Elevated Serum Xanthine Oxidase Activity Is Associated With the Development of Type 2 Diabetes: A Prospective Cohort Study

Xue Li,[1,2] Xing Meng,[1] Xiangchun Gao,[3] Xiuyu Pang,[1] Yanjiao Wang,[1] Xiaoyan Wu,[1] Xinrui Deng,[1] Qiao Zhang,[1] Changhao Sun,[1] and Ying Li[1]

Diabetes Care 2018;41:884–890 | https://doi.org/10.2337/dc17-1434

OBJECTIVE

We aimed to evaluate whether xanthine oxidase (XO), a key enzyme in uric acid (UA) metabolism and a major source of reactive oxygen species, plays a causal and important role in the development of type 2 diabetes mellitus (T2DM) in a large prospective cohort study.

RESEARCH DESIGN AND METHODS

A total of 4,412 diabetes-free adults (2,071 women and 2,341 men) aged 30–65 years at baseline in 2008 were involved. Participants were followed for incident change of glucose metabolism during an average of 4.7 years. At baseline, serum XO and UA, serum lipids, and glucose homeostasis indexes including fasting blood glucose (FBG), 2-h blood glucose (PBG), glycosylated hemoglobin A_{1c} (HbA_{1c}), and fasting insulin were tested for analysis.

RESULTS

During an average follow-up period of 4.7 years, 249 women and 360 men developed new-onset T2DM. Serum XO activity was positively associated with UA concentration (all *P* values <0.001). When XO activity and UA concentration were considered in the same model of the sex-specific analysis, only XO activity was significantly associated with the incidence of T2DM, with the hazard ratios from the bottom to the top quartile of XO activity being 1.00, 1.67 (95% CI 1.00–2.79), 1.86 (1.11–3.13), and 2.36 (1.43–3.90) in women and 1.00, 1.01 (0.68–1.52), 1.41 (0.98–2.03), and 1.90 (1.30–2.78) in men.

CONCLUSIONS

Elevated serum XO activity, but not UA concentration, was associated with an increased risk of developing T2DM in women and men with mutual adjustment for XO and UA. Further studies are needed to examine the underlying mechanisms.

Worldwide, type 2 diabetes mellitus (T2DM) is a major public health challenge owing to its high prevalence and increasing trend, associated morbidity and mortality, and huge economic burdens (1,2). If post-2000 trends in prevalence of T2DM continue, the number of adults with diabetes will surpass 700 million in 2025 (1). In China, the prevalence of T2DM has increased dramatically in the last two decades, affecting 10% of the adult population (3,4).

A high concentration of serum uric acid (UA) has frequently been associated with the risk of T2DM (5–8). UA metabolism is closely related to glucose and fructose

[1]Department of Nutrition and Food Hygiene, College of Public Health, Harbin Medical University, Harbin, China
[2]Department of Epidemiology and Biostatistics, College of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[3]Fourth Affiliated Hospital, Harbin Medical University, Harbin, China

Corresponding author: Ying Li, liying_helen@163.com, or Changhao Sun, changhaosun2002@163.com.

Received 17 July 2017 and accepted 11 January 2018.

Clinical trial reg. no. ChiCTR-ECH-12002938, www.chictr.org.cn.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc17-1434/-/DC1.

metabolism and obesity (9). However, not all studies support the association between UA and T2DM. In a large representative sample of the U.S. population, Bandaru and Shankar (10) reported that higher serum UA levels were inversely associated with diabetes. Such inconsistency raises doubts regarding the causal relationship between serum UA concentration and T2DM. Further, evidence from genetic studies does not support a causal relationship between UA levels and risk of T2DM (11,12). It has been suggested that xanthine oxidase (XO) may underlie the UA-T2DM association (9). XO is a metalloflavoenzyme that catalyzes oxidation of hypoxanthine to xanthine and then to UA (9). Apart from its role in UA production, XO also generates oxidants, which are key players in the T2DM development process (13–15). Although XO activity has been linked to cardiometabolic risk factors (16) and inhibition of XO activity leads to an improved cardiometabolic risk profile (17,18), no studies have examined the associations between serum XO activity and the risk of developing T2DM.

In the current study, we tested the hypothesis that elevated XO activity is a risk factor for T2DM in a large prospective study of 4,412 participants free of T2DM at baseline with a follow-up period of 4.7 years.

