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RESEARCH

The predictive value of plasma uridine for type 2 diabetes and its atherosclerotic complications

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Abstract

Objective: Uridine might be a common link between pathological pathways in diabetes and cardiovascular diseases. This study aimed to investigate the predictive value of plasma uridine for type 2 diabetes (T2D) and T2D with atherosclerosis.

Methods: Individuals with T2D and healthy controls (n = 218) were randomly enrolled in a cross-sectional study. Patients with T2D were divided into two groups based on carotid ultrasound: patients with carotid atherosclerosis (CA) (group DCA) and patients without CA (group D). Plasma uridine was determined using HPLC-MS/MS. Correlation and logistic regression analyses were used to analyze the results.

Results: Fasting and postprandial uridine were significantly increased in patients with T2D compared with healthy individuals. Logistic regression suggested that fasting and postprandial uridine were independent risk factors for T2D. The receiver operating characteristic (ROC) curve showed that fasting uridine had a predictive value on T2D (95% CI, 0.686–0.863, sensitivity 74.3%, specificity 71.8%). Fasting uridine was positively correlated with LDL-c, FBG, and PBG and negatively correlated with fasting C-peptide (CP-0h) and HOMA-IS. The change in postprandial uridine from fasting baseline (Δuridine) was smaller in T2D patients with CA compared with those without (0.80 (0.04–2.46) vs 2.01 (0.49–3.15), P = 0.010). Δuridine was also associated with T2D with CA and negatively correlated with BMI, CP-0h, and HOMA-IR.

Conclusion: Fasting uridine has potential as a predictor of diabetes. Δuridine is closely associated with carotid atherosclerosis in patients with T2D.

Keywords: uridine; diabetes; carotid atherosclerosis; cardiometabolic

Introduction

Type 2 diabetes (T2D) has become a pressing global public health concern in the 21st century due to its high morbidity and low treatment rate (1). China has the largest number of diabetes patients worldwide, and approximately half of all patients with diabetes die from cardiovascular events (2). Patients with diabetes are at a higher risk of developing atherosclerotic diseases compared to the general population. Arterial plaques in

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patients with diabetes differ pathologically from those in non-diabetic individuals (3, 4). Arterial plaque formation can be influenced by a high glucose environment, putting diabetics with concurrent carotid atherosclerosis (CA) at greater risk of cardiovascular events compared to the general population (5, 6). Therefore, investigating predictive markers for atherosclerosis holds clinical significance for the secondary prevention of stroke in patients with T2D.

Uridine is a natural nucleoside involved in substance synthesis and metabolism (7). It has garnered attention due to its anti-aging (8), anti-fibrotic (9), anti-inflammatory (10), and antioxidant properties (11). Uridine has been associated with metabolic diseases such as obesity (12) and diabetes (13). However, the relationship between uridine and glucose homeostasis is unclear. Some experiments show that short-term low-dose uridine supplementation increases the rate of glucose utilization during intraperitoneal glucose tolerance tests (14, 15). Conversely, other research suggests that chronically high levels of uridine promote the development of T2D by inhibiting insulin signaling through increasing glycosylation of key proteins, decreasing the ability of skeletal muscle to utilize glucose, and promoting hepatic gluconeogenesis (7, 16). Studies also indicate that uridine might induce vascular calcification through the actions of uridine adenosine tetraphosphate (Up4A) (17). Uridine contributes to elevated O-GlcNAcylation of regulators of vascular homeostasis, such as endothelial nitric oxide synthase (eNOS) (6, 18), which leads to chronic damage to the arteries and subsequent promotion of atherosclerosis. Elevated glucose might accelerate the progression of existing atherosclerotic lesions (19) or act as a trigger for their onset (20). Consequently, uridine is an important molecule in the common mechanisms underlying metabolic diseases such as diabetes and atherosclerosis.

This study aims to utilize a self-developed HPLC-MS/MS) method, which has high sensitivity and quantitative accuracy, to measure plasma uridine and explore the relationship between plasma uridine (fasting and postprandial) and diabetes with or without atherosclerosis. This research seeks to provide new insights into the common mechanisms of diabetes and atherosclerosis and shed light on the direction of future studies on cardio-metabolism.

Methods

Patients

This study recruited 179 patients with T2D admitted to the Endocrinology Department of Renmin Hospital, Wuhan University, from April 2022 to May 2023, and 39 healthy control subjects (group HC) registered at the hospital health examination center from May to July 2023. The diabetic status of the patients included in the study was identified by biochemical testing based on the American Diabetes Association (ADA) diagnostic criteria (21). Patients with T2D were categorized into two groups: patients without CA (group D) and patients with CA (group DCA) based on ultra-sound results. The enrolled patients continued their previous pharmacological regimens during this study: 48 received insulin, 110 received hypoglycemic agents, 22 received lipid-regulating agents, 68 received antihypertensives, and 19 received antiplatelet agents. Consent was obtained from each patient after a full explanation of the purpose and nature of all procedures used. This study was approved by the Ethics Committee of Renmin Hospital, Wuhan University.

