Original Article

Associations of circulating xanthine oxidoreductase activity with cardiometabolic risk markers in overweight and obese Japanese men: a cross-sectional pilot study

Keisei Kosaki,[1] Atsumu Yokota,[2] Koichiro Tanahashi,[3] Kanae Myoenzono,[2,4] Jiyeon Park,[1] Toru Yoshikawa,[5] Yasuko Yoshida,[6] Takayo Murase,[7] Seigo Akari,[7] Takashi Nakamura,[7] and Seiji Maeda[1,8,*]

[1]Faculty of Health and Sport Sciences and [2]Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8574, Japan
[3]Department of Health and Sports Sciences, Kyoto Pharmaceutical University, 5 Misasaginakauchi-cho, Yamashina-ku, Kyoto 607-8414, Japan
[4]Japan Institute of Sport Sciences, 3-15-1 Nishigaoka, Kita-ku, Tokyo 115-0056, Japan
[5]Faculty of Health & Sport Sciences, Ryutsu Keizai University, 120 Ryugasaki, Ibaraki 301-8555, Japan
[6]Faculty of Health Sciences, Tsukuba International University, 6-20-1 Manabe, Tsuchiura, Ibaraki 300-0051, Japan
[7]Sanwa Kagaku Kenkyusho Co., Ltd., Shiosaki, Hokusei-cho, Inabe, Mie 511-0406, Japan
[8]Faculty of Sport Sciences, Waseda University, 2-579-15 Mikajima, Tokorozawa, Saitama 359-1192, Japan

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Circulating xanthine oxidoreductase (XOR) activity may contribute to the pathogenesis of obesity-related adverse cardiometabolic profiles. This pilot study aimed to examine the cross-sectional associations between plasma XOR activity and cardiometabolic risk (CMR) markers in overweight and obese men. In 64 overweight and obese Japanese men (aged 31–63 years), plasma XOR activity and several CMR markers, such as homeostasis model assessment of insulin resistance (HOMA-IR), and clustered CMR score were measured in each participant. Clustered CMR score was constructed based on waist circumference, triglyceride, blood pressure, fasting plasma glucose, and high-density lipoprotein cholesterol. Plasma XOR activity in overweight and obese men was positively associated with the body mass index, waist circumference, visceral fat area, body fat mass, hemoglobin A1c, serum 8-hydroxy-2'-deoxyguanosine, HOMA-IR, and clustered CMR score and was inversely associated with handgrip strength and high-density lipoprotein cholesterol. Multiple linear regression analysis further demonstrated that the associations of plasma XOR activity with HOMA-IR and the clustered CMR score remained significant after adjustment for covariates including uric acid. Our data demonstrate that circulating XOR activity was independently associated, albeit modestly, with HOMA-IR and the clustered CMR score. These preliminary findings suggest that circulating XOR activity can potentially be one of the preventive targets and biomarkers of cardiometabolic disorders in overweight and obese men.

***Key Words*:** **purine metabolism, uric acid, clustered cardiometabolic risk, obesity, insulin resistance**

The rising prevalence of overweight and obese men has been recognized as a major global public health concern. Between 1980 and 2013, the proportion of men with a body mass index (BMI) of ≥25 kg/m^2 increased from 28.8% to 36.9%.[1] Overweight and obesity in the Japanese adult population are also increasing substantially,[2] and approximately 30% of men are overweight.[3] Accumulating evidence demonstrates that adverse cardiometabolic profiles, such as hypertension, dyslipidemia, and abnormal glucose metabolism, occur in the context of overweight/obesity.[4,5] With the growing epidemic of obesity, there is an urgent need to identify modifiable factors to prevent obesity-related cardiometabolic disorders.