RESEARCH DESIGN AND METHODS

Study Population

The Cohort Study on Purine Metabolism for the Risk of Chronic Non-Communicable Diseases was launched in 2008 in China and was registered at www.chictr.org.cn (ChiCTR-ECH-12002938). A total of 7,696 Chinese residents aged 30–65 years who finished the baseline survey in 2008 were recruited (19). The follow-up survey was completed in 2013. Participants with T2DM at baseline (n = 1,154), with a history of cardiovascular disease (n = 1,093) or stroke (n = 231), receiving medication for gout (n = 100), or taking diuretics (n = 439) were excluded. In addition, 267 were lost to follow-up. The final sample included 4,412 subjects (2,071 women and 2,341 men).

This study was approved by the ethics committee of Harbin Medical University. The investigations were conducted in accordance with the Declaration of Helsinki, and written informed consent was provided by all participants.

Data Collection

At baseline, physical examination, anthropometric measurements, and laboratory measurements were conducted, and information on health behavior, medical history, and dietary intake was also collected by using a validated questionnaire. Data on current drinking status included alcoholic beverage type, the frequency of alcohol intake weekly, and daily consumption amounts, which were calculated and converted into daily alcohol consumption (grams per day). Physical activity status was investigated by exercise frequency and intensity, and the proportion who achieved vigorous physical activity at least once a week was calculated. Current smokers were defined as participants who had smoked ≥100 cigarettes in their lifetime, and never smokers were defined as those who reported no smoking at all or <100 cigarettes in their lifetime (20). Anthropometric measurements were conducted by well-trained examiners. BMI was calculated as weight in kilograms divided by the square of height in meters. We measured blood pressure three times, and the mean values were used for analysis.

Participants were asked to fill in a semiquantitative food-frequency questionnaire. The validity and reliability of the food-frequency questionnaire were evaluated in our previous study (21). Energy intake was estimated using the Food Nutrition Calculator (V1.60; Chinese Center for Disease Control, Beijing, China). To estimate the status of purine food intake, we calculated the percentage of energy from purine-rich food to total energy. Foods that are rich in purines include meat (beef, pork, chicken, lamb, minced meat, meat sauce or sausages, and animal viscera), seafood (fish and shellfish) and vegetables (peas, beans, lentils, spinach, and cauliflower). Dairy products (skimmed, semiskimmed, and whole milk and yogurt) were also included.

Biochemical Measurements

Blood samples were collected from all participants at both the baseline and follow-up surveys. After an overnight fast, blood samples were collected and immediately centrifuged at 2,500g for 15 min to obtain serum and were then cooled and stored at −80°C. A standard 75-g oral glucose tolerance test (OGTT) was performed for each participant both at the baseline and at the follow-up survey. Fasting blood glucose (FBG), 2-h blood glucose (PBG), total cholesterol (TC), HDL cholesterol (HDL-C), triglycerides (TG), UA, and creatinine were measured using a Roche Modular P800 Automatic Biochemical Analyzer (Roche Diagnostics, Mannheim, Germany). The estimated glomerular filtration rate (eGFR) was estimated based on creatinine concentration, age, sex, and ethnicity using the equation of Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (22). Serum insulin and glycosylated hemoglobin A_{1c} (HbA_{1c}) were measured using an autoimmunoassay analyzer (AIA-2000 ST; Tosoh Corporation). Serum XO activity was measured using the Amplex Red reagent method (Molecular Probes, Invitrogen Detection Technologies, Eugene, OR). For assessment of the stability of XO activity in fresh and frozen serum samples, 50 random samples with a fresh status were measured, as were 1-month and 3-month cryostorage samples. The Friedman test showed that XO activity was stable in cryopreserved serum samples (P = 0.755). Data are shown in Supplementary Table 1.

Ascertainment of T2DM

T2DM was defined as meeting at least one of the following criteria established by the American Diabetes Association (23): *1*) HbA_{1c} ≥6.5% (48 mmol/mol), *2*) FBG ≥7.0 mmol/L, *3*) 2-h PBG ≥11.1 mmol/L, or *4*) a random plasma glucose ≥11.1 mmol/L with classic symptoms of hyperglycemia or hyperglycemic crisis. In the absence of unequivocal hyperglycemia, criteria 1–3 were confirmed by repeat testing. During the 20,883 person-years of follow-up, 609 cases were ascertained.

