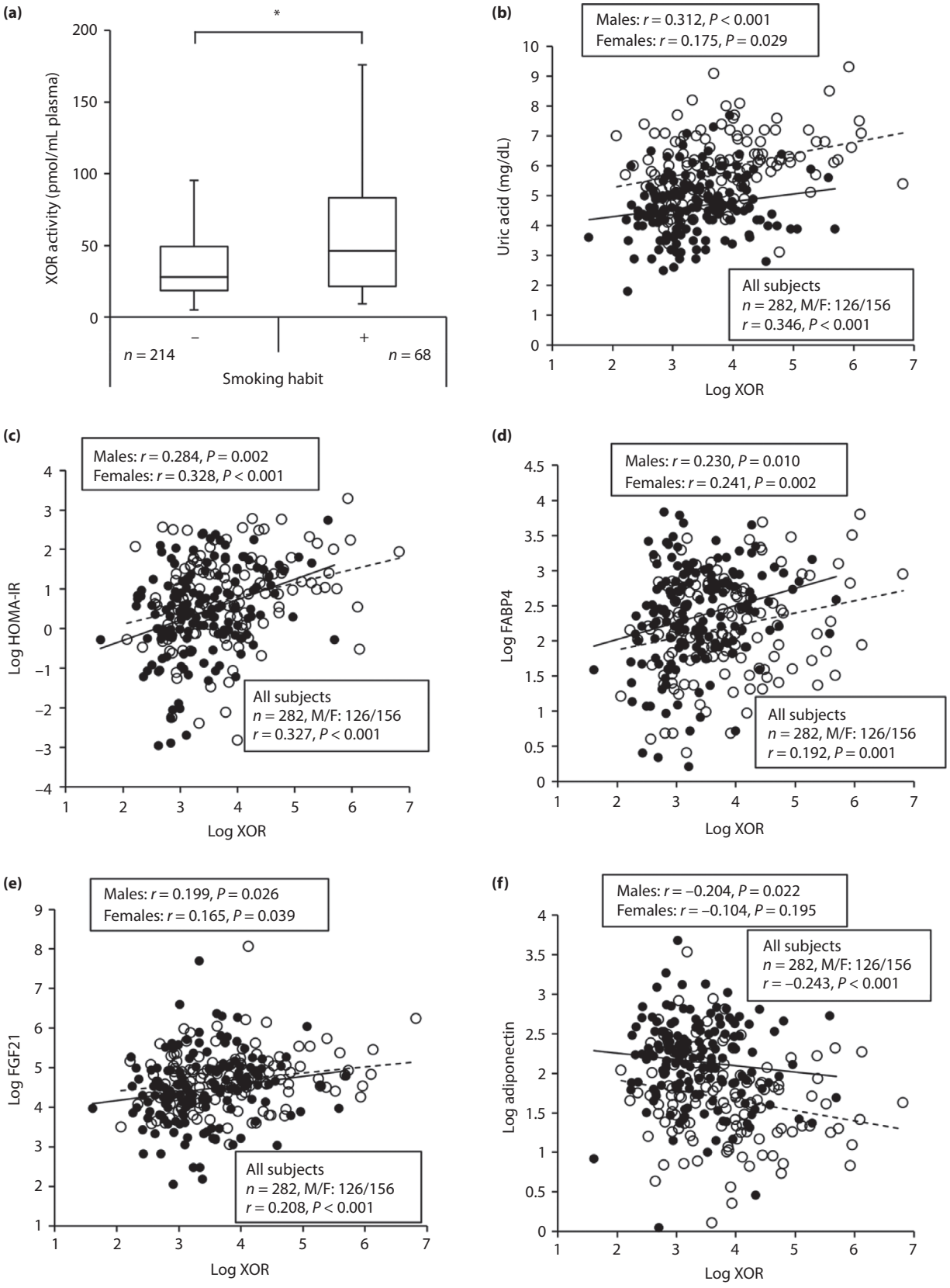

Figure 1 | Associations of plasma xanthine oxidoreductase (XOR) activity with metabolic parameters. (a) Comparison of plasma XOR activities (pmol/h/mL plasma) in participants with and those without a smoking habit shown by box plots. *$P < 0.05$. (b) Uric acid, (c) logarithmically transformed (log) homeostasis model assessment of insulin resistance (HOMA-IR), (d) log fatty acid-binding protein 4 (FABP4), (e) log fibroblast growth factor 21 (FGF21) and (f) log adiponectin were plotted against log plasma XOR activity for each participant (n = 282, male/female: 126/156). Open circles and broken regression line, men (n = 126); closed circles and solid regression line, women (n = 156).

Table 2 | Correlation analysis for log xanthine oxidoreductase activity

	Total (n = 282)		Male (n = 126)		Female (n = 156)	
	r	P	r	P	r	P
Age	−0.009	0.875	−0.104	0.243	0.126	0.117
Body mass index	0.416	<0.001	0.443	<0.001	0.328	<0.001
Waist circumference	0.412	<0.001	0.432	<0.001	0.338	<0.001
Systolic blood pressure	0.095	0.112	0.052	0.565	0.062	0.441
Diastolic blood pressure	0.127	0.034	0.138	0.125	0.059	0.468
Pulse rate	0.075	0.220	0.178	0.050	0.022	0.794
Log AST	0.623	<0.001	0.661	<0.001	0.526	<0.001
Log ALT	0.759	<0.001	0.798	<0.001	0.645	<0.001
Log γGTP	0.502	<0.001	0.470	<0.001	0.451	<0.001
Blood urea nitrogen	−0.022	0.709	−0.270	0.002	0.195	0.015
log Creatinine	0.085	0.153	−0.137	0.127	−0.043	0.595
eGFR	0.124	0.038	0.180	0.043	−0.037	0.650
Uric acid	0.346	<0.001	0.312	<0.001	0.175	0.029
Total cholesterol	0.054	0.368	0.074	0.413	0.213	0.008
LDL cholesterol	0.080	0.182	0.034	0.709	0.252	0.002
HDL cholesterol	−0.317	<0.001	−0.267	0.003	−0.211	0.008