Exclusion criteria

Patients with acute complications of diabetes, severe infections, acute cardiovascular or cerebrovascular events, liver dysfunction (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels exceeding twice the normal values), severe renal dysfunction (estimated glomerular filtration rate (eGFR) ≤60 mL/min), severe heart failure (New York Heart Association classes III–IV), malignant tumors or other debilitating diseases, or individuals undergoing major or moderate stress-inducing surgeries were excluded from the study.

Measurement of biochemical features

Clinical data were collected and biochemical parameters were measured by a biochemistry automatic analyzer (ADVIA 2400, Siemens Healthcare Diagnostics Inc). C-peptide index (CPI) levels were measured by an automatic chemiluminescence immunoassay analyzer (ADVIA XP, Siemens Healthcare Diagnostics Inc). Insulin resistance index (HOMA-IR) and insulin sensitivity index (HOMA-IS) were calculated based on fasting blood glucose (FBG) and C-peptide fasting level (Cp-0h) as follows: HOMA-IR = 1.5 + (FBG (mmol/L) × Cp-0h (pmol/L)) / 2800; HOMA-IS = 0.27 × Cp-0h (pmol/L) / (FBG (mmol/L) – 3.5) (22).

Carotid artery ultrasound measurement

The carotid artery ultrasound examination for all patients was conducted using the Philips EPIQ 5 ultrasound system. An experienced doctor who was blinded to clinical follow-up data analyzed the presence of atheromatous plaques. Atherosclerotic plaques were defined as having an intima–media thickness (IMT) ≥1.3 mm or a localized luminal protrusion with a thickness at least 50% greater than the adjacent intima–media complex.

Measurement of plasma uridine

Venous blood was collected in an EDTA anticoagulant tube, and the plasma was transferred to a polypropylene

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conical centrifuge tube after low-temperature centrifugation and then stored at –80°C until analysis. For the assay, 10 µL plasma were extracted and acetonitrile solution containing the internal standard (IS), uridine-2-^{13}C-1,3-$^{15}N_2$ (U829907-1 mg), was added to precipitate the proteins. After vortexing and centrifugation, the supernatant was taken for LC-MS/MS analysis.

LC-MS analysis was performed using an AB SCIEXION LC system and an AB SCIEX QTRAP 6500+ mass spectrometer. The samples were separated on a Waters ACQUITY UPLC BEH HILIC 1.7 µm (2.1 × 100 mm) chromatographic column operated at a column temperature of 40°C. The mobile phase A was water containing 0.2% formic acid (v/v), and the mobile phase B was acetonitrile containing 0.2% formic acid (v/v) at a flow rate of 0.5 mL/min. The gradient elution was used in the run, and the gradients were as follows: 0–0.8 min 95% B, 0.8–1.3 min 95–60% B, 1.3–3.3 min 60% B, 3.3–3.8 min 60–95% B, 3.8–5.5min 95% B.

Mass spectrometry analyses were performed in positive electrospray ionization (ESI) mode. The target substance was monitored using the multiple reaction monitoring (MRM) modes and analyzed based on the mass jump (precursor ions → product ions); uridine (245.1 → 113.0) and uridine-IS (246.0 → 113.0) ions monitored using and processing were performed using Analyst 1.6.3 software (Applied Biosystems SCIEX).

Uridine level was measured at fasting (uridine 0 h) and postprandial (uridine 2 h). Change in postprandial uridine from fasting baseline is denoted as Δuridine: Δuridine = uridine 0 h – uridine 2 h.

Method validation

Accuracy and imprecision

Methodological accuracy was validated using a spiked recovery assay, where the recovery of uridine was determined at different fortification levels (0.8, 2, 10, and 40 µmol/L), with three replicates per concentration. The imprecision was the coefficient of variation (CV) of the measured values. Accuracy and imprecision were studied at four peak levels (lower limit of quantification (LLOQ), low, medium, and high) at a concentration of 0.8, 2, 10, and 40 µmol/L on 1 day (intra-day) and 6 consecutive days (inter-day). Accuracy was defined as 85–115% (80–120% for LLOQ levels). Imprecision should not be higher than 15% (20% for LLOQ levels).

Linearity and sensitivity

The samples were quantitatively analyzed by the internal standard method. The uridine gradient concentration standard solution containing a fixed concentration of IS was prepared. The sample was injected according to the optimized method, and chromatograms were recorded to calculate the peak areas of the standard and the internal standard. The concentration gradient of the standard was taken as the horizontal coordinate, and the peak area ratio between the standard and the internal standard was taken as the vertical coordinate to make the standard curve. The available curve was taken as $r^2 > 0.99$. Under the same conditions, the sample solution with the internal standard was injected and analyzed, the chromatogram was recorded, the peak areas of the sample peaks and the internal standard were calculated, and the ratio of the peak areas of the sample and the IS peaks was used to calculate the concentration of the samples from the calibration curve. The signal-to-noise ratio of the LLOQ was ≥5:1.