Statistical Analyses

Selected baseline characteristics are presented as mean (SD) for continuous variables and percentages for categorical variables. For serum XO activity and UA concentrations, the cutoff points of concentration quartiles for women and men were calculated separately. Serum XO and UA concentration were categorized by quartiles, respectively, and the lowest quartile was used as the reference category. Baseline characteristics were compared using ANCOVA for continuous variables and Pearson χ^2 test for categorical variables across serum XO quartiles of women and men. Pearson correlation analysis was performed to evaluate the association between XO and UA. The

χ^2 test of independence was performed to analyze the interaction. The adjusted hazard ratios (HRs) and their 95% CIs were calculated to evaluate the effect of serum XO or UA on T2DM incidence using Cox proportional hazards models. In the sex-specific analysis, we first evaluated the association between serum XO activity and T2DM or UA concentration and T2DM separately in women and men, adjusted for age, menopause (for women only), BMI, systolic blood pressure (SBP), alcohol use, physical activity, TC, TG, HDL-C, FBG, fasting insulin, and eGFR. Then, we included serum XO activity and UA concentration in a mutually adjusted model, with additional adjustment for the variables mentioned above.

All analyses were conducted using SAS (version 9.4; SAS Institute Inc., Cary, NC). A two-sided P value <0.05 was considered to be statistically significant.

RESULTS

Baseline Characteristics of Participants According to Sex-Specific Quartile of Serum XO Activity

In this study, stratified analyses by sex were conducted because interactions with sex were significant in overall analyses (all $P < 0.001$). In consideration of the difference in distributions, the quartiles of XO and UA in women and men were analyzed separately (Table 1). During an average follow-up period of 4.7 years, the incidence of T2DM was 29.2 per 1,000 person-years (609 of 20,883 person-years), including 25.3 per 1,000 person-years (249 of 9,853 person-years) in women and 32.6 per 1,000 person-years (360 of 11,031 person-years) in men. For women, as XO activity at baseline increased from the bottom to the top quartile, age at recruitment, BMI, diastolic blood pressure (DBP), daily alcohol intake, FBG, 2-h PBG, TC, UA, fasting insulin, and HbA_{1c} all increased significantly and HDL-C decreased significantly ($P < 0.001$ in all cases). Significant differences were also observed between XO quartiles in SBP and TG ($P < 0.05$ in all cases). For men, as XO activity at baseline increased, BMI, DBP, FBG, TG, UA, and HbA_{1c} all increased significantly; HDL-C and eGFR decreased significantly ($P < 0.05$ in all cases). Significant differences were also observed between XO quartiles in age at recruitment, SBP, daily alcohol intake, physical activity, 2-h PBG, TC, and fasting insulin ($P < 0.05$ in all cases).

Association Between Serum XO Activity and UA Concentration and T2DM Incidence

Pearson correlation was conducted to analyze the association between XO and UA. The coefficients were 0.414, 0.348, and 0.450 in the total population, in women, and in men, respectively (all P values <0.001) (data not shown).

The cutoff points of the sex-specific groups of XO activity and UA concentration are shown, respectively, in Tables 2 and 3. Table 2 shows that when UA concentrations were not in the regression model, XO was observed to be associated with increased risk of T2DM after adjustment for age, menopause status (for women only), BMI, SBP, alcohol use, physical activity, TC, TG, HDL-C, FBG, fasting insulin, and eGFR. The association of XO activity with T2DM was similar in women ($P_{trend} < 0.001$) and men ($P_{trend} < 0.001$). For the analysis of women, compared with participants in the first quartile of XO activity, the HRs for those in the second, third, and fourth quartile were 1.68 (95% CI 1.01–2.81), 1.96 (1.18–3.26), and 2.54 (1.55–4.16), respectively ($P_{trend} < 0.001$); for the analysis of men, they were 1.10 (0.75–1.62), 1.45 (1.02–2.05), and 1.88 (1.32–2.67) ($P_{trend} < 0.001$).

When both serum UA concentration and XO activity were included in the same model, serum XO activity was still observed to be statistically significantly associated with T2DM after adjustment for potential confounders. The HRs (95% CIs) were 1.00 (reference), 1.67 (1.00–2.79), 1.86 (1.11–3.13), and 2.36 (1.43–3.90) for women ($P_{trend} < 0.001$) and 1.00 (reference), 1.01 (0.68–1.52), 1.41 (0.98–2.03), and 1.90 (1.30–2.78) for men ($P_{trend} < 0.001$), according to their respective quartiles.

Table 3 shows that when XO activity was not in the regression model, UA was observed to be associated with increased risk of T2DM in women after adjustment for age, menopause status, BMI, SBP, alcohol use, physical activity, TC, TG, HDL-C, FBG, fasting insulin, and eGFR. For the analysis of women, compared with participants in the first quartile of XO activity, the HRs for those in the second, third, and fourth quartiles were 1.28 (95% CI 0.84–1.96), 1.42 (0.96–2.10), and 1.56 (1.06–2.30), respectively ($P_{trend} < 0.05$); but for the analysis in men, the association was found to be not significant ($P_{trend} > 0.05$). When adjusted for XO activity and the other covariates, UA concentration was no longer associated with T2DM in both women and men (all $P_{trend} > 0.05$).