Xanthine oxidoreductase (XOR) is the enzyme localized abundantly in the liver and intestines,[6] and is responsible for the formation of uric acid in the purine metabolic pathway.[7] There are two interconvertible XOR forms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO); the latter produces superoxide by exclusively consuming the oxygen molecule as an electron acceptor.[8] Although circulating XOR activity is normally very low in humans, their levels can become elevated in several pathological conditions, including obesity, because XOR is released from severely damaged cells into the circulation before the XDH/XO conversion occurred.[9] A growing body of evidence suggests that increased circulating XOR activity can increase reactive oxygen species, resulting in several adverse health outcomes.[10–12] Moreover, recent studies are examining in detail the pathophysiological role of increased XOR activity in human plasma, building on the development of a sensitive and accurate method for measuring plasma XOR activity in humans.[13]

Hyperuricemia is a well-known risk factor for the onset and progression of cardiometabolic diseases.[14] However, it has been demonstrated that lowering uric acid levels did not always reduce cardiometabolic events.[15–17] In this context, a previous study reported that high-dose allopurinol, an XOR inhibitor, improves endothelial function by decreasing oxidative stress but not by reducing uric acid levels.[18] Therefore, these findings indicate one possibility that XOR activity, rather than uric acid, can be a novel preventive target for cardiometabolic diseases.

Previous studies have shown the independent associations of plasma XOR activity with several cardiometabolic risk (CMR) markers in young and disease-free volunteers,[19] as well as in the Japanese general population.[20,21] Furthermore, plasma XOR activity has been reported to be independently associated with adipokines in a general population not taking medications.[22] These findings suggest that circulating XOR activity can be a novel metabolic biomarker in the general population. However,

*To whom correspondence should be addressed.
E-mail: maeda.seiji.gn@u.tsukuba.ac.jp

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it remains unclear whether plasma XOR activity can be a target for intervention to prevent obesity-related cardiometabolic disorders because there are no studies examining its associations in overweight/obese population. Thus, we investigated the cross-sectional associations of circulating XOR activity with several CMR markers in overweight and obese men.

Materials and Methods

Participants. This cross-sectional pilot study used data collected during a community-based medical checkup conducted between 2017 and 2018 at the University of Tsukuba. Men with a BMI ≥25 kg/m^2 (overweight/obese) were included in the study. A total of 64 overweight/obese men (aged 31–63 years) were included in this study. Participants were excluded if they were treated with XOR inhibitors, including allopurinol, febuxostat, and topiroxostat, or had missing data on any of the covariates of interest. All procedures were approved by the Institutional Review Board of the University of Tsukuba (Tai 019-19) and performed according to the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Procedures. All measurements were performed in an environmentally controlled room after adequate rest. Participants were instructed to abstain from large meals and strenuous exercise for at least 12 h before visiting our laboratory. After arrival, the participants underwent anthropometric assessments (barefoot and in light clothing), following which antecubital venous blood samples were collected for blood biochemistry. Subsequently, blood pressure and heart rate were measured in the supine position. Medication use, such as antihypertensives, lipid-lowering agents, and glucose-lowering agents were surveyed using a self-administered questionnaire.

Measurements. Height was evaluated to the nearest 0.1 cm using a wall-mounted stadiometer (YG-200; Yagami, Aichi, Japan). Weight was measured to the nearest 0.1 kg on a calibrated digital scale (InBody 770; InBody Japan, Tokyo, Japan), and adjusted for the estimated clothing mass by subtracting 0.5 kg. BMI was calculated as the weight divided by the height squared (kg/m^2). Waist circumference was measured in duplicate directly on the skin at the level of the umbilicus in the standing position to the nearest 0.1 cm. Skeletal muscle mass and body fat mass were measured using multi-frequency (1, 5, 50, 250, 500, and 1,000 kHz) bioelectrical impedance analysis with a tetrapolar eight-point tactile electrode system (InBody 770, InBody Japan). The visceral fat area was roughly estimated using the dual-impedance analysis method (HSD-2000; Omron Healthcare, Kyoto, Japan). Handgrip strength (kgf per body weight) was measured using a Smedley-type handgrip dynamometer (TKK5401; Takei Kiki Kogyo, Niigata, Japan). The average of the left and right maximum values was used in the analysis. Brachial blood pressure and heart rate were evaluated using a semi-automated vascular testing device (Form PWV/ABI, Colin Medical Technology, Aichi, Japan).