Carry-over effects and matrix effects

Carry-over effects were evaluated by analyzing the blank matrix before and after injection of the upper limit of the quantification sample ($n = 6$). The residual should be less than 15% of the LLOQ. Six different sources of plasma matrices were used, and the response value of the internal standard in the matrix divided by the response value of the internal standard in the non-matrix was used to determine the matrix effect of the compounds to be tested at low and high concentrations, respectively, with a calculated CV ≤15%.

Stability

Plasma ($n = 3$) was assayed immediately after collection and each sample assay was repeated four times. The mean value was used as the expected concentration. The stability of the plasma samples was investigated at room temperature for 0.5, 1, and 4 h and at 4°C for 2, 4, and 8 h. The samples were frozen at 80°C for 30, 60, and 180 days, freeze-thawed repeatedly at −80°C for three times, and placed in the autosampler for 24 h. Relative error (RE) indicates the result.

Statistical analysis

The data is presented as forms of mean ± S.D. or median (25th–75th percentile). Between-group differences were determined using *t*-test analysis (for normally distributed numerical data), chi-square test (categorical data), or Mann–Whitney *U* test (for non-normally distributed numerical data). The relationship between uridine and other variables was analyzed using Spearman's correlation coefficient. Logistic regression analysis was used to assess significant independent factors associated with diabetes and group DCA. Graphs were created using Origin software (Origin Lab Corporation, MA, USA). All statistical analyses, including the receiver operating characteristic (ROC) curve, were conducted using SPSS version 26 (IBM Corporation). All tests were two-tailed, and a *P*-value < 0.05 was considered statistically significant.

Table 1 Accuracy and recovery of uridine in plasma.

	Intra-day		Inter-day		
Concentration (μmol/L)	**Measured (μmol/L, x ± s)**	**CV (%)**	**Measured (μmol/L, x ± s)**	**CV (%)**	**Recovery** (%)
0.8	0.80 ± 0.13	16.8	0.80 ± 0.139	17.2	81–117%
2	2.05 ± 0.17	8.4	1.93 ± 0.18	9.3	86–111%
10	10.2 ± 0.54	5.3	10.1 ± 0.94	9.3	89–111%
40	40.9 ± 1.84	4.5	39.3 ± 1.83	4.6	93–107%

Results

Method validation

Accuracy and imprecision

The spiked recovery experiments were performed at 0.8, 2, 10, and 40 μmol/L, and the recoveries were above 86% (LLOQ 81%). Six different samples were subjected to intra-day and inter-day assays, and the CVs were <15% (LLOQ < 20%) in both cases (Table 1).

Linearity and sensitivity

Uridine showed good linearity from 0.8–60 μmol/L with a standard curve equation of $y = 0.1708x + 0.3443$ ($r^2 = 0.9981$). The LLOQ was 0.8 μmol/L and the signal-to-noise ratio of the LLOQ was 10.

Carry-over effects and matrix effects

No residues were found near the peak positions of uridine and internal standard before and after analysis of the samples at the upper limit of quantification, and the results indicate that there were no significant carry-over effects (Fig. 1). For all mass concentration levels, two internal standard peak area ratios resulted in a CV of 7.89%. This shows that different plasma matrices did not affect the quantitative determination of uridine, which benefits from the addition of the internal standard.

Stability

The REs of low- and high-concentration plasma samples left at room temperature for 0.5, 1, and 4 h ranged from −4.2% to −6.6%. The REs of 2, 4, and 8 h at 4°C were −3.9% to −6.9%. The REs of 30, 60, and 180 days of freezing at 80°C were −5.2 to −11.6%. The REs of three repeated freezes and thawing at −80°C were −5.7% to −8.4%. The REs was −4.4% to −6.2% before and after 24 h in the preconditioner. These results showed that the stability of plasma uridine was good.

The clinical characteristics and biochemical features at baseline for healthy controls vs diabetes

Body mass index (BMI), systolic blood pressure (SBP), ALT, urea, FBG, PBG, and triglyceride (TG) were higher in T2D patients compared with the control group. T2D patients had significantly lower total bilirubin (TBIL) and high-density lipoprotein cholesterol (HDL-Ch) than the control group. Regardless of pre-meal or post-meal conditions, the uridine level in T2D was significantly higher than in healthy subjects (uridine 0 h: 6.22 (5.29–7.23) vs 4.57 (3.46–5.69) μmol/L, $P < 0.001$; uridine 2 h: 4.7 (3.7–5.82) vs 2.59 (1.61–3.81) μmol/L, $P < 0.001$). In both the HC group and T2D group, postprandial uridine showed a significant decrease compared to fasting uridine (HC: 4.57 (3.46–5.69) vs 2.59 (1.61–3.81) μmol/L, $P < 0.001$; T2D: 6.22 (5.29–7.23) vs 4.7(3.7–5.82) μmol/L, $P < 0.001$). No significant differences were observed in other biochemical parameters (Table 2).