CONCLUSIONS

In this large prospective study, we observed that serum XO activity was associated with the incidence of T2DM in both women and men. XO activity also drove the association between serum UA concentration and T2DM, given that UA concentration was no longer significantly associated with T2DM after adjustment for XO activity. Further, the association of T2DM with XO activity was independent of other risk factors, in particular, insulin and eGFR. These novel findings suggest that previously observed association between UA concentration and T2DM may not be causal or at least not directly causal. To our knowledge, the current study is the first to directly address the association between XO activity and T2DM and provides an important piece of evidence that the UA-T2DM association may not be directly causal.

The most important finding of the current study is that serum XO activity is a risk factor for T2DM, independent of other traditional risk factors for T2DM. This observation is consistent with cross-sectional studies showing that XO activity is associated with cardiometabolic risk factors in both children and adults (16,24) and with the observation that serum XO activity is higher in T2DM patients (25). The observation is also consistent with the fact the XO inhibitors improve the cardiometabolic risk profile (18,26,27). Considering that serum UA concentration is modulated both by XO activity and renal clearance, and that glomerular filtration rate is judged as the best overall index of kidney function, we calculated eGFR using CKD-EPI, a commonly used equation to estimate glomerular filtration rate (22). The results, indicating that the effect of XO activity on the risk of T2DM is not affected by eGFR, may provide clues to the underlying mechanism. Further, our findings indicate that the effect of serum XO activity on T2DM risk is independent of fasting insulin. As supporting proofs of the relationship between XO activity and T2DM, HbA_{1c} and OGTT at the end point of

Table 1—Baseline characteristics of participants by sex-specific quartiles of serum XO activity

	Serum XO activity				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
Women					
N	497	439	477	658	
Age at recruitment (years)	42.4 (9.2)	44.3 (10.1)	46.9 (10.5)	49.3 (10.9)	<0.001
BMI (kg/m^2)	23.7 (3.2)	23.8 (3.1)	24.8 (3.4)	25.4 (3.2)	<0.001
SBP (mmHg)	114.7 (22.1)	124.8 (22.1)	134.2 (19.6)	133.4 (20.7)	<0.001
DBP (mmHg)	73.6 (9.4)	73.2 (7.9)	74.4 (9.1)	77.1 (9.8)	<0.001
Current smoker, *N* (%)	67 (13.5)	69 (15.7)	60 (12.6)	87 (13.2)	0.537
Alcohol intake (g/day)	2.54 (8.73)	2.84 (10.88)	5.12 (14.22)	9.69 (21.73)	<0.001
Physical activity at least once a week, *N* (%)	174 (35.0)	166 (37.8)	197 (41.3)	268 (40.7)	0.141
FBG (mmol/L)	4.49 (0.64)	4.49 (0.62)	4.54 (0.63)	4.69 (0.69)	<0.001
2-h PBG (mmol/L)	5.42 (1.40)	5.54 (1.47)	5.72 (1.61)	5.90 (1.79)	<0.001
TC (mmol/L)	4.67 (1.54)	4.82 (2.73)	5.09 (3.31)	5.24 (2.10)	0.001
TG (mmol/L)	1.29 (2.89)	1.21 (0.83)	1.35 (0.99)	2.09 (4.09)	<0.001
HDL-C (mmol/L)	1.33 (0.29)	1.33 (0.32)	1.26 (0.32)	1.15 (0.27)	<0.001
UA (μmol/L)	235.7 (128.6)	241.8 (113.6)	273.1 (99.1)	329.0 (122.6)	<0.001
eGFR (mL/min/1.73 m^2)	84.2 (15.6)	83.6 (15.0)	84.2 (15.4)	83.3 (14.8)	0.670
Fasting insulin (μU/mL)	4.62 (3.80)	5.16 (4.14)	5.16 (5.10)	5.76 (4.83)	<0.001
HbA_{1c} (%)	4.53 (0.49)	4.55 (0.44)	4.62 (0.38)	4.68 (0.55)	<0.001
Total energy (kcal/day)	2,086.2 (478.7)	2,056.1 (468.8)	2,097.2 (491.8)	2,095.5 (454.7)	0.516
Purine-rich food intake, *N* (%)	33.2 (13.0)	31.4 (13.1)	33.5 (14.6)	33.0 (13.3)	0.097
Men					
N	543	521	689	588	
Age at recruitment (years)	46.0 (10.5)	47.7 (11.2)	47.1 (11.2)	47.5 (10.9)	0.049
BMI (kg/m^2)	24.0 (3.4)	24.7 (3.2)	25.1 (3.3)	25.9 (3.6)	<0.001
SBP (mmHg)	123.7 (24.0)	135.1 (19.3)	135.9 (19.2)	131.9 (21.0)	<0.001
DBP (mmHg)	73.0 (8.4)	74.7 (9.7)	75.8 (9.2)	78.0 (10.3)	<0.001
Current smoker (%)	88 (16.2)	81 (15.5)	106 (15.4)	91 (15.5)	0.976
Alcohol intake (g/day)	3.87 (10.67)	5.44 (15.40)	10.79 (20.91)	10.72 (19.83)	<0.001
Physical activity at least once a week, *N* (%)	204 (37.6)	238 (45.7)	291 (42.2)	283 (48.1)	0.002
FBG (mmol/L)	4.52 (0.62)	4.57 (0.63)	4.65 (0.71)	4.65 (0.68)	0.001
2-h PBG (mmol/L)	5.46 (1.44)	5.73 (1.64)	5.83 (1.65)	5.82 (1.75)	<0.001
TC (mmol/L)	4.78 (1.27)	4.90 (0.96)	4.62 (0.99)	5.32 (2.92)	<0.001
TG (mmol/L)	1.28 (1.34)	1.50 (1.28)	1.73 (1.44)	2.39 (2.48)	<0.001
HDL-C (mmol/L)	1.35 (0.32)	1.29 (0.32)	1.19 (0.33)	1.18 (0.33)	<0.001
UA (μmol/L)	214.4 (128.9)	266.2 (96.0)	309.0 (104.6)	381.0 (153.9)	<0.001
eGFR (mL/min/1.73 m^2)	95.8 (15.5)	94.8 (16.7)	93.9 (14.9)	92.3 (15.0)	0.001
Fasting insulin (μU/mL)	5.09 (4.25)	4.72 (3.81)	5.12 (4.07)	6.12 (5.52)	<0.001
HbA_{1c} (%)	4.57 (0.49)	4.64 (0.31)	4.68 (0.52)	4.72 (0.62)	<0.001
Total energy (kcal/day)	2,069.8 (441.0)	2,041.9 (431.6)	2,090.5 (401.0)	2,106.2 (401.3)	0.061
Purine-rich food intake, *N* (%)	34.2 (14.1)	32.5 (13.8)	32.7 (12.6)	32.2 (13.5)	0.055