Serum concentrations of high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, 8-OHdG (8-hydroxy-2'-deoxyguanosine) (n = 55), and plasma concentrations of glucose, hemoglobin A1c, and insulin were measured using antecubital venous blood samples in the morning following overnight fasting. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from the fasting plasma glucose and insulin levels using the following equation: HOMA-IR = fasting plasma glucose (mg/dl) × fasting plasma insulin (μU/ml)/405. A continuous clustered CMR score was calculated using waist circumference, blood pressure (average of the systolic and diastolic blood pressure), HDL cholesterol, log-transformed triglyceride, and fasting plasma glucose, as previously described.[23,24] Briefly, these cardiometabolic variables were standardized (z-scores were computed), and then all z-scores were summed and divided by five to compile the clustered CMR score. The z-score of HDL cholesterol was inverted and included in this calculation because of its protective effects.

Plasma XOR activity. Plasma XOR activity was measured using a highly sensitive assay utilizing a combination of [$^{13}C_2$,$^{15}N_2$] xanthine and liquid chromatography-triple quadrupole mass spectrometry (LC-TQMS), which was developed by Murase *et al.*,[13] with minor modifications. Specifically, 100 μl plasma samples (purified by Sephadex G-25 column) were mixed with Tris buffer (pH 8.5) containing [$^{13}C_2$,$^{15}N_2$] uric acid as an internal standard. The mixtures were incubated at 37°C for 90 min. Subsequently, the mixtures were mixed with 500 μl methanol and centrifuged at 3,000 × 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. The amount of [$^{13}C_2$,$^{15}N_2$] uric acid production in plasma samples was quantified from the calibration curve obtained by measuring the calibration standards. XOR activity was expressed as [$^{13}C_2$,$^{15}N_2$] uric acid in pmol/h/ml.

Statistical analysis. Data are reported as the mean ± SD for normal distribution, median [interquartile range (IQR)] for skewed distribution, or frequency counts (%) for categorical data. Simple correlations were examined using the Pearson product-moment correlation analysis. Multiple linear regression analysis was also used to determine the associations of plasma XOR activity with HOMA-IR and the clustered CMR score. Each model was forcibly adjusted for age, height, weight, medication use (antihypertensives, lipid-lowering agents, and glucose-lowering agents), and uric acid. Moreover, a two-way analysis of variance (ANOVA) with pairwise comparisons (Bonferroni method) was performed to assess the effects of combined exposure to standardized BMI (zBMI) and plasma XOR activity (four groups categorized according to each median value) on HOMA-IR and the clustered CMR score. All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Inc., Armonk, NY). Statistical significance was set a priori at p<0.05.

Results

Participant characteristics are summarized in Table 1. The mean (± SD) age was 47 ± 9 years, and the mean (± SD) BMI was 28.0 ± 2.2 kg/m^2. Some of the participants were on medications, including antihypertensives (5%), lipid-lowering agents (2%), and/or glucose-lowering agents (2%).

The results of the simple correlation analyses are presented in Fig. 1 and 2. Plasma XOR activity showed a positive correlation with BMI, waist circumference, visceral fat area, and body fat mass, and an inverse correlation with handgrip strength; however, there was no correlation with skeletal muscle mass (Fig. 1). Additionally, there were positive correlations between plasma XOR activity and hemoglobin A1c, HOMA-IR, serum 8-OHdG, and the clustered CMR score, as well as inverse correlations with HDL cholesterol (Fig. 2). Weight (r = 0.225, p = 0.074), triglyceride (r = 0.226, p = 0.072), fasting plasma glucose (r = 0.205, p = 0.104), and uric acid (r = 0.208, p = 0.100) tended to be positively correlated with the plasma XOR activity. Age, height, LDL cholesterol, systolic and diastolic blood pressure, and heart rate were not significantly associated with the plasma XOR activity. Multiple linear regression analysis demonstrated that the association between plasma XOR activity and HOMA-IR and the clustered CMR score remained significant after adjustment for potential covariates including age, height, weight, medication use (antihypertensives, lipid-lowering agents, and glucose-