Log Triglycerides	0.402	<0.001	0.389	<0.001	0.325	<0.001
Log Fasting glucose	0.265	<0.001	0.227	0.011	0.224	0.005
Log Insulin	0.302	<0.001	0.259	0.004	0.313	<0.001
Log HOMA-IR	0.327	<0.001	0.284	0.002	0.328	<0.001
Log HbA1c	0.222	<0.001	0.193	0.030	0.208	0.009
Log Adiponectin	−0.243	<0.001	−0.204	0.022	−0.104	0.195
Log FABP4	0.192	0.001	0.230	0.010	0.241	0.002
Log FGF21	0.208	<0.001	0.199	0.026	0.165	0.039

γGTP, γ-glutamyl transpeptidase; ALT, alanine transaminase; AST, aspartate transaminase; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; FGF21, fibroblast growth factor 21; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin resistance; XOR, xanthine oxidoreductase.

of aspartate transaminase (AST), ALT, low-density lipoprotein cholesterol, total cholesterol, triglycerides, parameters of glucose metabolism, FGF21 and plasma XOR activity, and was negatively correlated with eGFR and HDL cholesterol level. Similar correlations of FABP4 level with adiposity, systolic blood pressure, and levels of AST, ALT, HDL cholesterol, triglycerides, hemoglobin A1c, FGF21 and plasma XOR activity were found when sex was separately analyzed.

As shown in Table S3, FGF21 concentration was positively correlated with age, adiposity, pulse rate, blood pressure, and levels of AST, ALT, γ-glutamyl transpeptidase, uric acid, triglycerides, FABP4 and plasma XOR activity, and was negatively correlated with levels of blood urea nitrogen and HDL cholesterol. Similar correlations of FGF21 level with diastolic blood pressure, and levels of triglycerides, AST, γ-glutamyl transpeptidase, FABP4 and plasma XOR activity were found when sex was separately analyzed.

