Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.7101-26
Intern Med Advance Publication
http://internmed.jp

【 ORIGINAL ARTICLE 】

The urinary Isoxanthopterin/pterin Ratio Reflects Mild Fasting Hyperglycemia in Obese Patients with Type 2 Diabetes Mellitus: An Exploratory Cross-sectional Study

Rei Chinen[1], Takashi Shirakura[2], Ryoma Hashimoto[1], Yohei Ishiki[1], Atsuko Tamaki[1], Ken-Ichiro Honma[1], Chie Horiguchi[1], Tsugumi Uema[1], Koshi Nakamura[3] and Hiroaki Masuzaki[1]

Abstract:

Objective The exaggerated activation of xanthine oxidase (XO) provokes oxidative stress, a feature of type 2 diabetes mellitus (T2DM) and obesity. Because measuring the plasma XO activity is complicated, we tested whether urinary isoxanthopterin (IXP), the end product of pterin metabolism catalyzed by XO, could serve as an alternative marker.

Methods We enrolled 51 patients with mild T2DM and obesity (BMI ≥25 kg/m^2; median HbA1c 6.7%) and 9 healthy individuals. Urinary IXP level was determined by HPLC-FL. Correlations between 24-hour and spot urinary IXP were examined in healthy individuals to validate spot urine, followed by analyses in patients stratified by the median IXP/Pterin ratio.

Results In healthy individuals, spot urinary IXP correlated with 24-h measurements (r=0.795, p=0.010); however, both urinary IXP levels and the IXP/Pterin ratio were not correlated with the XO activity. In patients with mild T2DM and obesity, the urinary IXP/pterin ratio was significantly associated with fasting plasma glucose (p=0.030). Among patients with higher urinary IXP/Pterin ratios, this association was tightly independent of other clinical parameters (p=0.028).

Conclusion The level of spot urinary IXP was correlated with that of 24-h excretion, but not with circulating XO activity. The ratio of IXP/Pterin was associated with fasting hyperglycemia and provides a novel avenue to evaluate glucose dysmetabolism in obese patients with mild T2DM.

Key words: fasting plasma glucose, isoxanthopterin, obesity disease, pterin, type 2 diabetes mellitus, xanthine oxidase

(Intern Med Advance Publication)
(DOI: 10.2169/internalmedicine.7101-26)

Introduction

Xanthine oxidase (XO), a rate-limiting enzyme in uric acid (UA) synthesis, simultaneously produces reactive oxygen species (ROS), such as H_2O_2 and O_2^- (1, 2). Along with NADPH oxidase, XO is a major source of oxidative stress throughout the body (3). We previously demonstrated that the plasma XO activity is well correlated with insulin resistance, body mass index (BMI), and liver dysfunction in patients with type 2 diabetes mellitus (T2DM) and obesity (4). Metabolic abnormalities associated with XO-derived oxidative stress considerably exacerbate vascular damage, highlighting the importance of an early assessment of residual cardiovascular risk (5, 6).

In general, the serum UA level is regarded as a surrogate

[1]Division of Endocrinology, Diabetes and Metabolism, Hematology and Rheumatology, Second Department of Internal Medicine, Graduate School of Medicine, University of the Ryukyus, Japan, [2]Teijin Pharma Limited, Japan and [3]Department of Public Health and Epidemiology Graduate School of Medicine, University of the Ryukyus, Japan
Received: January 8, 2026; Accepted: February 25, 2026; Advance Publication by J-STAGE: April 14, 2026
Correspondence to Dr. Hiroaki Masuzaki, hiroaki@cs.u-ryukyu.ac.jp

marker for obesity (7-9). Indeed, this level is influenced by various factors, including dehydration, purine-rich diet, alcohol consumption, and impaired excretion from the kidneys and intestines (10). In accordance with this notion, our previous data suggested that patients with normal serum UA levels but elevated plasma XO activity represent an increased risk for cardiovascular complications (4). However, the measurement of the plasma XO activity requires complex procedures and strict temperature control, thus limiting its clinical applicability (7).

In contrast, isoxanthopterin (IXP), which is mainly excreted in urine, is a stable end product derived from pterin by XO (1, 2, 11, 12). Unlike plasma XO activity measurement, urinary sample collection for IXP is feasible in clinical settings, thereby offering a practical advantage over plasma XO measurement (13, 14). Limited studies have reported that the urinary excretion of IXP is increased in patients with neuromuscular diseases, bladder cancer, heart failure (13, 15, 16), and children with type 1 diabetes (17). However, the clinical implications of urinary IXP in patients with T2DM or obesity remain unclear. Therefore, we explored the potential benefits of urinary IXP and pterin concentrations in assessing the pathophysiology of obese patients with mild T2DM.

Materials and Methods

Study design

This study was designed to explore the clinical implications of urinary IXP and pterin in patients with T2DM and obesity in a single center, cross-sectional study. The study was approved by the Research Ethics Review Committee of the Faculty of Medicine at the University of the Ryukyus on April 22, 2023 (approval number 23-2119-00-00-00) and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Based on our previous work (4), the present study was conducted to investigate whether the urinary indices related to the IXP metabolism, particularly the urinary IXP/Pterin ratio (hereafter referred to as the IXP/Pterin ratio), are associated with the metabolic parameters known to correlate with plasma xanthine oxidase activity. Such parameters include indices of insulin resistance (homeostatic model assessment of insulin resistance: HOMA-IR) and the circulating levels of liver transaminases. We further explored the possible association between a dichotomized urinary IXP/Pterin ratio and fasting plasma glucose (FPG).