Table 1. Participant characteristics

Variable	All (n = 64)
Age, years	47 ± 9
Height, cm	171 ± 6
Weight, kg	82.1 ± 8.3
Body mass index, kg/m^2	28 ± 2.2
Waist circumference, cm	96 ± 6
Visceral fat, cm^2	102 ± 25
Body fat mass, kg	23.3 ± 5.8
Skeletal muscle mass, kg	33.1 ± 3.2
Handgrip strength, kgf/kg	0.5 ± 0.09
HDL cholesterol, mg/dl	52 ± 10
LDL cholesterol, mg/dl	127 ± 26
Triglyceride, mg/dl	115 [81–172]
Hemoglobin A1c, mg/dl	5.6 [5.4–6.0]
Fasting plasma glucose, mg/dl	102 [95–108]
Fasting plasma insulin, μU/ml	8.1 [5.7–10.8]
HOMA-IR, U	2.0 [1.4–2.7]
Systolic blood pressure, mmHg	136 ± 15
Diastolic blood pressure, mmHg	88 ± 11
Heart rate, bpm	64 ± 9
Clustered CMR score, z	0 ± 0.5
Serum 8-OHdG, ng/ml	0.068 [0.038–0.108]
Uric acid, mg/dl	6 ± 1.3
Plasma XOR activity, pmol/h/ml plasma	51 [33–113]
Antihypertensive use, n (%)	5 (8)
Renin-angiotensin system inhibitor, n (%)	3 (5)
Calcium channel blocker, n (%)	3 (5)
Unknown, n (%)	2 (3)
Lipid-lowering use, n (%)	2 (3)
Statins, n (%)	2 (3)
Glucose-lowering use, n (%)	2 (3)
Metformin, n (%)	2 (3)
Sulfonylureas, n (%)	1 (2)
Dipeptidyl peptidase-4 inhibitors, n (%)	1 (2)

Data are presented as the means ± SD, median [interquartile range], or frequency counts (%) as appropriate. HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; CMR, cardiometabolic risk, 8-OHdG; 8-hydroxy-2'-deoxyguanosine, XOR, xanthine oxidoreductase.

lowering agents), and uric acid levels (Table 2).

Figure 3 shows the effects of combined exposure to zBMI and plasma XOR activity (four groups categorized according to each median value) on HOMA-IR and the clustered CMR score. Although there were no significant interaction effects of zBMI and XOR groups on HOMA-IR ($p = 0.151$) and the clustered CMR score ($p = 0.052$), the significant main effects of XOR group were confirmed respectively. Specifically, there were significant differences in HOMA-IR ($p = 0.003$) and the clustered CMR score ($p = 0.004$) between lower and higher plasma XOR activity in the lower zBMI group. The mean values of log-transformed HOMA-IR and the clustered CMR score were lowest in those engaged in both lower zBMI and plasma XOR activity.

Discussion

In this cross-sectional pilot study of overweight and obese Japanese men, we found that the plasma XOR activity was correlated with several CMR markers, including BMI, waist circumference, visceral fat area, body fat mass, handgrip strength, HDL cholesterol, hemoglobin A1c, HOMA-IR, serum 8-OHdG, and the clustered CMR score. Moreover, higher plasma XOR activity was independently associated, albeit modestly, with higher HOMA-IR and the clustered CMR score after adjustment for covariates, including uric acid. Also, the mean values of HOMA-IR and the clustered CMR score were lowest in those engaged in both lower zBMI and plasma XOR activity. These findings extend our knowledge of XOR activity in human plasma and suggest the possibility that circulating XOR activity, but not uric acid, is one of the preventive targets and biomarkers for the development of obesity-related cardiometabolic disorders.

The median value of plasma XOR activity observed in the present study was 51(IQR: 33–113) pmol/h/ml plasma. Kotozaki *et al.*[20] detected plasma XOR activity in 1,605 participants and reported its median value was 35 (IQR: 21–63) pmol/h/ml plasma. In addition, Furuhashi *et al.*[21] reported the median value of plasma XOR activity in the general Japanese population was 36 (IQR: 21–66) pmol/h/ml plasma. Although the sample size of this study is considerably smaller than that of these previous studies, our data indicate that plasma XOR activity in the overweight/obese population is approximately 1.5 times higher than the general population.

