



# Fasting Serum Fructose Levels Are Associated With Risk of Incident Type 2 Diabetes in Middle-Aged and Older Chinese Population

Diabetes Care 2020;43:2217–2225 | <https://doi.org/10.2337/dc19-2494>

Ying Chen,<sup>1</sup> Huandong Lin,<sup>1</sup> Li Qin,<sup>2</sup>  
Youli Lu,<sup>3,4</sup> Lin Zhao,<sup>1</sup> Mingfeng Xia,<sup>1</sup>  
Jingjing Jiang,<sup>1</sup> Xiaomu Li,<sup>1</sup> Chen Yu,<sup>3,4</sup>  
Geng Zong,<sup>5</sup> Yan Zheng,<sup>6</sup> Xin Gao,<sup>1</sup>  
Qing Su,<sup>2</sup> and Xiaoying Li<sup>1</sup>

## OBJECTIVE

We investigated the relationship between fasting serum fructose levels and the risk of incident type 2 diabetes in a prospective Chinese cohort.

## RESEARCH DESIGN AND METHODS

Among 949 community-based participants aged  $\geq 40$  years without diabetes at baseline, fasting serum fructose levels were measured using liquid chromatography–tandem mass spectrometry. The participants were followed up for the occurrence of diabetes. Cox regression models were performed to analyze the effect of fasting serum fructose levels on risk of incident diabetes.

## RESULTS

During a median of 3.5 years' follow-up, 179 of 949 (18.9%) participants developed type 2 diabetes. Elevated fasting serum fructose levels were associated with an increased risk of incident diabetes in a dose-response manner. After adjustment for age, sex, BMI, lipid profiles, blood pressure, liver function, smoking and drinking status, baseline glucose level, and sugar-sweetened beverage consumption, a 1-SD increased fasting fructose level was associated with a 35% (95% CI 1.08–1.67) increased risk of developing diabetes. After further adjustment for serum uric acid and estimated glomerular filtration rate, the association was partially attenuated (hazard ratio 1.33 [95% CI 1.07–1.65]). The association was similar by age, prediabetes status, BMI, and family history of diabetes but attenuated in women ( $P$  for heterogeneity = 0.037).

## CONCLUSIONS

Elevated fasting serum fructose levels were independently associated with increased risk of incident type 2 diabetes in a middle-aged and older Chinese population. Our data suggest that higher fasting serum fructose levels might serve as a biomarker and/or a contributor to incident diabetes.

Type 2 diabetes is globally epidemic (1) and associated with an increase in cardiovascular diseases and mortality (2,3), which pose a heavy public burden. In Asia, type 2 diabetes has become prevalent over the past few decades, and the number of people with type 2 diabetes continues to rise (4). Therefore, identifying risk factors and/or early markers for future diabetes is of major importance. Dietary factors have been well acknowledged to play a key role in triggering diabetes (5). Accumulating evidence demonstrates that dietary fructose consumption primarily from sugar-sweetened

<sup>1</sup>Ministry of Education Key Laboratory of Metabolism and Molecular Medicine, Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, Shanghai, China

<sup>2</sup>Department of Endocrinology and Metabolism, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

<sup>3</sup>Shanghai Xuhui Central Hospital/Zhongshan-Xuhui Hospital, Fudan University, Shanghai, China

<sup>4</sup>Shanghai Clinical Center, Chinese Academy of Science, Shanghai, China

<sup>5</sup>CAS Key Laboratory of Nutrition, Metabolism and Food Safety, Institute of Nutrition and Health, Shanghai Institutes for Biological Sciences, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China

<sup>6</sup>State Key Laboratory of Genetic Engineering, School of Life Sciences and Zhongshan Hospital, Fudan University, Shanghai, China

Corresponding author: Xiaoying Li, li.xiaoying@zs-hospital.sh.cn, or Qing Su, suqingxinhua@163.com

Received 11 December 2019 and accepted 24 May 2020

Clinical trial reg. no. NCT01506869, clinicaltrials.gov

This article contains supplementary material online at <https://doi.org/10.2337/figshare.12385463>.

Y.C., H.L., and L.Q. equally contributed to this work.

© 2020 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at <https://www.diabetesjournals.org/content/license>.

beverages (SSBs) and fruit juice is associated with an increase in diabetes incidence and poor glycemic control in patients with diabetes (6,7).