Participants

Between June 2023 and February 2024, 51 patients aged ≥18 years with type 2 diabetes mellitus and obesity were enrolled. All patients had a body mass index (BMI) ≥25 kg/m^2, estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2, without serious liver disease, active infection, or malignancy. We selectively included patients with mild type 2 diabetes mellitus, defined as a hemoglobin A1c (HbA1c) level of 6.0-7.0%, managed by diet therapy with or without oral anti-diabetic medication. In the present study, obesity disease refers to the definition in the 2022 Japanese Society for the Study of Obesity Guidelines (18). Patients treated with insulin or GLP-1 receptor agonists were excluded; however, the use of other oral antidiabetic agents, including metformin, SGLT2 inhibitors, and thiazolidinediones, was approved. None of the participants were receiving uric acid-lowering agents. Of the 56 enrolled participants, two withdrew, and three patients with pre-existing heart disease were excluded. We also included nine healthy individuals (four men aged 30-49 years and five women aged 40-50 years) to validate the urinary IXP measured in spot urine samples as an alternative to that measured in 24-h urine samples.

Sample collection

Fasting morning void urine samples were collected at 09:00, and fasting blood samples were collected to measure clinical parameters, plasma XO activity, urinary IXP, and pterin concentrations. To avoid hypoxanthine leakage from red blood cells into the plasma (4), blood samples were centrifuged within 1 h after collection. The supernatant was transferred to a new tube and stored at -80°C as plasma samples.

For healthy individuals, urinary creatinine and IXP concentrations, as well as 24-h total urine volume, were measured from 24-h pooled urine samples. The 24-h IXP excretion was calculated based on urine volume assessed using urine collection containers (MD-63350, SB-KAWASUMI LABORATORIES, Inc., Kanagawa, Japan). Urine samples were stored at -30°C until further analysis.

Measurement of metabolic parameters

Body composition was measured using a body composition analyzer (Inbody 770; Inbody Japan Inc.). The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γ-GTP), triglycerides (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), creatinine, FPG, and fasting immunoreactive insulin (FIRI) were measured using an automatic analyzer. High-sensitivity C-reactive protein (hs-CRP) and high-molecular-weight adiponectin levels were measured using the latex agglutination method (automated analyzer 07E1X8000, JEOL Ltd., Tokyo, Japan) and an electrochemiluminescence enzyme immunoassay (LUMIPASS).

Measurement of urinary IXP and pterin

Urinary IXP and pterin concentrations were measured using high-performance liquid chromatography (HPLC) with a fluorescence detector (4, 7, 12, 13). Fifty μL of urine was mixed with 50 μL of 0.05 mol/L Tris-HCl buffer (pH 9.0), 100 μL of 4% $HClO_4$ was added, and centrifuged at 15,000× g for 10 min at room temperature. The supernatant (150 μL)

was neutralized with 6 μL of 5 mol/L K_2CO_3 and centrifuged again at 15,000× g for 10 min at room temperature. The final supernatant was used for the HPLC-FL analysis. HPLC was performed on a YMC-Triart C18 column (150× 4.6 mm I.D., S-3 μm, 12 nm) (YMC, Kyoto, Japan) using a Nexera X2 (Shimadzu Corporation, Kyoto, Japan) and a fluorescence detector RF-20Axs (Shimadzu Corporation). The mobile phase was 0.05 mol/L phosphate buffer (pH 7.0) with a flow rate of 0.5 mL/min, the excitation wavelength for both pterin and IXP was 345 nm, and the fluorescence wavelength was 450 nm. Standard solutions were 31.25-2,000 nmol/L of pterin and IXP solutions diluted in 0.1N NaOH or 0.05 mol/L Tris-HCl buffer (pH 9.0), respectively. The area under the curve (AUC) of the urine was calculated, and the concentrations were calibrated using standard curves. The urinary IXP and pterin concentrations are expressed in nmol/L and corrected for the urinary creatinine levels (μmol/g Cr).

Measurement of plasma XO activity

The plasma XO activity was measured using a highly sensitive fluorescence assay (4, 7) as previously described. The enzyme reaction was initiated by mixing 50 μL of plasma with 50 μL of 0.05 mol/L Tris-HCl buffer (pH 9.0) containing 100 μmol/L pterin and 1% dimethyl sulfoxide. For the blank sample, 0.05 mol/L Tris-HCl buffer (pH 9.0) was used instead of plasma. After incubation at 37°C for 3 h, the reaction was stopped by the addition of 100 μL of 4% $HClO_4$. The resulting mixture was shaken vigorously and centrifuged at 15,000× *g* for 10 min at room temperature. Next, 150 μL of the supernatant was neutralized with 6 μL of 5 mol/L K_2CO_3 and centrifuged at 15,000× *g* for 10 min at room temperature. Ten microliters of the supernatant were subjected to fluorometric analysis. The plasma XO activity was measured using the same method as described previously (4). The plasma XO activity is expressed as pmol isoxanthopterin/min/mL (pmol IXP/min/mL).