Previous studies have reported that plasma XOR activity assessed using the same assay in our study was correlated with BMI, waist circumference, blood pressure, smoking, alanine aminotransferase, uric acid, HDL cholesterol, triglycerides, hemoglobin A1c, fasting glucose and insulin, HOMA-IR, and several adipokines in the Japanese general population.[21,22] Similarly, another previous study in a small number of young adults showed that plasma XOR activity was positively correlated with BMI, uric acid, high-sensitivity C-reactive protein, and negatively correlated with the quantitative insulin sensitivity check index and adiponectin.[19] Our previous study also demonstrated that plasma XOR activity was significantly correlated with BMI, visceral fat area, waist circumference, systolic blood pressure, fasting glucose, and daily step counts in middle-aged and older women.[25] In addition to these findings, the present observations confirmed that plasma XOR activity was significantly correlated with handgrip strength, serum 8-OHdG, and the clustered CMR score in overweight and obese men. To the best of our knowledge, this pilot study provides one of the first pieces of evidence that plasma XOR activity can be associated with muscular strength, oxidative stress, and clustered CMR in overweight/obesity.

There is literature that may partly explain a positive correlation of plasma XOR activity with handgrip strength and serum 8-OHdG observed in this study. Ryan *et al.*[26] demonstrated that xanthine oxidase inhibition by allopurinol reduced H_2O_2 levels, lipid peroxidation, and caspase-3 activity and improved maximal isometric force in the plantar flexor muscles in aged mice. Moreover, xanthine oxidase-derived oxidant production has been reported to be involved in muscle atrophy via the activation of the p38MAPK-MAFbx pathway.[27] Since the participants of this study were relatively young and healthy overweight and obese men, a significant association was not observed between skeletal muscle mass and plasma XOR activity. However, these findings collectively suggest that XOR activity is one of the putative factors responsible for altering skeletal muscle physiology and function.

Increased uric acid levels (hyperuricemia) are known to be independently associated with the development of cardiometabolic diseases.[14] However, the causal inferences between hyperuricemia and cardiometabolic disorders remain highly controversial. Indeed, the treatment of lowering uric acid levels does not necessarily lead to an improved prognosis.[28] In this context, XOR activity, but not uric acid levels, was recently

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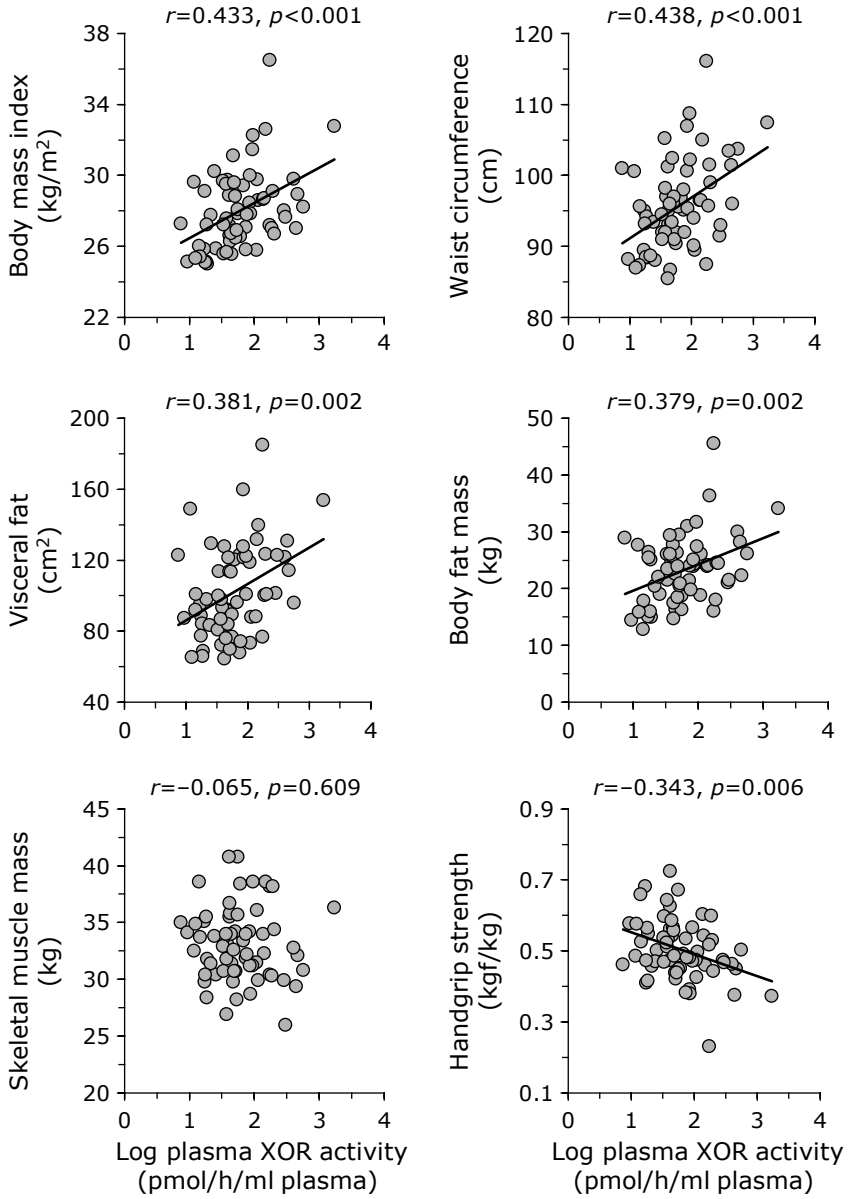