Continuous variables are presented as mean (SD). Categorical variables are presented as *N* (%).

follow-up were both examined. Similar results have been reached by several studies, such as that long-term and high-dose XO inhibitor therapy contributes to lower HbA_{1c} levels in normotensive patients with diabetes (28). The underlying mechanisms for the observed association between serum XO activity and T2DM could be supported by several studies. It suggests that increased oxidative stress as a result of elevated XO activity may be an important link, as XO also produces oxidants (29). Oxidative stress is a well-established risk factor for T2DM (15,30). Whether the association between serum XO activity and T2DM is mediated by oxidative stress or other means needs to be addressed by future population and experimental studies.

Another important finding of the current study is that serum UA is not an independent risk factor for T2DM. In fact, XO and UA were closely correlated in the metabolic process. UA is the final oxidation product of purine catabolism and catalyzed from xanthine by the XO (31). We examined the relationship between serum XO activity and UA, and there was a significant and positive association. With UA as a potential confounding factor in the regression model, the relationships between elevated XO activity and the high risk of incident T2DM was still significant. This is consistent with findings from genetic studies (11,12). These studies demonstrate that a genetic score for serum UA levels, derived from multiple genetic markers identified by recent genome-wide associations studies, does not influence T2DM risk, suggesting that the association between serum UA levels and T2DM risk from observational studies is likely confounded and thus not causal. Another study reports that the beneficial effects of XO inhibitors on chronic kidney disease associated with increased cardiovascular risk are due to the reduction of oxidative stress, independent of UA levels (32). Taken together, accumulating evidence suggest that serum UA levels may not be directly causally associated with T2DM.