Statistical analysis

We analyzed the IXP-to-creatinine ratio, urinary pterin-to-creatinine ratio, and IXP/Pterin ratio to evaluate their associations with a line of metabolic parameters. All values are expressed as the mean ± standard deviation, median, interquartile range (IQR), or percentage (%), appropriately. Missing data were handled using multiple imputations. To examine intergroup differences in characteristics, the Mann-Whitney U test was used for numerical variables with a non-normal distribution, the unpaired t-test for numerical variables with a normal distribution, and the chi-squared test for categorical variables. In healthy individuals, we examined the correlations between 24-h urine and fasting morning samples for urinary IXP/Pterin via Spearman's rank correlation coefficient analysis. In patients, we investigated the urinary IXP-to-creatinine ratio, urinary pterin-to-creatinine ratio, and urinary IXP/Pterin ratio. Histograms were constructed to visualize the distribution of each data point of the urinary IXP-to-creatinine ratio, urinary pterin-to-creatinine ratio, and urinary IXP/Pterin ratio. We examined the correlations between each outcome variable and various metabolic parameters using a Spearman rank correlation coefficient analysis. We compared each metabolic parameter for the patients grouped according to the lower or higher levels of urinary IXP/Pterin ratio based on the median value using the Mann-Whitney U test or unpaired t-test. Because the urinary IXP/Pterin ratios showed a wide and overlapping distribution among the patients, and to date, no established clinical cutoff has been available, we used the median value to stratify the patient group into two groups.

We then performed a logistic regression analysis in which the dichotomous variable of the urinary IXP/Pterin ratio was used as the dependent variable, and the FPG, Fib-4 index, UA, eGFR, and plasma XO activity were used as independent variables. Because IXP is a product of the enzymatic conversion of pterin by XO (19), plasma XO activity and the variables associated with plasma XO activity demonstrated in our previous study (4) were selected. The receiver operating characteristic (ROC) curve illustrated by this model yielded an area under the curve (AUC). We further performed a logistic regression analysis after adjusting for the presence of fatty liver and diabetes duration. As a sensitivity analysis, we conducted bootstrap resampling (2,000 iterations) based on the same multivariate logistic regression model.

Statistical significance was set at $p<0.05$. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (20), a graphical user interface for R (Ver 4.5.1: The R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of Participants

The characteristics of the patients included in the study are summarized in Table 1. In the nine healthy individuals, the urinary IXP-to-creatinine ratio measured in the 24-h urine sample showed a strong correlation with that in the first-morning void sample ($r=0.795$, $p=0.010$) (Fig. 1A). Plasma XO activity was significantly higher in the patient group than in the healthy individual group (Fig. 1B). In contrast, no significant differences were noted in the urinary IXP level (Fig. 1C) or the IXP/Pterin ratio between the patient and healthy individual groups (Fig. 1D). In healthy individuals, we examined the relationships between the urinary IXP/Pterin ratio and various metabolic parameters (Table S1). There were no correlations between plasma XO activity and the urinary IXP-to-creatinine ratio (Fig. S1A) or the IXP/Pterin ratio (Fig. S1B).

Associations Between FPG, eGFR, and Urinary IXP/Pterin Ratio

In 51 patients, the distributions of the urinary IXP-to-

Table 1. Clinical Characteristics of Studied Participants.

Anthropometric and clinical parameters *(median [IQR] or mean ± SD)*	Patients *(median [IQR] or mean ± SD)*	Healthy individuals *(median [IQR] or mean ± SD)*	p value
N (M:F)	51 (31:20)	9 (4:5)	
Age (years)	58 (52–64)	42 (40–45)	<0.001[a]
Duration of diabetes (years)	9 (5–12)	NA	
Waist circumference (cm)	98 (92–105.5)	74 (67.8–80.2)	0.002[a]
BMI (kg/m^2)	28 (26.5–32.4)	22.9 (21.1–24.4)	<0.001[a]
HbA1c NGSP (%)	6.7 (6.4–7.0)	5.2 (5.2–5.3)	<0.001[a]
HbA1c IFCC (mmol/mol)	50 (46–52)	33 (33–34)	<0.001[a]
FPG (mmol/L)	6.8 (6.1–7.4)	5.5 (5.2–5.8)	0.001[a]
FIRI (μIU/mL)	9.5 (5.9–15.5)	10.0 (5.67–17.9)	0.709[a]
HOMA-IR	2.8 (1.9–4.6)	2.4 (1.2–4.0)	0.717[a]
C-peptide (ng/mL)	2.2 (1.6–2.9)	2.3 (1.1–2.5)	0.627[a]
ALT (U/L)	22 (16–31.5)	18 (13–20)	0.229[a]
AST (U/L)	20 (17–24.5)	20 (17–22)	0.844[a]
γ-GTP (U/L)	30 (20.5–46)	24 (18–38)	0.330[a]
Fib-4 index	1.094±0.5	0.8±0.3	0.090[b]
Cre (mg/dL)	0.73 (0.65–0.83)	0.79 (0.65–0.88)	0.626[a]
eGFR (mL/min/1.73m^2)	77 (69–83.5)	76 (73–83.1)	0.975[a]
Urinary microalbumin (mg/g/Cr)	8.7 (1.3–26.1)	–	
UA (mg/dL)	5.4 (4.4–6.1)	4.4 (4.2–5.2)	0.118[a]
HDL (mg/dL)	50 (43.5–57)	68 (60–82)	0.004[a]
TG (mg/dL)	133 (93–179.5)	84 (67–94)	0.021[a]
LDL (mg/dL)	110±31	113±27	0.826[b]
hs-CRP (mg/dL)	0.08 (0.04–0.2)	0.03 (0.02–0.04)	0.008[a]
Body fat percentage (%)	34.4 (29.1–40.1)	–	
Fatty liver (+), n (%)	44 (86.3)	NA	
Urinary creatinine (mg/dL)	82.9 (49.8–121.6)	93.0 (70.7–107.2)	0.836[a]
Plasma XO activity (pmol IXP/min/mL)	0.34 (0.21–0.74)	0.16 (0.14–0.28)	0.024[a]
Urinary IXP concentration (nmol/L)	351 (239–564)	195 (131–432)	0.199[a]
Urinary IXP to creatinine ratio (μmol/g Cr)	0.5 (0.3–0.8)	0.2 (0.2–0.5)	0.185[a]
Urinary Pterin concentration (nmol/L)	322 (259–533)	309 (224–471)	0.494[a]
Urinary Pterin to creatinine ratio (μmol/g Cr)	0.4 (0.3–0.6)	0.3 (0.3–0.6)	0.203[a]
Urinary IXP / Pterin ratio	0.96 (0.64–1.30)	0.82 (0.58–1.23)	0.562[a]