Fig. 1. Scatter plots showing the associations of plasma XOR activity with anthropometric measurements and handgrip strength. XOR, xanthine oxidoreductase.

suspected to be a true risk factor for hyperuricemia-induced diseases.[29] Our study also showed that plasma XOR activity, but not uric acid levels, was an independent determinant of HOMA-IR and the clustered CMR score in overweight and obese men who did not take an XOR inhibitor. Therefore, these preliminary findings indicate that lowering the plasma XOR activity, rather than lowering the uric acid levels, can be more important in preventing the development of cardiometabolic disease in overweight/obesity.

The clinical usefulness of measuring redox stress (e.g., XOR activity) in predicting future cardiometabolic events has been indicated in recent literature. For example, Otaki *et al.*[10] reported that XOR activity in plasma was significantly associated with severity and cardiac events in patients with chronic heart failure. Moreover, previous research investigating the association between plasma XOR activity and the Framingham Risk Score showed that elevated XOR activity in plasma had approximately 80% chances of predicting the high risk for cardiovascular disease (Framingham Risk Score ≥15).[20] Similarly, our cross-sectional data demonstrated that higher plasma XOR activity was independently associated with a higher clustered CMR score. In the perspective of risk assessment, therefore, these findings collectively suggest that close monitoring of circulating XOR activity may help the primary prevention of cardiometabolic disease in clinical settings.

Our previous study reported that the plasma XOR activity was inversely associated with daily step counts and decreased by 12-week exercise training in middle-aged and older women [25]. Moreover, the present study showed that handgrip strength (i.e., muscular strength) was inversely associated with the plasma XOR activity in overweight and obese men. Although future interventional studies are needed to confirm whether exercise training decreases plasma XOR activity in overweight/obesity, these findings collectively suggest that lifestyle modifications, especially exercise, can potentially lead to a decrease in plasma XOR activity.