Fasting uridine is an independent risk factor for the occurrence of T2D

Logistic regression analysis was performed to assess the independent predictors of T2D. The statistically different variables (BMI, AST, SBP, TBTL, urea, TG, HDL-Ch, fasting uridine, postprandial uridine) were entered into the full regression model. As demonstrated in Table 3, the results confirmed that BMI, SBP, HDL-C, fasting uridine and postprandial uridine were significantly associated with T2D. The odds ratio (OR) values of fasting uridine and postprandial uridine were 1.552 (1.068–2.254), $P = 0.021$ and 1.697 (1.304–2.966), $P = 0.001$ (Table 3).

The predictive value of plasma fasting uridine and its association with other risk factors for T2D

To explore the predictive value of fasting uridine and postprandial uridine for T2D, we analyzed the ROC curves. The results revealed that the area under the curve (AUC) of fasting uridine in identifying the risk of T2D was 0.775 (95% CI, 0.686–0.863, $P < 0.001$) (Fig. 2A). The optimal cutoff of fasting uridine was 5.33 μmol/L, with a sensitivity of 74.3% and specificity of 71.8%. The optimal cutoff for the postprandial uridine to distinguish patients with T2D was 3.29 μmol/L, with a sensitivity of 84.4%, a specificity of 30.8%, and the highest AUC equal to 0.822 (95%CI, 0.744–0.900, $P < 0.001$) (Fig. 2B).

To find the factors affecting the fasting and postprandial uridine in T2D, the correlation of uridine with other

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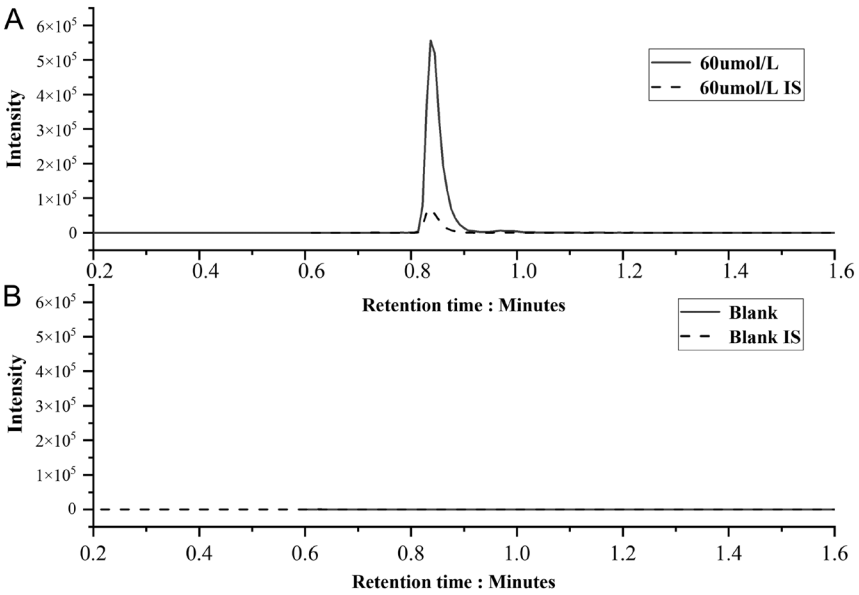

Figure 1

Carry-over effects of uridine by LC-MS measurement. (A) LS-MS/MS measurement for upper limit of quantification sample. (B) LS-MS/MS measurement for blank matrices after upper limit of quantification sample.

clinical parameters was analyzed using Spearman's correlation analysis. As shown in Table 4, fasting uridine was positively correlated with ALT, FBG, PBG, LDL-C (r_s = 0.190, P = 0.005), uridine 2 h, and Δuridine. It was negatively correlated with CP-0h (r_s = –0.204, P = 0.006) and HOMA-IS (r_s = –0.159, P = 0.033). Postprandial uridine was positively correlated with ALT, FBG, PBG, and uridine-0h. Δuridine was negatively correlated with postprandial uridine (Table 4).

The comparison of clinical characteristics and biochemical features among the three subgroups (HC vs. D vs. DCA)

SBP, ALT, TBIL, FGB, PBG, TG, HDL-C, fasting uridine, and postprandial uridine were significantly lower in group HC than in group DCA and group D. In group DCA: age, percentage of males, mean patient duration, and urea, Cr, and UA levels were higher, compared to group D. However, Δuridine was significantly lower in group DCA (2.01 (0.29–3.15) vs 0.8 (0.04–2.46), P = 0.010). The proportion of smokers in the DCA group was significantly higher than in healthy individuals but was not substantially different between the DCA and D groups. The median and interquartile range of BMI, SBP, urea, Cr, and FBP in the HC, D, and DCA groups was increased, and the eGFR was decreased (Table 5).