Continuous variables are expressed as a median (1st quartile–3rd quartile) or a mean ± standard deviation. Numbers of participants are shown as n (men:women). –, not assessed; NA, not applicable. ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMI: body mass index, eGFR: estimated glomerular filtration rate, FIRI: fasting immunoreactive insulin, FPG: fasting plasma glucose, γ-GTP: γ-glutamyltransferase, HbA1c: glycated hemoglobin, HDL: high-density lipoprotein cholesterol, HOMA-IR: homeostatic model assessment of insulin resistance, hs-CRP: high-sensitivity C-reactive protein, IFCC: International Federation of Clinical Chemistry, LDL: low-density lipoprotein cholesterol, NGSP: National Glycohemoglobin Standardization Programme, TG: triglycerides, UA: uric acid, XO: xanthine oxidase. [a] Mann-Whitney U test; [b] unpaired t-test.

creatinine ratio (Fig. 2A), pterin-to-creatinine ratio (Fig. 2B), and IXP/Pterin ratio (Fig. 2C) were non-normal. In contrast, there was a significant correlation between the urinary IXP-to-creatinine ratio and the urinary pterin-to-creatinine ratio (Fig. 2D).

A correlation analysis within the patients did not reveal any significant correlations between the urinary IXP-to-creatinine ratio and body mass index, homeostasis model assessment of insulin resistance, hepatic enzyme levels, or UA. No correlation was observed between the urinary pterin-to-creatinine ratio and any metabolic parameters.

A significant positive relationship was found between the urinary IXP/Pterin ratio and FPG (r=0.313, p=0.030) (Fig. 3A), as well as a significant negative association between the urinary IXP/Pterin ratio and eGFR (r=–0.314, p=0.010) (Fig. 3B). There were no correlations between the plasma XO activity and the urinary IXP-to-creatinine ratio (Fig. S1C) or the IXP/Pterin ratio (Fig. S1D) in patients with T2DM and obesity. Among the scatter plot showing the significant relationship between the urinary IXP/Pterin ratio and FPG (Fig. 3C), patients with a urinary IXP/Pterin ratio ≥0.96 showed significantly lower eGFR (p<0.05) as well as significantly higher FPG values (p<0.05) compared to the group with a urinary IXP/Pterin ratio <0.96 (Table S2).

The plasma XO activity did not differ between the two groups (p=0.88; Table S2). Similarly, no significant correlation between FPG and the urinary IXP/Pterin ratio was observed within each group (the urinary IXP/Pterin ratio over