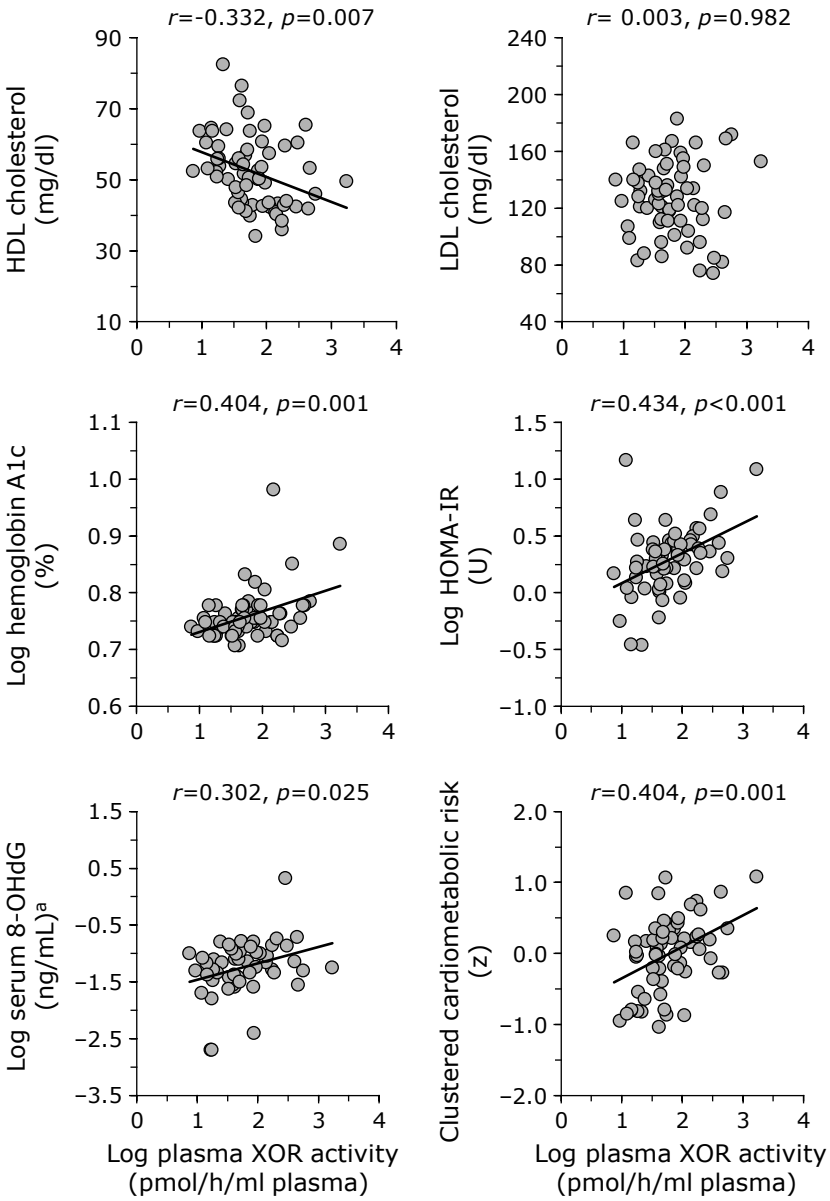

Fig. 2. Scatter plots showing the associations of plasma XOR activity with several cardiometabolic risk markers. [a]Data were available in 55 participants. XOR, xanthine oxidoreductase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

The present study had several noteworthy limitations. First, this was a cross-sectional pilot study with a small sample size, which strongly limits our ability to assess the precise causal associations between plasma XOR activity and CMR markers. Thus, we cannot exclude the possibility that the cardiometabolic status may affect plasma XOR activity. Second, generalizing our results is considerably challenging because our study was a single-center study with a selective population (overweight and obese Japanese men). Third, the participants of this study were relatively young and healthy overweight and obese men and few individuals had a BMI $\geq$30 kg/m^2 (n = 8). In addition, few participants were taking medication such as antihypertensives, lipid-lowering agents, and/or glucose-lowering agents. It has been recently considered that overweight/obesity under certain conditions (i.e., metabolically healthy obesity) may not be associated with a substantially increased risk of cardiometabolic diseases.[30] Therefore, the present findings are preliminary and must be carefully interpreted and be confirmed by more large samples which included healthy normal-weight, and/or severe obese individuals. Finally, the pathophysiological mechanisms underlying the association between plasma XOR activity and CMR markers were not investigated in this study. Therefore, future prospective or interventional studies and basic experimental studies are needed to identify the causal structure of associations and potential mechanistic pathways linking circulating XOR activity with adverse cardiometabolic outcomes in overweight/obesity.