Associations Between Plasma XOR Activity and Adipokines

As shown in Table 3, multivariate regression analyses showed that the level of adiponectin, FABP4 or FGF21 was independently related to plasma XOR activity after adjusting age and sex (model 1). When BMI was incorporated into the adjustment (model 2), the level of FGF21, but not that of adiponectin or FABP4, was an independent predictor of plasma XOR activity. When uric acid (model 3) or uric acid and HOMA-IR (model 4) instead of BMI were incorporated into the adjustment in model 1, the level of adiponectin, FABP4 or FGF21 was an independent predictor of plasma XOR activity. With additional adjustment of uric acid, HOMA-IR and smoking

Table 3 | Multivariate regression analysis for log xanthine oxidoreductase activity

	Log adiponectin			Log FABP4			Log FGF21		
	β	*P*	R^2	β	*P*	R^2	β	*P*	R^2
Model 1	−0.183	0.005	0.098	0.233	<0.001	0.126	0.175	0.003	0.104
Model 2	−0.089	0.157	0.214	0.090	0.136	0.216	0.146	0.007	0.227
Model 3	−0.161	0.011	0.151	0.189	0.001	0.166	0.146	0.011	0.152
Model 4	−0.133	0.033	0.225	0.149	0.010	0.236	0.126	0.027	0.226
Model 5	−0.124	0.052	0.242	0.135	0.022	0.250	0.068	0.255	0.234

Standardized regression coefficient (β). Model 1, adjusted for age and sex. Model 2, adjusted for model 1 + body mass index. Model 3, adjusted for model 1 + uric acid. Model 4, adjusted for model 3 + log homeostasis model assessment of insulin resistance. Model 5, adjusted for model 4 + smoking habit. FABP4, fatty acid-binding protein 4; FGF21, fibroblast growth factor 21.

habit (model 5), the level of FABP4, but not that of FGF21 or adiponectin, remained as an independent determinant of plasma XOR activity, explaining 25.0% of the variance (R^2 = 0.250).

DISCUSSION

Plasma XOR activity was independently linked to levels of adipokines in the general population of participants with no medication. Levels of adiponectin, FABP4 and FGF21 were independently correlated with plasma XOR activity after adjusting age, sex, insulin resistance and uric acid level. After additional adjustment of smoking habit, the level of FABP4, but not that of adiponectin or FGF21, was still an independent predictor of plasma XOR activity. It has been previously and preliminarily reported that plasma XOR activity measured by the same assay was positively correlated with insulin resistance and negatively correlated with adiponectin level in 29 young participants (mean age 25.9 years)[6]. In the present study using a large number of participants not taking any medication (n = 282), we showed independent links between plasma XOR activity and levels of adipokines, not only