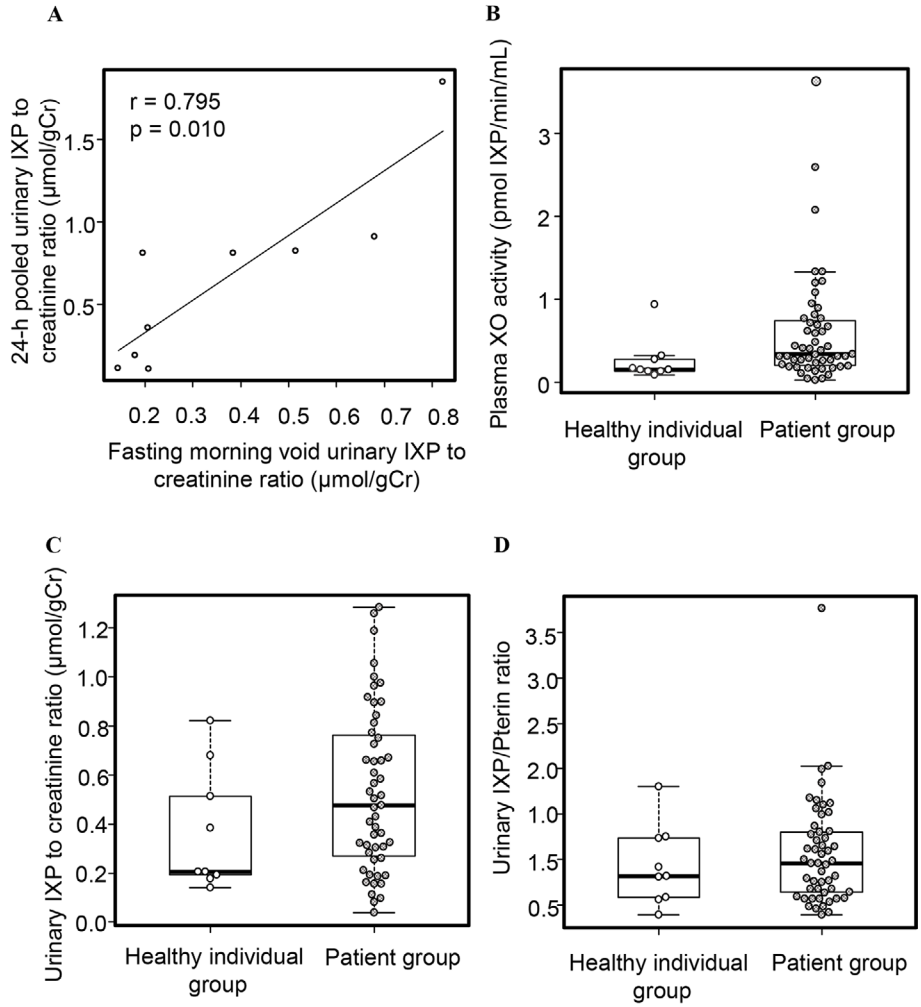

Figure 1. Validation of urinary IXP measurement and comparison between the healthy individuals and patients with type 2 diabetes mellitus. (A) Correlation between 24-hour urinary isoxanthopterin (IXP) and spot urinary IXP in healthy individuals (n=9). A significant correlation was observed (r=0.795, p=0.010). Data are shown as individual values with regression lines. A statistical analysis was performed using Spearman rank correlation coefficient. (B-D) Comparison of the plasma XO activity, urinary IXP to creatinine ratio and urinary IXP/Pterin ratio between healthy individuals and patients with type 2 diabetes mellitus and obesity. (B) The plasma XO activity was significantly higher in the patient group compared to the healthy individual group (p=0.020, Mann-Whitney U test). (C) Urinary IXP to creatinine ratio and (D) urinary IXP/Pterin ratio did not differ significantly between the two groups. Cre: creatinine, IXP: isoxanthopterin, XO: xanthine oxidase

0.96 group: r=0.119, p=0.560; the urinary IXP/Pterin ratio under 0.96 group: r=0.110, p=0.601; Fig. 3C).

A logistic regression analysis with the dichotomized urinary IXP/Pterin ratio as the dependent variable demonstrated that higher FPG was associated with a higher urinary IXP/Pterin ratio (odds ratio [OR]: 1.84; 95% Confidence Interval [CI]: 1.07-3.17; p=0.028; Table 2). In contrast, elevated urinary IXP/Pterin ratios were neither significantly associated with eGFR (p=0.196) nor the plasma XO activity (p=0.561; Table 2). The ROC curve derived from this model yielded an area under the curve (AUC) of 0.732 (95% CI: 0.59-0.87; Fig. S2).

The association between the IXP/Pterin ratio and FPG remained significant even after adjusting for fatty liver and diabetes duration (OR:1.66; 95% CI: 1.00-2.75; p=0.048; Table S3). Bootstrap resampling analyses (OR, 1.84; 95% CI, 1.22-6.36; Table S4) yielded estimates and confidence intervals that were largely comparable to those in the primary analysis (OR, 1.84; 95% CI, 1.07-3.17; Table 2).

Discussion

To our knowledge, the present study is the first to demonstrate that the urinary IXP/Pterin ratio is significantly associated with elevated FPG levels in patients with obese T2DM.

Among patients with a higher urinary IXP/Pterin ratio, this association was strongly independent of other clinical parameters (Fig. 3A, Table 2). XO converts pterin into IXP (11, 18). Therefore, we examined whether urinary IXP reflected the plasma XO activity and utilized an alternative

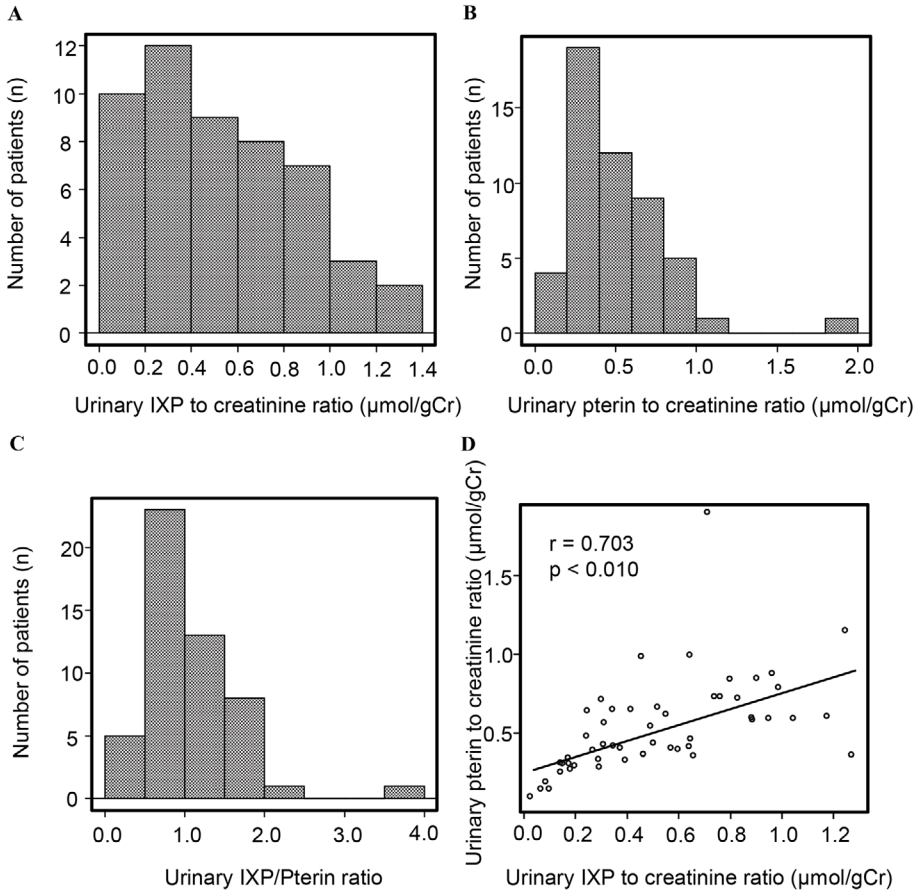

Figure 2. Histograms of urinary IXP-related values in patients with type 2 diabetes mellitus and obesity. Histograms of urinary IXP to creatinine ratio (A), urinary pterin to creatinine ratio (B), and urinary IXP/Pterin ratio (C) in patients with type 2 diabetes mellitus and obesity. There was a significant correlation between the urinary IXP to creatinine ratio and urinary pterin to creatinine ratio (r=0.703, p<0.01, Spearman rank correlation coefficient; D). Cre: creatinine, IXP: isoxanthopterin