Fructose, a six-carbon monosaccharide with similar chemical structure and the same caloric value as glucose, is one of the major ingredients of SSBs and fruit juice. While the majority of metabolized fructose is derived from dietary sources of sugar, animals and humans are capable of synthesizing fructose endogenously. Fructose is produced endogenously through activation of the aldose reductase-sorbitol dehydrogenase pathway. This is the only known mechanism whereby humans and most mammals generate fructose. Although fructose can be metabolized by hexokinase similarly to glucose (8), the relative affinity for fructose is substantially less. Furthermore, under most conditions, fructose is metabolized by fructokinase (ketohexokinase [KHK]), an enzyme that is specific for fructose. KHK phosphorylates fructose to fructose-1-phosphate, resulting in ATP depletion and further generation of uric acid, oxidants, and inflammatory proteins (9). Moreover, Lanaspá et al. (10) found that with fructose-free diet intake, endogenous fructose in liver could induce insulin resistance and fatty liver through KHK, and the effect is independent of energy intake.

In micromolar concentrations, serum fructose is much more reactive *in vivo* and plays a pivotal role in mediating the pathology process. Therefore, quantification of serum fructose levels is of clinical importance to investigate the effect of fructose on metabolic disease in human. There is evidence that fructose levels are elevated in patients with diabetic nephropathy, retinopathy, and neuropathy (11–13). However, serum fructose has rarely been explored in individuals with and without diabetes, and the results are controversial (14,15). Despite compelling data *in vitro* and in animal studies, to our knowledge, the association of fasting serum fructose with incident diabetes has not been investigated. In the current study, we examined the association between fasting serum fructose concentrations and the risk of incident diabetes in a prospective cohort.

## RESEARCH DESIGN AND METHODS

### Participants and Study Design

We designed and performed a prospective cohort study, which was approved by the Ethical Committee of Zhongshan

Hospital Fudan University. Each participant provided written informed consent.

The prospective cohort study was conducted in the Shanghai Chongming District, where 9,930 subjects participated in the baseline survey from 2011 to 2012 and 7,707 participants completed the follow-up survey in 2014 (16). One thousand individuals who were free of diabetes at baseline were randomly selected for fasting serum fructose measurement. Fasting serum fructose concentration was finally measured in 949 individuals who remained after exclusion of participants with missing blood samples at baseline ( $n = 3$ ), missing blood glucose values at follow-up ( $n = 1$ ), or extreme serum fructose values ( $<0.43 \mu\text{g/mL}$  or  $>1.17 \mu\text{g/mL}$ ) ( $n = 47$ ).

Standard questionnaires were used to obtain information about demographic characteristics, lifestyle, history of diseases, and medication by face-to-face interviews conducted by trained investigators. Body weight and height were obtained in light clothing and bare feet to the nearest 0.1 kg and 0.1 cm, respectively. BMI was derived from weight in kilograms divided by the square of height in meters. Blood pressure was measured in the nondominant arm with the subject in a seated position three consecutive times with a 1-min interval and at least a 10-min rest using an automated electronic sphygmomanometer. The average value of three readings was used. Smokers were defined as participants regularly consuming cigarettes (duration  $>6$  months) right before the survey. Drinkers were defined as participants regularly consuming alcohol (duration  $>6$  months) right before the survey. Fasting venous blood samples were collected after at least a 10-h fast. Plasma glucose levels were measured by glucose oxidase method. Serum insulin was measured using an electrochemiluminescence assay. The index of HOMA of insulin resistance (HOMA-IR) was calculated as fasting insulin ( $\mu\text{IU/mL}$ )  $\times$  fasting glucose (mmol/L) / 22.5. The index HOMA of insulin sensitivity (HOMA-IS) was calculated as  $1 / (\text{fasting glucose} \times \text{fasting insulin})$ . Serum triglycerides (TG), total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), ALT, AST, and serum creatinine were measured on the Auto-Analyzer. Estimated glomerular filtration rate (eGFR) was calculated by the following equations (Chronic Kidney

Disease Epidemiology Collaboration): 1) for females,  $\text{creatinine (Cr)} \leq 0.7 \text{ mg/dL}$ ,  $\text{eGFR} = 144 \times (\text{Cr} / 0.7)^{-0.329} \times (0.993)^{\text{age}}$  and  $\text{Cr} > 0.7 \text{ mg/dL}$ ,  $\text{eGFR} = 144 \times (\text{Cr} / 0.7)^{-1.209} \times (0.993)^{\text{age}}$ ; 2) for males,  $\text{Cr} \leq 0.9 \text{ mg/dL}$ ,  $\text{eGFR} = 141 \times (\text{Cr} / 0.7)^{-0.411} \times (0.993)^{\text{age}}$  and  $\text{Cr} > 0.9 \text{ mg/dL}$ ,  $\text{eGFR} = 141 \times (\text{Cr} / 0.7)^{-1.209} \times (0.993)^{\text{age}}$  (17).