These findings have important implications. A causal relationship between XO activity and T2DM suggests that inhibiting

Table 2—HRs (95% CI) of the incidence of T2DM across quartiles of serum XO activity in women and men

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Women					
Serum XO activity, mU/mL	<0.14	0.46–0.25	0.26–0.40	≥0.41	
N	497	439	477	658	
No. of cases	24	42	56	127	
Total person-years	2,411	2,127	2,226	3,089	
Incidence density (per 1,000 person-years)	10.0	19.7	25.2	41.1	
Model 1	1 (Ref.)	1.69 (1.02–2.79)	2.02 (1.24–3.30)	2.73 (1.74–4.29)	<0.001
Model 2	1 (Ref.)	1.68 (1.01–2.81)	1.96 (1.18–3.26)	2.54 (1.55–4.16)	<0.001
Model 3	1 (Ref.)	1.67 (1.00–2.79)	1.86 (1.11–3.13)	2.36 (1.43–3.90)	<0.001
Men					
Serum XO activity, mU/mL	<0.25	0.25–0.29	0.30–0.47	≥0.47	
N	543	521	689	588	
No. of cases	51	62	117	130	
Total person-years	2,591	2,460	3,291	2,689	
Incidence density (per 1,000 person-years)	19.7	25.2	35.6	48.3	
Model 1	1 (Ref.)	1.16 (0.80–1.68)	1.66 (1.20–2.31)	2.33 (1.68–3.22)	<0.001
Model 2	1 (Ref.)	1.10 (0.75–1.62)	1.45 (1.02–2.05)	1.88 (1.32–2.67)	<0.001
Model 3	1 (Ref.)	1.01 (0.68–1.52)	1.41 (0.98–2.03)	1.90 (1.30–2.78)	<0.001

Model 1, adjusted for age and menopause status (for women only); model 2, additionally adjusted for BMI, SBP, alcohol use, physical activity, TC, TG, HDL-C, FBG, fasting insulin, and eGFR; and model 3, additionally adjusted for UA concentrations.

XO activity, by XO inhibitors or other means, may likely reduce the risk of T2DM to some extent. Another implication is that reducing serum UA levels is less likely to be effective if the association between serum UA levels and T2DM is indeed not directly causal. It should be noted that our study does not nullify the associations between UA levels and other conditions, for example, hypertension (33). Finally, if the causal relationship between serum XO activity and T2DM is confirmed, future studies should examine the utility of serum XO activity as a predictor for future T2DM risk and as a marker for treatment assessment, as previously suggested (25).

Our study has several strengths. First, this is a prospective study with a relatively large sample size. Second, we used a comprehensive approach to identifying T2DM incidence cases including FBG, 2-h PBG, and HbA_{1c}. OGTTs were conducted, as a "gold standard" for diagnosis of diabetes (34), and HbA_{1c} is an indicator of chronic sustained hyperglycemia, used to measure glycemic control (35). To be clear, this study adopted diagnostic American Diabetes Association 2010 criteria for T2DM. With these, patients with normal FBG levels but excess PBG or HbA_{1c} levels accounted for a considerable portion, and the incidence, according to new diagnostic criteria, would be greater than that with diagnosis by FBG alone. Third, the observed association between serum XO activity and T2DM in women and men was robust because it persisted after adjustment for a wide range of available confounding factors.

We also recognize that our study has certain limitations. First, the study was observational in nature, and we cannot rule out the influence of unmeasured confounding factors. Besides, no amount of

Table 3—HRs (95% CI) of the incidence of T2DM across quartiles of serum UA levels in women and men

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Women					
Serum UA concentrations, μmol/L	<168.7	168.7–255.1	255.2–354.3	≥354.4	
N	517	516	520	518	
No. of cases	40	50	74	85	
Total person-years	2,479	2,461	2,444	2,469	
Incidence density (per 1,000 person-years)	16.1	20.3	30.3	34.4	
Model 1	1 (Ref.)	1.33 (0.88–2.02)	1.55 (1.05–2.28)	1.78 (1.22–2.59)	<0.001
Model 2	1 (Ref.)	1.28 (0.84–1.96)	1.42 (0.96–2.10)	1.56 (1.06–2.30)	0.001
Model 3	1 (Ref.)	1.20 (0.78–1.84)	1.24 (0.83–1.86)	1.35 (0.91–2.00)	0.189
Men					
Serum UA concentrations, μmol/L	<202.0	202.0–279.9	280.0–387.5	≥387.5	
N	584	577	592	588	
No. of cases	70	86	102	102	
Total person-years	2,776	2,710	2,800	2,744	
Incidence density (per 1,000 person-years)	25.2	31.7	36.4	37.2	
Model 1	1 (Ref.)	1.24 (0.90–1.70)	1.38 (1.02–1.88)	1.43 (1.05–1.93)	0.001
Model 2	1 (Ref.)	1.18 (0.85–1.63)	1.23 (0.90–1.69)	1.29 (0.94–1.77)	0.115
Model 3	1 (Ref.)	1.25 (0.89–1.76)	1.13 (0.82–1.57)	1.03 (0.73–1.44)	0.739