biomarker of the plasma XO activity. In healthy individuals, the urinary IXP concentrations in spot urine were strongly correlated with those in 24-h urine samples (Fig. 1A), indicating that spot urine measurements reliably reflect daily urinary IXP excretion. Therefore, spot urine samples were used for subsequent analyses in the present study.

In the present study, the plasma XO activity levels were significantly higher in patients with obese T2DM than in healthy individuals (Fig. 1B), consistent with our previous report (4). However, no significant differences were observed in the urinary IXP-to-creatinine ratio and pterin-to-creatinine ratios between patients with obese T2DM and healthy individuals (Table 1). In addition, no significant association was found between the plasma XO activity and the urinary IXP levels in either group (Fig. S1). Such a lack of association may be due to the overlap in the interquartile ranges of the urinary IXP-to-creatinine ratio (IQR; patients with obese T2DM: IQR 0.3-0.8; healthy individuals: IQR 0.2-0.5; Table 1). As the plasma XO activity is largely derived from hepatic sources (4), these findings reflect physiological differences in the regulation of purine metabolism between systemic circulation and urine. Because urinary IXP represents an end-stage metabolite excreted into the urine, these findings may also suggest that the urinary IXP/Pterin ratio reflects a more complex metabolic state than XO enzymatic activity *per se*. A recent study in patients with gout demonstrated that the value of urinary IXP is helpful in identifying clinically occult gout (12). However, to date, no clear relationship has been reported between urinary IXP and plasma XO activity.

In the present study, no significant differences were observed in the concentrations of urinary IXP and pterin between patients and healthy individuals. Pterin is oxidized to IXP by xanthine oxidoreductase (XOR). Therefore, this conversion reflects the activity of XOR, and importantly, this conversion has been shown to be stable in healthy individuals (11). Based on this notion, we focused on the urinary IXP/Pterin ratio. Pterin is a substrate of XO, and IXP is a metabolite of this reaction. In the present study, a significant correlation was observed between urine IXP and urine pterin (Fig. 2D). Based on this finding, we focused on the urinary IXP/Pterin ratio for subsequent analyses. However, no correlation was found between the urinary IXP/Pterin ratio and plasma XO activity, and simple regression analysis did not demonstrate any significant relationship. Given the wide and overlapping distribution of urinary IXP levels between pa-