Analysis of risk factors related to carotid atherosclerosis in patients with diabetes

Significant variables (age, gender, TBTL, duration of diabetes, SBP, urea, Cr, UA, eGRF, and Δuridine) were used as independent variables in a binary logistic regression analysis to determine the presence of CA in T2D. The results indicated that age, gender, SBP, and Δuridine significantly impact the presence of CA in T2D (Table 6).

ΔUridine is predictive for CA in T2D

To explore the predictive value of Δuridine for T2D with CA, we analyzed the ROC curves of Δuridine (Fig. 2C). The results revealed that the AUC of Δuridine in identifying the probability of T2D with CA was 0.622 (95% CI, 0.547–0.699), P = 0.002. The optimal cutoff of Δuridine was 0.79 μmol/L, with a sensitivity of 50.0% and specificity of 72.0%.

The association of Δuridine with other risk factors for atherosclerosis in T2D

There was a significant difference in Δuridine in patients with T2D with CA and without CA. Further Spearman's exploration of the correlates revealed that Δuridine was negatively correlated with BMI (r_s = −0.204, P = 0.003), CP-0h (r_s = −0.198, P =), and HOMA-IR (r_s = −0.176, P = 0.019) (Table 7).

Discussion

Uridine is a precursor for many physiologically important substances such as nucleic acids and uridine diphosphate (UDP), maintains a dynamic homeostasis in the blood, and is regulated by food intake (23, 24). This study showed that fasting and postprandial uridine were significantly higher in T2D compared to healthy individuals. Elevated fasting and postprandial uridine were independently associated with the presence of T2D. When fasting uridine exceeds 5.33 μmol/L, it may serve as an indicative marker for diagnosing T2D. Higher concentrations of uridine promote appetite

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Table 2 Baseline characteristics of healthy controls and patients with type 2 diabetes.

Variables	Healthy control ($n = 39$)	Type 2 diabetes ($n = 179$)	*P*
Age (years)	53 (48–59)	56 (47–65)	0.138
Gender (male)	23 (58.9%)	117 (65.3%)	0.451
Duration of diabetes (years)	–	5 (0.39–13)	–
Smokers	9 (23%)	65 (36.3%)	0.114
BMI (kg/m^2)	23.18 (21.45–26.57)	25.30 (22.58–27.39)	0.042*
SBP (mm Hg)	120 (114–126)	130 (118–143)	0.001*
DBP (mm Hg)	76 (70–83)	78 (69–86)	0.474
ALT (U/L)	17 (11–23)	21 (15–30)	0.007*
AST (U/L)	20 (17–24)	19 (15–24)	0.354
TBIL (μmol/L)	14.0 (11.9–17.1)	12.50 (8.90–15.50)	0.019*
DBIL (μmol/L)	3.60 (2.7–4.3)	3.5 (2.50–4.70)	0.604
Urea (mmol/L)	4.87 (4.30–5.80)	5.70 (4.70–7.30)	0.001*
Cr (μmol/L)	62 (52–75)	63 (53–77)	0.650
UA (μmol/L)	318 (254–370)	338 (275–416)	0.074
FBG (mmol/L)	5.70 (5.08–6.20)	9.33 (6.70–12.60)	0.000*
PBG (mmol/L)	6.30 (5.40–8.06)	13.91 (9.8–17.59)	0.000*
Tch (mmol/L)	4.60 (4.14–5.04)	4.53 (3.69–5.44)	0.618
TG (mmol/L)	1.27 (0.95–1.68)	1.74 (1.15–2.92)	0.001*
HDL-C (mmol/L)	1.21 (1.06–1.45)	0.95 (0.80–1.15)	0.000*
LDL-C (mmol/L)	2.80 (2.27–3.15)	2.78 (2.13–3.52)	0.834
eGFR (mL/min)	101.74 (95.39–107.92)	100.89 (88.63–113.55)	0.463
Uridine 0h (μmol/L)	4.57 (3.46–5.69)	6.22 (5.29–7.23)	0.000*
Uridine 2h (μmol/L)	2.59 (1.61–3.81)	4.70 (3.7–5.82)	0.000*
Δuridine (μmol/L)	1.65 (0.86–2.69)	1.45 (0.23–2.78)	0.128

*$P < 0.05$. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Cr, creatinine; DBIL, direct bilirubin; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PBG, postprandial blood glucose; SBP, systolic blood pressure; TBIL, total bilirubin; Tch, total cholesterol; TG, triglycerides; UA, uric acid.

and modulate ingestion behavior by stimulating agouti-related peptide/neuropeptide Y (AgRP/NPY) neurons in the hypothalamic arcuate nucleus. This induces higher caloric intake which may contribute to the development of T2D (25). Moreover, increased uridine can lead to metabolic abnormalities by promoting O-glycosylation of functional proteins in metabolic pathways such as FOXO1 (16) and FABP1 (26), mediated by accelerated hepatic gluconeogenesis.