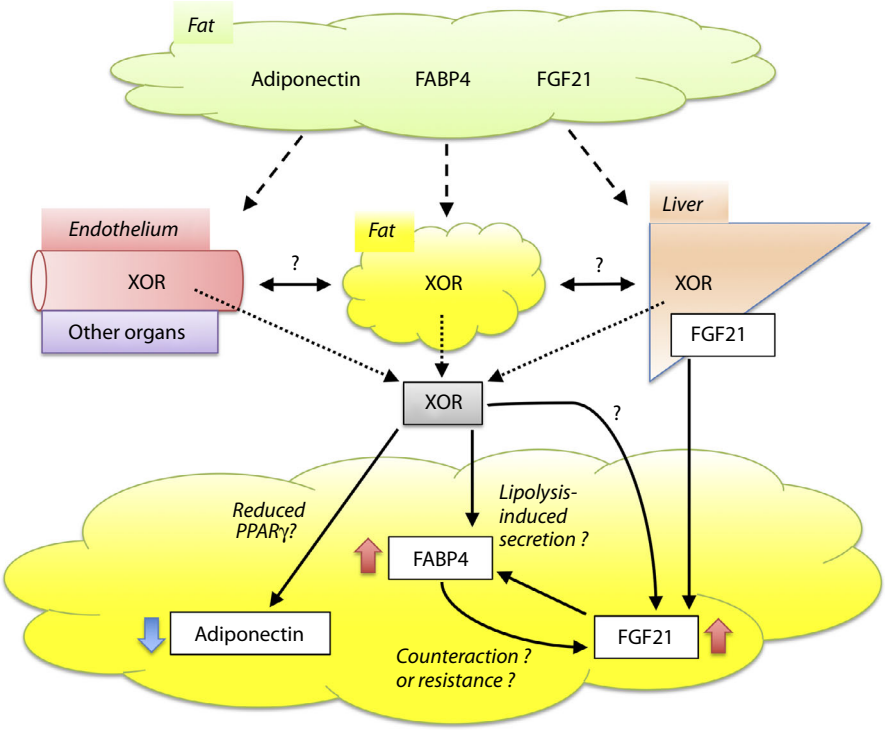

Figure 2 | Possible mechanisms about the association between plasma xanthine oxidoreductase (XOR) activity and adipokines. Adipokines, including adiponectin, fatty acid-binding protein 4 (FABP4) and fibroblast growth factor 21 (FGF21), as bioactive molecules derived from adipocytes might directly affect the expression and activity of XOR in several tissues. Activation of XOR might also modulate circulating levels of adipokines. PPARγ, proliferator-activated receptor γ.

adiponectin, but also FABP4 and FGF21. Speculation of possible mechanisms about the association between XOR and adipokines is shown in Figure 2.

Adiponectin, a favorable adipokine, is a molecule regulated by peroxisome proliferator-activated receptor γ (PPARγ). Treatment with thiazolidinediones, PPARγ agonists, increases the expression and circulating level of adiponectin[21]. It has been shown that gene expression of XOR is abundant in fat tissue of mice and is increased in visceral fat in the obese condition[19]. XOR has also been shown to regulate adipocyte differentiation in an early stage by the activation of PPARγ[25]. Knockdown of XOR decreased the expression of PPARγ and inhibited adipocyte differentiation, whereas overexpression of XOR increased PPARγ activity, but decreased the expression of PPARγ, resulting in inhibition of adipogenesis. One possible reason for the inverse correlation between XOR activity and adiponectin level is that robust activation of XOR might decrease the expression of adiponectin through reduced expression of PPARγ in adipose tissue (Figure 2).

It has previously been showed that a small molecule FABP4 inhibitor might be a new therapy for insulin resistance and atherosclerosis[26]. FABP4 is non-classically secreted from adipocytes in connection with lipolysis[27,28], although the amino acid sequence of FABP4 has no signal peptides for secretion[10]. Previous studies showed that circulating FABP4 can act as an adipokine, and directly develop insulin resistance and atherosclerosis *in vitro* and *in vivo*[28–30], suggesting that circulating FABP4 might directly affect plasma XOR activity. Conversely, increased XOR activity might increase circulating FABP4 concentration through augmentation of lipolysis (Figure 2), as it has recently been reported that febuxostat, an XOR inhibitor, attenuates lipolysis in adipose tissue[31].

FGF21 is an endocrine molecule for regulating glucose and lipid metabolism[12,13]. Treatment with FGF21 has been shown to improve glucose and lipid homeostasis, increase the number of brown adipocytes[16], preserve β-cell functions[32], ameliorate hepatic steatosis,[33] and decrease atherosclerosis[17]. FGF21 concentration has been shown to be increased in several aspects of metabolic syndrome[34,35], indicating the presence of a compensatory response to higher metabolic stress or resistance to FGF21. As FGF21 is widely expressed and secreted in metabolic organs including fat tissue, liver and skeletal muscle, FGF21 has been proposed as a complex biological molecule, such as an adipokine, hepatokine or myokine[12]. In the present study, the level of FGF21, but not that of adiponectin or FABP4, was independently associated with plasma XOR activity after adjustment of age, sex and BMI (Table 3). Furthermore, XOR activity was strongly correlated with liver enzymes, AST and ALT (Table 2). FGF21 derived from the liver might be mainly associated with XOR activity (Figure 2). The present study also showed that FGF21 concentration was positively correlated with FABP4 concentration, as shown in a previous study[36]. It has been reported that FGF21 induces lipolysis during normal feeding in white fat tissue[37], probably leading to an increased circulating FABP4 level caused by lipolysis-related secretion of FABP4 from adipocytes in a non-classical pathway[27,28]. FABP4 might participate in the counteraction of FGF21 or resistance to FGF21.