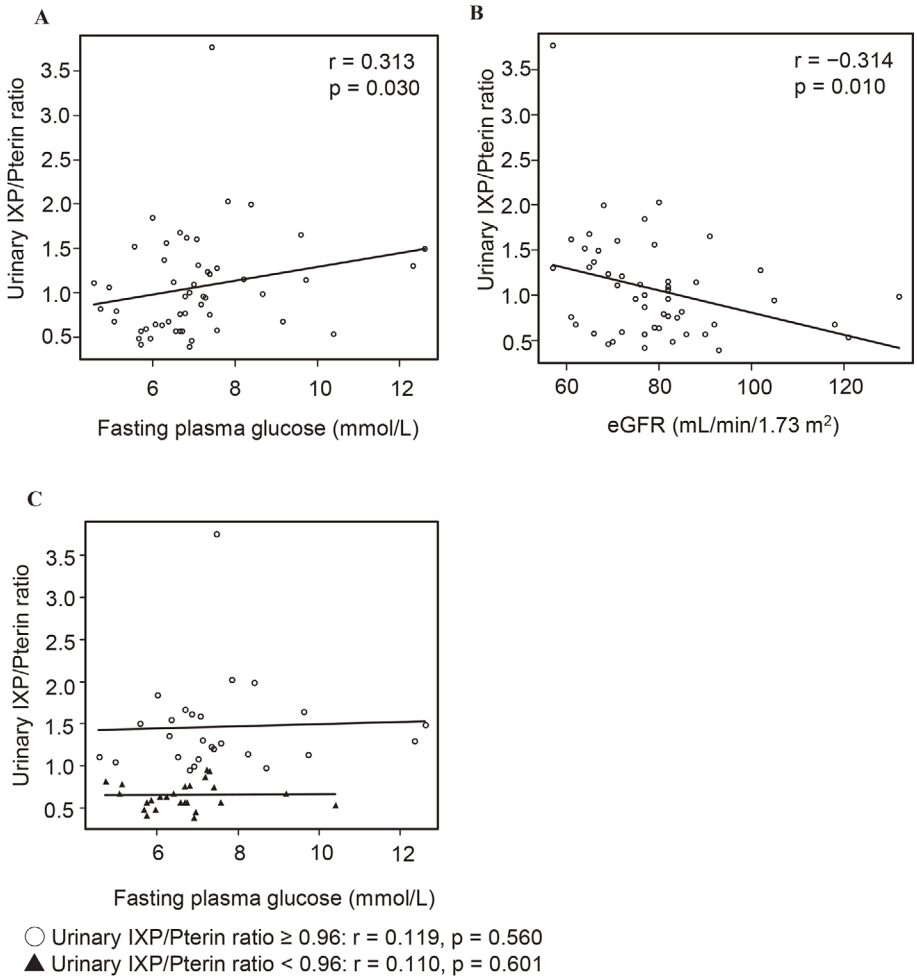

Figure 3. Correlations between urinary IXP/Pterin ratio and FPG or eGFR in type 2 diabetes patients with obesity. (A) FPG: correlation coefficient r=0.313, p=0.030. (B) eGFR: r=-0.314, p=0.010. Spearman rank correlation coefficients were used for both analyses. (C) Scatter plot of FPG versus the urinary IXP/Pterin ratio, stratified by the median urinary IXP/Pterin ratio (0.96): ≥0.96 (○) and <0.96 (▲). Regression lines for each group are shown. No significant correlation was observed within each group (the urinary IXP/Pterin ratio ≥0.96 group: r=0.119, p=0.560; the urinary IXP/Pterin ratio <0.96 group: r=0.110, p=0.601). Cre: creatinine, eGFR: estimated glomerular filtration rate, FPG: fasting plasma glucose, IXP: isoxanthopterin

Table 2. Logistic Regression Analysis was Performed with Urinary IXP/Pterin Ratio as the Dependent Variable.

Variable	Odds ratio	95% CI	p value
FPG	1.84	1.07–3.17	0.028
Fib-4index	1.02	0.21–4.88	0.980
UA	1.29	0.70–2.39	0.422
eGFR	0.96	0.91–1.02	0.196
Plasma XO activity	0.71	0.22–2.29	0.561

The urinary IXP/Pterin ratio in the patient group was divided into low (<0.96) and high (≥0.96) groups based on the median values (0.96), and logistic regression analysis was performed with urinary IXP/Pterin ratio divided into two groups as the dependent variable. CI: confidence interval, eGFR: estimated glomerular filtration rate, FPG: fasting plasma glucose, IXP: isoxanthopterin, ROC: receiver operating characteristic, UA: uric acid, XO: xanthine oxidase

tients and healthy individuals, we attempted to stratify the patient group into two groups based on the median IXP/Pterin ratio (Fig. 3C). Consequently, logistic regression analyses demonstrated that the IXP/Pterin ratio was associated with FPG levels (Table 2). In the logistic regression analysis, in which fatty liver and duration of diabetes were included as covariates and missing data were handled using multiple imputation (Table S3), the OR was 1.66 (95% CI, 1.00-2.75; p=0.048; Table S3), which was consistent with the findings observed in the primary analysis (OR, 1.84; 95% CI, 1.07-3.17; p=0.028; Table 2). To overcome the limited sample size, bootstrap resampling was performed using the same model as in Table 2, and the results are presented in Table S4. The OR for FPG obtained from the bootstrap analysis was 1.84 with a 95% confidence interval of 1.22-6.36 (Table S4), which was comparable to the results of the primary analysis (OR, 1.84; 95% CI, 1.07-3.17; Table 2).

When logistic regression was performed using the same model as in Table S3 and restricted to complete-case data (Table S5), the direction and magnitude of the association between FPG and the outcome (OR, 1.88; 95% CI, 1.05-3.37; P=0.034, respectively in Table S5) remained consistent with those observed in the multiple imputation analysis (OR,1.66; 95% CI, 1.00-2.75; p=0.048; Table S3).

It has been suggested that obesity aggravates endothelial dysfunction through impaired pterin metabolism (21). Intriguingly, such dysmetabolism of pterin, characterized by low-grade inflammation and impairment in cellular redox state, has been shown to be associated with glucose intolerance (22). In this context, a significant association between a higher urinary IXP/Pterin ratio and elevated FPG levels (Fig. 3A, Table 2) may represent a facet of the metabolic milieu independent of XO.

We fully acknowledge that the present study has several limitations. First, owing to its cross-sectional study design, the temporal direction of the association between the urinary IXP/Pterin ratio and FPG could not be determined, and it remains unclear whether an elevated IXP/Pterin ratio precedes dysglycemia or merely reflects existing metabolic alterations. Second, because of the extremely limited sample size, the present study should be considered exploratory. Third, the HbA1c level in most participants was within 6-7%, warranting further analyses in patients with a wider range of HbA1c values. Although patients treated with insulin or GLP-1 receptor agonists were excluded, some of the participants were taking oral antidiabetic agents, including metformin, SGLT2 inhibitors, or thiazolidinediones, all of which may influence the serum insulin levels (23-25).

Although eGFR was not significant in the multivariable models, the observed association between the urinary IXP/Pterin ratio and renal function raises the possibility that renal handling of IXP or pterin may influence urinary measurements. However, it should be noted that the study population largely consisted of individuals with a preserved renal function (eGFR >60 mL/min/1.73 m^2). In this context, further validation in larger, multicenter cohorts with more diverse clinical characteristics is warranted.

Despite these limitations, the following points merit consideration. Our data suggest that the urinary IXP/Pterin ratio can contribute to identifying residual risks that cannot be predicted by XO activity. It should also be noted that the urinary IXP/Pterin ratio can be measured noninvasively and conveniently. This measurement requires only routine urine collection and basic analytical procedures. Therefore, the ratio is advantageous for assessing metabolic risk in primary care settings with limited resources.

In obese patients with mild T2DM, whose HbA1c levels were well controlled (6-7%), but exhibited elevated FPG, the urinary IXP/Pterin ratio was significantly associated with FPG, providing a potentially novel clue to evaluate metabolic derangement independent of plasma XO activity.

The authors state that they have no Conflict of Interest (COI).

Funding: The present study was supported in part by Teijin Pharma Limited and its designated research project (Project Code: 20E7000010).