Table 3 Independent factors for T2D by multivariable logistic regression analysis.

Covariables	B	OR (95% CI)	*P*
BMI (kg/m^2)	0.122	0.885 (0.749–1.046)	0.152
SBP (mm Hg)	0.035	1.036 (1.003–1.069)	0.032*
ALT (U/L)	0.012	1.012 (0.935–1.089)	0.494
TBTL (μmol/L)	0.008	0.986 (0.978–1.047)	0.740
Urea (mmol/L)	0.579	1.784 (1.190–2.676)	0.005*
TG (mmol/L)	0.524	1.689 (0.937–3.044)	0.081
HDL-C (mmol/L)	−2.633	0.072 (0.011–0.454)	0.005*
Uridine 0h (μmol/L)	0.439	1.552 (1.068–2.254)	0.021*
Uridine 2h (μmol/L)	0.676	1.697 (1.304–2.966)	0.001*

*$P < 0.05$.ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; UA, uric acid.

The liver is the main metabolizing organ for uridine as well as glucose and could be an important site for studying their interactions. In the mouse liver, when there are high uridine levels, the uridine catabolic gene UPP1 is downregulated, and no significant changes are seen in the uridine synthesis gene CAD. Decreased catabolism of uridine may be the cause of elevated fasting uridine (27). Fasting uridine was positively correlated with PBG, FBG, and LDL-c, but negatively correlated with HOMA-IS and CP-0h. Some studies have found a positive correlation between fasting uridine and the insulin resistance index, which may be linked to T2D through the regulation of glucose metabolism in skeletal muscle (28, 29). This is similar to our findings, and the subtle differences may be due to differences in sample size and the influence of medications. Although causality is unclear, these results indicate that fasting uridine is closely related to glucose and lipid metabolism. Therefore, it may play a role in the development of T2D by promoting insulin resistance and β-cell function failure. This further supports uridine as an independent risk factor and predictor of T2D.

Similar to Deng *et al.* (23), who reported a significant decrease in postprandial uridine in healthy individuals, our study showed that this phenomenon is also present in patients with T2D. Δuridine was not

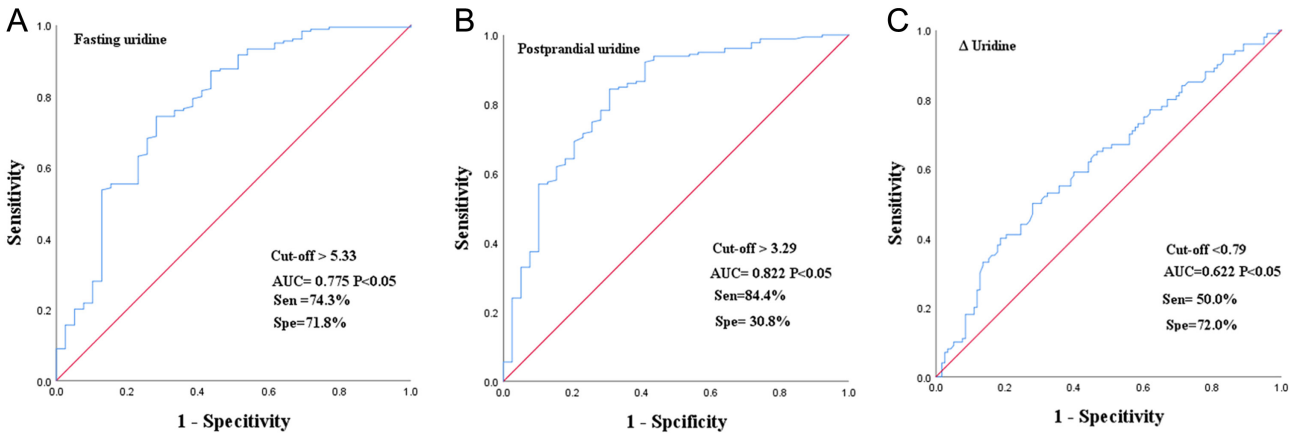

Figure 2

Uridine and ΔUridine as predictive factors for T2D and T2D without CA. (A) The ROC curve of fasting uridine for T2D; (B)The ROC curve of postprandial uridine for T2D; (C) The ROC curve of ΔUridine for T2D with CA.

significantly decreased in T2D patients compared to healthy individuals. Due to the rise in fasting uridine in patients with T2D, the percentage reduction from baseline to 2 h after a meal was significantly smaller than in healthy controls. This also suggests an altered capacity for uridine regulation under chronic hyperglycemic conditions.

After dividing the T2D patients into subgroups based on the presence of CA, we found that T2D patients with atherosclerosis had significantly smaller Δuridine levels than healthy individuals and T2D patients without CA. It appears that a decrease in Δuridine is associated with a higher risk of CA in patients with T2D. We found no significant difference in Δuridine between healthy individuals and T2D patients without CA, which may be due to a small sample size. But T2D patients without CA had higher fasting uridine compared to healthy subjects. Although Δuridine was not significantly different between healthy subjects and T2D (without CA), the percentage change in postprandial uridine from fasting baseline is statistically decreased, further suggesting that uridine response or signaling is impaired as disease progression (exacerbation of metabolism).