Model 1, adjusted for age and menopause status (for women only); model 2, additionally adjusted for BMI, SBP, alcohol use, physical activity, TC, TG, HDL-C, FBG, fasting insulin, and eGFR; model 3, additionally adjusted for XO activity.

adjustment can deal completely with confounding in an observational setting. Second, there are limitations to using epidemiology to address causality. We were not able to address the underlying mechanisms for the observed XO-T2DM association. The evidence of the linkage between UA and the induction of insulin resistance is growing. In differentiated adipocytes, UA induces an activation of NADPH oxidase (NOX) followed by the activation of redox-dependent proinflammatory signaling via protein kinase p38 (36). The effect of hyperuricemia has been proposed to be responsible, at least in part, for the low-grade inflammation and insulin resistance in the adipose tissue (37). In our study, UA was not associated with the risk of incident T2DM independently, but our study does not exclude that the relationship between UA and T2DM might be mediated by XO activity. Third, in this study, serum UA concentration was measured but in the absence of data on intracellular values. The results from this cohort study is one clue indicating that the relationship between UA and incident T2DM is not independent, but further research is still needed to support this finding because serum UA is an indirect reflection of intracellular urate, which is postulated to be the direct cause of insulin resistance (9) and organ damage (38). Finally, the study participants were of Han Chinese ethnicity only, which limits the generalizability of the findings. Studies of other populations are needed to confirm the findings from the current study.

In conclusion, elevated serum XO activity is a risk factor for T2DM in women and men, independent of serum UA concentration. Our study does not support an independent association between serum UA concentration and T2DM. These findings may have implications for the possible modifiable pathways to T2DM.

Acknowledgments. The authors thank all of the study participants.