Author's Disclosure of Potential Conflict of Interest

Author A received research funding from Teijin Pharma Limited.

Takashi Shirakura is an employee of Teijin Pharma Limited. The authors declare no conflicts of interest.

Financial Support

This study was funded by Teijin Pharma Limited and supported by the project entitled "Collaborate Research: In Vivo Xanthine Oxidase in the Pathogenesis of Various Autoimmune Diseases including Rheumatoid Arthritis and Autoinflammatory Syndromes such as Behcet's Disease and Familial Mediterranean Fever" (Project Code: 20E7000010).

References

1. Kelley EE. Dispelling dogma and misconceptions regarding the most pharmacologically targetable source of reactive species in inflammatory disease, xanthine oxidoreductase. Arch Toxicol **89**: 1193-1207, 2015.
2. Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol **555**: 589-606, 2004.
3. Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Arch Toxicol **98**: 1323-1367, 2024.
4. Sunagawa S, Shirakura T, Hokama N, et al. Activity of xanthine oxidase in plasma correlates with indices of insulin resistance and liver dysfunction in patients with type 2 diabetes mellitus and metabolic syndrome: A pilot exploratory study. J Diabetes Investig **10**: 94-103, 2019.
5. Sheehan JP. Fasting hyperglycemia: etiology, diagnosis, and treatment. Diabetes Technol Ther **6**: 525-533, 2004.
6. Hanefeld M, Fischer S, Julius U, et al. Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. Diabetologia **39**: 1577-1583, 1996.
7. Hokama N, Shirakura T, Sunagawa S, et al. A pilot assessment of xanthine oxidase activity in plasma from patients with hematological malignancies using a highly sensitive assay. Hematol Oncol **37**: 527-530, 2019.
8. Yamamoto T, Moriwaki Y, Takahashi S. Effect of ethanol on metabolism of purine bases (hypoxanthine, xanthine, and uric acid). Clin Chim Acta **356**: 35-57, 2005.
9. Corso LML, Wing RR, Tate DF, Espeland MA, Blanchard BE, McCaffery JM. Uric acid as a predictor of weight gain and cardiometabolic health in the Study of Novel Approaches to Weight Gain Prevention (SNAP) study. Int J Obes (Lond) **46**: 1556-1559, 2022.
10. Lytvyn Y, Perkins BA, Cherney DZ. Uric acid as a biomarker and a therapeutic target in diabetes. Can J Diabetes **39**: 239-246, 2015.
11. Blau N, de Klerk JB, Thöny B, et al. Tetrahydrobiopterin loading test in xanthine dehydrogenase and molybdenum cofactor deficiencies. Biochem Mol Med **58**: 199-203, 1996.
12. Li Q, Wei S, Wu D, Wen C, Zhou J. Urinary Metabolomics Study of Patients with Gout Using Gas Chromatography-Mass Spectrometry. Biomed Res Int **2018**: 3461572, 2018.
13. Lindsay A, McCourt PM, Karachunski P, Lowe DA, Ervasti JM.

Xanthine oxidase is hyper-active in Duchenne muscular dystrophy. Free Radic Biol Med **129**: 364-371, 2018.

14. Ma Y, Burton C. Pteridine detection in urine: the future of cancer diagnostics? Biomark Med **7**: 679-681, 2013.

15. Kośliński P, Pluskota R, Mądra-Gackowska K, et al. Comparison of Pteridine Normalization Methods in Urine for Detection of Bladder Cancer. Diagnostics (Basel) **10**: 612, 2020.

16. Otaki Y, Watanabe T, Kinoshita D, et al. Association of plasma xanthine oxidoreductase activity with severity and clinical outcome in patients with chronic heart failure. Int J Cardiol **228**: 151-157, 2017.

17. Özer Y, Cansever M, Turan H, et al. Pteridine and tryptophan pathways in children with type 1 diabetes: Isoxanthopterin as an indicator of endothelial dysfunction. J Pharm Biomed Anal **243**: 116072, 2024.

18. Japan Society for the Study of Obesity. Guidelines for the management of obesity disease 2022ed. Life Science Publishing CO., Ltd., Tokyo, 2022.

19. Walter R, Blau N, Schaffner A, et al. Inhalation of the nitric oxide synthase cofactor tetrahydrobiopterin in healthy volunteers. Am J Respir Crit Care Med **156**: 2006-2010, 1997.

20. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant **48**: 452-458, 2013.

21. Nyström T, Nygren A, Sjöholm A. Tetrahydrobiopterin increases insulin sensitivity in patients with type 2 diabetes and coronary heart disease. Am J Physiol Endocrinol Metab **287**: E919-E925, 2004.

22. Di Bonito P, Valerio G, Licenziati MR, et al. Uric acid, impaired fasting glucose and impaired glucose tolerance in youth with overweight and obesity. Nutr Metab Cardiovasc Dis **31**: 675-680, 2021.

23. Usui R, Hamamoto Y, Imura M, et al. Differential effects of imeglimin and metformin on insulin and incretin secretion-An exploratory randomized controlled trial. Diabetes Obes Metab **27**: 856-865, 2025.

24. Cuatrecasas G, De Cabo F, Coves MJ, et al. Dapagliflozin added to metformin reduces perirenal fat layer in type 2 diabetic patients with obesity. Sci Rep **14**: 10832, 2024.

25. Bril F, Kalavalapalli S, Clark VC, et al. Response to Pioglitazone in Patients With Nonalcoholic Steatohepatitis With vs Without Type 2 Diabetes. Clin Gastroenterol Hepatol **16**: 558-566.e552, 2018.