Xanthine oxidoreductase is expressed as the xanthine dehydrogenase form in tissues, and it leaks into the blood and consequently converts to the xanthine oxidase form[38,39]. Xanthine oxidase is shed by an organ without non-specific membrane damage into plasma, and is partially bound to sulfated glycosaminoglycans on the surface of vascular endothelial cells[2,3]. It has been reported that activation of endothelium-bound XOR can inhibit endothelial nitric oxide production and impair vasodilatory reaction[40]. In contrast, T-cadherin-mediated accumulation of adiponectin in the endothelium plays a protective role against neointimal and atherosclerotic plaque formation[41]. Ectopic expression of FABP4 in endothelial cells has also been reported to contribute to neointima formation and organ damage[30,42]. Plasma XOR activity might reflect endothelial dysfunction in association with adipokines in endothelial cells.

It has been suggested that high plasma XOR activity is a metabolic parameter that is superior to uric acid level, and that adequately inhibiting plasma XOR activity unless lowering uric acid would be a new therapeutic strategy for treatment of metabolic and cardiovascular diseases[7]. It has also been reported that unexpected high plasma XOR activities possibly associated with liver dysfunction and insulin resistance are found in some women with a relatively low level of uric acid in a general population[43]. There have been some interventional investigations on the effects of XOR inhibitors, including allopurinol, febuxostat and topiroxostat, on adipokine levels in humans, but results showed that XOR inhibitors did not significantly change levels of adipokines[44–46]. Possible interventions for adipokines, including adiponectin receptor agonists[47], FABP4 inhibitors[10,26] and FGF21 analogs[48], have been postulated in metabolic and cardiovascular diseases. It is possible that modulations of adipokines using adiponectin receptor agonists, FABP4 inhibitors or FGF21 analogs might contribute to the regulation of XOR activity, and the prognosis of metabolic and cardiovascular diseases in humans.

The present study had some limitations. First, the results in the present study do not prove causal relations between plasma XOR activity and correlated biomarkers because of a cross-sectional study. Second, as only Japanese people were enrolled, the results in the present study might not correspond to other races. Third, several related biomarkers, including oxidative stress, other adipokines and free fatty acids as ligands of FABP4, were not examined in the present study owing to the lack of remaining blood samples. Finally, measurement of plasma XOR activity is varied in laboratories. Values of plasma XOR activity are not comparable with those measured in other laboratories using different assay protocols.

In conclusion, plasma XOR activity is independently linked to several adipokines, including FABP4, adiponectin and FGF21, in the general population of individuals not taking medication. Measurement of XOR activity might contribute to

finding potentially high-risk patients with metabolic disorders and/or cardiovascular diseases. Further understanding of the associations between plasma XOR activity and levels of adipokines might enable the development of novel therapies for metabolic and cardiovascular diseases.

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DISCLOSURE

Takayo Murase and Takashi Nakamura at Sanwa Kagaku Kenkyusho Co., Ltd. developed the assay of plasma XOR activity and measured the activity. This does not alter our adherence about sharing data and materials. The other authors declare no conflict of interest.

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SUPPORTING INFORMATION

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

Table S1 | Correlation analysis for log adiponectin.
Table S2 | Correlation analysis for log fatty acid-binding protein 4.
Table S3 | Correlation analysis for log fibroblast growth factor 21.