Table 4 Correlation of uridine and clinical variables.

	Fasting uridine		Postprandial uridine	
Parameters	r_s	*P*	r_s	*P*
ALT (U/L)	0.184	0.013*	0.137	0.043*
FBG (mmol/L)	0.180	0.008*	0.188	0.005*
PBG (mmol/L)	0.186	0.006*	0.235	0.000*
LDL-C (mmol/L)	0.190	0.005*	0.071	0.296
CP-0h (pmol/L)	−0.204	0.006*	0.038	0.609
HOMA-IS	−0.159	0.033*	0.006	0.938
Uridine-0h (μmol/L)	1.000	–	0.468	0.000*
Uridine-2h (μmol/L)	0.468	0.000*	1.000	–
Δuridine (μmol/L)	0.418*	0.000*	−0.554	0.000*

*P < 0.05.ALT, alanine aminotransferase; CP-0h, fasting C-peptide; FBG, fasting blood glucose; HOMA-IS, insulin sensitivity index; LDL-C, low-density lipoprotein cholesterol; PBG, postprandial blood glucose.

Animal experiments showed that mice on a high-fat diet had decreased Δuridine. Uridine is cleared via the adipo−biliary−uridine axis, so uridine levels drop after a meal because food intake promotes the clearance of uridine (23). Impaired uridine clearance after a meal can contribute to smaller Δuridine. Therefore, we speculate that a diminished clearance of uridine after a meal may contribute to the development of atherosclerosis in patients with T2D.

Δuridine has also been linked to energy metabolism (30). Unusually, postprandial uridine levels are increased rather than decreased in extremely obese patients. This indicates that obesity (or very large caloric intake) can alter the physiological decline in postprandial uridine (31). We found that there is a negative correlation of Δuridine with BMI and HOMA-IR, which suggests that decreased Δuridine and dynamic regulation of uridine may be involved in the development of atherosclerosis in T2D by promoting obesity and insulin resistance. From the findings of our study, we believe that the dynamic regulation of diet-related uridine may promote atherosclerosis by stimulating caloric intake and insulin resistance.

This study has several limitations. As a single-center, small, cross-sectional study, larger multicenter prospective studies are needed to establish a causal relationship between uridine metabolism and T2D or cardiometabolic diseases. In this study, all enrolled patients continued their current treatment regimens, especially lipid-regulating agents, which may have led to negative results in lipid indicators between the groups. Therefore, patients with T2D and newly identified CA

Table 5 Baseline characteristics of healthy controls and type 2 diabetes patients W/O carotid atherosclerosis.