Conclusion

Higher plasma XOR activity was negatively associated, albeit modestly, with several CMR markers, including HOMA-IR and the clustered CMR score in overweight and obese Japanese men. These cross-sectional findings suggest that circulating XOR

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Table 2. Multivariate-adjusted associations of plasma XOR activity with HOMA-IR and clustered CMR score

Variable	B ± SE	β	p value
Dependent variable: HOMA-IR* (R^2 = 0.264, p = 0.024)			
Age, years	−0.003 ± 0.004	−0.091	0.481
Height, cm	−0.002 ± 0.008	−0.048	0.769
Weight, kg	0.005 ± 0.006	0.148	0.387
Antihypertensive use, yes	0.122 ± 0.144	0.114	0.4
Lipid-lowering use, yes	−0.128 ± 0.222	−0.078	0.566
Glucose-lowering use, yes	0.342 ± 0.234	0.208	0.149
Uric acid, mg/dl	0.012 ± 0.027	0.056	0.654
Plasma XOR activity, pmol/h/ml plasma*	0.206 ± 0.083	0.338	0.017
Dependent variable: Clustered CMR score (R^2 = 0.278, p = 0.016)			
Age, years	0.003 ± 0.008	0.045	0.725
Height, cm	0.007 ± 0.014	0.078	0.636
Weight, kg	0.009 ± 0.011	0.137	0.42
Antihypertensive use, yes	−0.236 ± 0.257	−0.123	0.362
Lipid-lowering use, yes	−0.515 ± 0.397	−0.174	0.199
Glucose-lowering use, yes	0.474 ± 0.418	0.16	0.261
Uric acid, mg/dl	0.052 ± 0.048	0.133	0.284
Plasma XOR activity, pmol/h/ml plasma*	0.371 ± 0.149	0.338	0.016

β indicate unstandardized and standardized regression coefficients respectively. HOMA-IR, homeostatic model assessment of insulin resistance; CMR, cardiometabolic risk, XOR, xanthine oxidoreductase. *Log transformed.

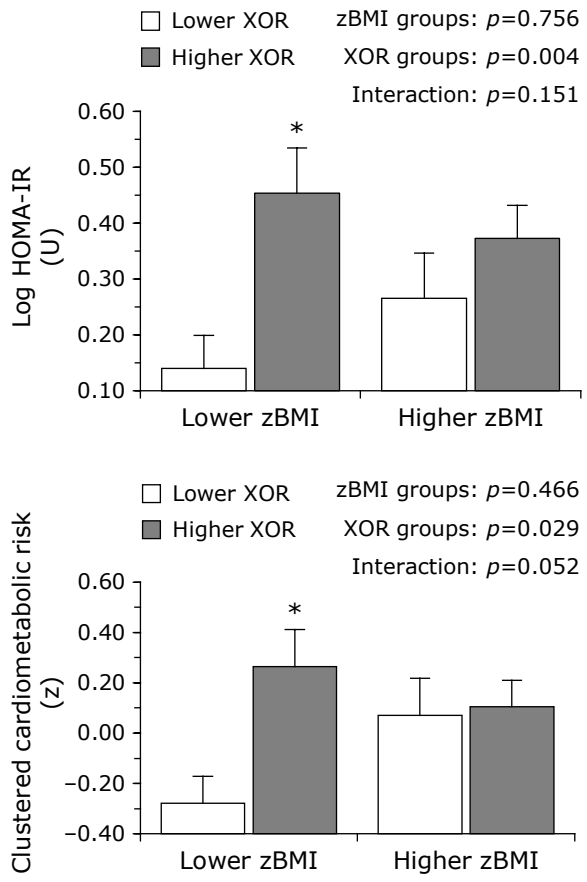

Fig. 3. Log-transformed HOMA-IR and clustered cardiometabolic risk compared among zBMI and XOR groups. *P* values were calculated by two-way analysis of variance. Data are presented as mean ± SE. *p<0.05 vs Lower XOR group. Multiple pairwise comparisons were corrected by the Bonferroni method. zBMI, standardized body mass index; XOR, xanthine oxidoreductase; HOMA-IR, homeostasis model assessment of insulin resistance.

activity can be one of the targets for intervention to prevent obesity-related cardiometabolic disorders.

Author Contributions

KK: study concept and design. AY, KT, KM, JP, and TY: acquisition of data. KK, YY, TM, SA, and TN: analysis and interpretation of data. KK: drafting of the manuscript. KK, AY, KT, KM, JP, TY, YY, TM, SA, TN, and SM: critical revision of the manuscript for important intellectual content. KK: statistical analysis; KK, SM: obtained funding. YY, TM, SA, and TN: administrative, technical, or material support. SM: study supervision.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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