Funding. This work was financially supported by National Natural Science Foundation of China (grant no. 81472980) and Chinese Postdoctoral Science Foundation (grant no. 2017M621506).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. X.L., Y.L., and C.S. conceived and designed the study. X.L. and X.W. analyzed data. X.L., X.M., X.G., X.P., Y.W., X.D., and Q.Z. collected data. X.L., X.M., and X.P. carried out the experiments. All authors were involved in writing and revising the paper and had final approval of the submitted and published versions. Y.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet 2016;387:1513–1530
2. Dall TM, Yang W, Halder P, et al. The economic burden of elevated blood glucose levels in 2012: diagnosed and undiagnosed diabetes, gestational diabetes mellitus, and prediabetes. Diabetes Care 2014;37:3172–3179
3. Xu Y, Wang L, He J, et al.; 2010 China Noncommunicable Disease Surveillance Group. Prevalence and control of diabetes in Chinese adults. JAMA 2013;310:948–959
4. Yang W, Lu J, Weng J, et al.; China National Diabetes and Metabolic Disorders Study Group. Prevalence of diabetes among men and women in China. N Engl J Med 2010;362:1090–1101
5. Kodama S, Saito K, Yachi Y, et al. Association between serum uric acid and development of type 2 diabetes. Diabetes Care 2009;32:1737–1742
6. Viazzi F, Leoncini G, Vercelli M, Deferrari G, Pontremoli R. Serum uric acid levels predict new-onset type 2 diabetes in hospitalized patients with primary hypertension: the MAGIC study. Diabetes Care 2011;34:126–128
7. Krishnan E, Pandya BJ, Chung L, Hariri A, Dabbous O. Hyperuricemia in young adults and risk of insulin resistance, prediabetes, and diabetes: a 15-year follow-up study. Am J Epidemiol 2012;176:108–116
8. Juraschek SP, McAdams-Demarco M, Miller ER, et al. Temporal relationship between uric acid concentration and risk of diabetes in a community-based study population. Am J Epidemiol 2014;179:684–691
9. Johnson RJ, Nakagawa T, Sanchez-Lozada LG, et al. Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes 2013;62:3307–3315
10. Bandaru P, Shankar A. Association between serum uric acid levels and diabetes mellitus. Int J Endocrinol 2011;2011:604715
11. Sluijs I, Holmes MV, van der Schouw YT, et al.; InterAct Consortium. A Mendelian randomization study of circulating uric acid and type 2 diabetes. Diabetes 2015;64:3028–3036
12. Pfister R, Barnes D, Luben R, et al. No evidence for a causal link between uric acid and type 2 diabetes: a Mendelian randomisation approach. Diabetologia 2011;54:2561–2569
13. Henriksen EJ, Diamond-Stanic MK, Marchionne EM. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. Free Radic Biol Med 2011;51:993–999
14. Domingueti CP, Dusse LM, Carvalho Md, de Sousa LP, Gomes KB, Fernandes AP. Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. J Diabetes Complications 2016;30:738–745
15. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004;24:816–823
16. Feoli AM, Macagnan FE, Piovesan CH, Bodanese LC, Siqueira IR. Xanthine oxidase activity is associated with risk factors for cardiovascular disease and inflammatory and oxidative status markers in metabolic syndrome: effects of a single exercise session. Oxid Med Cell Longev 2014;2014:587083
17. Zhang J, Dierckx R, Cleland JG. Xanthine oxidase inhibition for the treatment of cardiovascular disease: a systematic review and meta-analysis. Cardiovasc Ther 2014;32:57–58
18. Doehner W, Schoene N, Rauchhaus M, et al. Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: results from 2 placebo-controlled studies. Circulation 2002;105:2619–2624
19. Li X, Lin L, Lv L, et al. U-shaped relationships between sleep duration and metabolic syndrome and metabolic syndrome components in males: a prospective cohort study. Sleep Med 2015;16:949–954
20. Lindbohm JV, Kaprio J, Jousilahti P, Salomaa V, Korja M. Sex, smoking, and risk for subarachnoid hemorrhage. Stroke 2016;47:1975–1981
21. Huang L, Xue J, He Y, et al. Dietary calcium but not elemental calcium from supplements is associated with body composition and obesity in Chinese women. PLoS One 2011;6:e27703
22. Levey AS, Stevens LA, Schmid CH, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–612
23. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33(Suppl. 1):S62–S69
24. Tam HK, Kelly AS, Metzig AM, Steinberger J, Johnson LA. Xanthine oxidase and cardiovascular risk in obese children. Child Obes 2014;10:175–180
25. Kuppusamy UR, Indran M, Rokiah P. Glycaemic control in relation to xanthine oxidase and antioxidant indices in Malaysian type 2 diabetes patients. Diabet Med 2005;22:1343–1346
26. Higgins P, Dawson J, Lees KR, McArthur K, Quinn TJ, Walters MR. Xanthine oxidase inhibition for the treatment of cardiovascular disease: a systematic review and meta-analysis. Cardiovasc Ther 2012;30:217–226
27. Kamatani N, Fujimori S, Hada T, et al. Placebo-controlled, double-blind study of the non-purine-selective xanthine oxidase inhibitor Febuxostat (TMX-67) in patients with hyperuricemia including those with gout in Japan: phase 3 clinical study. J Clin Rheumatol 2011;17(Suppl. 2):S19–S26
28. Dogan A, Yarlioglues M, Kaya MG, et al. Effect of long-term and high-dose allopurinol therapy on endothelial function in normotensive diabetic patients. Blood Press 2011;20:182–187
29. Berry C, Hamilton CA, Brosnan MJ, et al. Investigation into the sources of superoxide in human blood vessels: angiotensin II increases superoxide production in human internal mammary arteries. Circulation 2000;101:2206–2212

30. Maiese K. New insights for oxidative stress and diabetes mellitus. Oxid Med Cell Longev 2015;2015:875961
31. Gurel A, Altinyazar HC, Unalacak M, Armutcu F, Koca R. Purine catabolic enzymes and nitric oxide in patients with recurrent aphthous ulceration. Oral Dis 2007;13:570–574
32. Gondouin B, Jourde-Chiche N, Sallee M, et al. Plasma xanthine oxidase activity is predictive of cardiovascular disease in patients with chronic kidney disease, independently of uric acid levels. Nephron 2015;131:167–174
33. Soletsky B, Feig DI. Uric acid reduction rectifies prehypertension in obese adolescents. Hypertension 2012;60:1148–1156
34. Genuth S, Alberti KG, Bennett P, et al.; The Expert Committee on the Diagnosis of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160–3167
35. Kohnert KD, Heinke P, Vogt L, Salzsieder E. Utility of different glycemic control metrics for optimizing management of diabetes. World J Diabetes 2015;6:17–29
36. Sautin YY, Nakagawa T, Zharikov S, Johnson RJ. Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress. Am J Physiol Cell Physiol 2007;293:C584–C596
37. Baldwin W, McRae S, Marek G, et al. Hyperuricemia as a mediator of the proinflammatory endocrine imbalance in the adipose tissue in a murine model of the metabolic syndrome. Diabetes 2011;60:1258–1269
38. Verzola D, Ratto E, Villaggio B, et al. Uric acid promotes apoptosis in human proximal tubule cells by oxidative stress and the activation of NADPH oxidase NOX 4. PLoS One 2014;9:e115210