Variables	Healthy control (n = 39)	P^1	T2D without CA (n = 79)	P^2	T2D with CA (n = 100)	P^3
Age (years)	53 (48–59)	0.292	51 (42–58)	0.000*	59 (53–66)	0.001*
Gender (male)	23 (58.9%)	0.735	44 (55.6%)	0.004*	76 (76%)	0.046*
Disease duration (years)	–	–	3.00 (0.27–7.00)	0.000*	7.5 (1.25-14)	–
Smokers	9 (23%)	0.406	24 (30.3%)	0.142	41 (41%)	0.048*
BMI (kg/m^2)	23.18 (21.45–26.57)	0.098	24.80 (22.86–27.54)	0.701	25.59 (22.51–27.13)	0.041*
SBP (mm Hg)	120 (114–126)	0.056	126.77 ± 16.74	0.006*	133.91 ± 17.228	0.000*
DBP (mm Hg)	76 (70–83)	0.723	78.15 ± 13.21	0.820	78.59 ± 12.44	0.364
ALT(U/L)	17 (11–23)	0.004*	23 (16–36) a	0.165	20 (15–28)	0.030*
AST(U/L)	20 (17–24)	0.559	19 (14–26)	0.828	19 (15–23)	0.283
TBIL (μmol/L)	14.0 (11.9–17.1)	0.025*	12.50 (8.80–15.50)	0.918	12.50 (8.90–15.45)	0.036*
DBIL (μmol/L)	3.60 (2.7–4.3)	0.857	3.40 (2.50–4.50)	0.580	3.6 (2.50–4.85)	0.471
Urea (mmol/L)	4.87 (4.30–5.80)	0.026*	5.48 (4.46–6.50)	0.014*	5.95 (4.90–7.90)	0.000*
Cr (μmol/L)	62 (52–75)	0.338	60.00 (50.00–72.00)	0.003*	66.00 (56.00–82.00)	0.122
UA (μmol/L)	318 (254–370)	0.817	307 (271–400)	0.011*	362 (286–437)	0.005*
FBG (mmol/L)	5.70 (5.08–6.20)	0.000*	10.14 (7.44–12.75)	0.248	9.04 (6.18–12.47)	0.000*
PBG (mmol/L)	6.30 (5.40–8.06)	0.000*	13.91 (9.80–17.24)	0.881	13.85 (9.82–17.94)	0.000*
Tch (mmol/L)	4.60 (4.14–5.04)	0.823	4.63 (3.65–5.51)	0.860	4.51 (3.70–5.40)	0.515
TG (mmol/L)	1.27 (0.95–1.68)	0.001*	1.97 (1.10–3.18)	0.264	1.50 (1.16–2.80)	0.004*
HDL-C (mmol/L)	1.21 (1.06–1.45)	0.000*	0.95 (0.81–1.12)	0.907	0.95 (0.79–1.22)	0.000*
LDL-C (mmol/L)	2.80 (2.27–3.15)	0.952	2.81 (2.12–3.40)	0.602	2.75 (2.13–3.58)	0.689*
eGFR (mL/min)	101.74 (95.39–107.92)	0.160	105.91 (96.15–118.21)	0.000*	95.77 (85.28–107.99)	0.017*
Uridine 0h (μmol/L)	4.57 (3.46–5.69)	0.000*	6.29 (5.29–7.59)	0.166	6.11 (5.23–6.91)	0.000*
Uridine 2h (μmol/L)	2.59 (1.61–3.81)	0.000*	4.59 (3.57–5.80)	0.277	4.82 (3.89–5.93)	0.000*
Δuridine (μmol/L)	1.65 (0.86–2.69)	0.970	2.01 (0.49–3.15)	0.010*	0.80 (0.04–2.46)	0.010*
CP-0h (pmol/L)	–	–	423.33 (290.00–673.33)	0.868	431.66 (250.00–655.00)	–
CP-2h (pmol/L)	–	–	896.6 (486.6–1463.3)	0.758	943.3 (552.5–1483.3)	–
HOMA-IR	–	–	2.93 (2.43–3.77)	0.733	2.83 (2.28–3.83)	–
HOMA-IS	–	–	19.28 (8.86–33.95)	0.254	22.88 (10.91–38.21)	–

*P < 0.05; 1healthy control vs. T2D without CA; 2T2D without CA vs. T2D with CA; 3healthy control vs. T2D with CA. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CP-0h, fasting C-peptide; CP-2h, postprandial C-peptide; Cr, creatinine; DBP, diastolic blood pressure; DBIL, direct bilirubin; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, insulin resistance index; HOMA-IS, insulin sensitivity index; LDL-C, low-density lipoprotein cholesterol; PBG, postprandial blood glucose; SBP, systolic blood pressure; TBIL, total bilirubin; Tch, total cholesterol; TG, triglycerides; UA, uric acid.

who have not been on lipid-regulating agents are better candidates for future studies. Increasing the sample size, particularly of healthy subjects, and assessing insulin function could provide a more accurate picture of the role of uridine metabolism in T2D. Lastly, atherosclerosis (especially under high glucose conditions) involves complex pathological processes with numerous molecular changes. A single biomarker can be helpful but is not sufficient to fully explain the progress of atherosclerosis.

Table 6 Independent factors for CA in T2D by multivariable logistic regression analysis.

Covariables	*B*	OR (95% CI)	*P*
Age (years)	0.074	1.077 (1.004–1.155)	0.039*
Gender	–2.139	0.118 (0.033–0.418)	0.001*
Disease duration (years)	0.045	1.046 (0.987–1.108)	0.127
SBP (mm Hg)	0.030	1.030 (1.008–1.053)	0.008*
Urea (mmol/L)	0.145	1.156 (0.936–1.427)	0.179
UA (μmol/L)	0.002	1.002 (0.998–1.007)	0.311
Cr (μmol/L)	–0.028	0.973 (0.916–1.071)	0.367
eGFR (mL/min)	–0.010	0.980 (0.909–1.073)	0.771
Δuridine (μmol/L)	–0.214	0.808 (0.658–0.991)	0.041*

*P < 0.05. Cr, creatinine; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; UA, uric acid.

Table 7 Correlation of Δuridine and clinical variables.

	Δuridine	
Parameters	r_s	P
BMI (kg/m^2)	−0.167	0.025*
CP-0h (pmol/L)	−0.198	0.008*
HOMA-IR	−0.176	0.019*
Uridine-0h (μmol/L)	0.516	0.000*
Uridine-2h (μmol/L)	−0.603	0.000*

*P < 0.05. BMI, body mass index; CP-0h, fasting C-peptide; HOMA-IR, insulin resistance index.

Conclusion

Fasting uridine is an effective predictor of the onset of T2D. The decrease of uridine decline after a meal is associated with carotid atherosclerosis in patients with T2D.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

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Author contributions
Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used. This study has obtained approval from the Ethics Committee of Renmin Hospital, Wuhan University.

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