Diet information was assessed using a food frequency questionnaire at baseline, which was designed to assess usual diet over the previous year. Participants were asked how often they consumed each food item included in the questionnaire. The frequency response ranged from times per day to times per week, month, or year. In addition to frequency of consumption, participants were asked to estimate the usual quantity of SSB consumed as a standard bottle (250 mL). A typical serving of SSB was defined as 12 oz (360 mL) (18).

### Definition of Incident Diabetes

A 75-g oral glucose tolerance test was conducted, and blood samples were collected at 0 and 2 h postload. Glycated hemoglobin ( $\text{HbA}_{1c}$ ) was measured by high-performance liquid chromatography. According to American Diabetes Association 2010 criteria, diabetes was defined as 1) self-reported diabetes by health care professionals, and/or 2) fasting plasma glucose  $\geq 7.0 \text{ mmol/L}$ , and/or 3) 2-h oral glucose tolerance test plasma glucose  $\geq 11.1 \text{ mmol/L}$ , and/or 4)  $\text{HbA}_{1c}$  concentration  $\geq 6.5\%$  (48 mmol/mol). Incident diabetes was evaluated at the date of first diagnosis or the latest assessed date of follow-up, whichever occurred first. The duration of follow-up was calculated from the date of baseline blood draw to the date of assessment.

### Quantitation of Serum Fructose Concentrations

Peripheral venous blood samples were collected after at least a 10-h overnight fast and stored at  $-80^\circ\text{C}$  until assay. Serum fructose concentration was measured by a validated liquid chromatography–tandem mass spectrometry method as described previously with some modifications (19).

Briefly, 50- $\mu\text{L}$  serum samples were mixed with 10  $\mu\text{L}$  internal standard (*o*-fructose- $^{13}\text{C}_6$ ) and 150  $\mu\text{L}$  methanol, vortexed for 10 s, and centrifuged. Fifty microliters of supernatant were transferred

to a tube and mixed with 100  $\mu$ L of 75% aqueous methanol containing 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (200 mmol/L) (Sigma-Aldrich) and 3-nitrophenylhydrazine hydrochloride (150 mmol/L) (Sigma-Aldrich). The mixtures were reacted at 50°C for 60 min. After reaction, 350  $\mu$ L water was added, and the solutions were stored at  $-15^{\circ}\text{C}$  until assay.

The samples were analyzed by negative-ion ultraperformance liquid chromatography–electrospray ionization–mass spectrometry in a multiple reaction monitor mode performed on an UltiMate 3000 RSLC System (Thermo Fisher Scientific, Sunnyvale, CA) coupled to an API 4000 mass spectrometer (SCIEX, Concord, ON, Canada). Chromatographic separations were performed on a Waters ACQUITY UPLC HSS PFP ( $2.1 \times 150$  mm,  $1.7 \mu\text{m}$ ) column using water:formic acid (100:0.1 v/v; solvent A) and acetonitrile:formic acid (100:0.1 v/v; solvent B) as the mobile phase for gradient elution at a flow rate of 0.2 mL/min. The mass transitions were charge/mass ratio  $314.1 \rightarrow 236.0$  for fructose and  $320.1 \rightarrow 240.0$  for D-fructose- $^{13}\text{C}_6$ , respectively. All the data were acquired and processed using Analyst 1.6 software (SCIEX). Coefficients of variation of the assay were assessed by repeatedly analyzing quality control samples. Intra-assay coefficients of variation were 2.1% for fructose measurements.

### Oral SSB Challenge Test

We enrolled 11 healthy men and women aged 18–35 years who had no chronic illness (including no known history of diabetes, heart, renal, pulmonary, or hematologic disease) or active infections and were not pregnant or lactating. Participants were instructed not to consume any SSB or fruit juice 7 days before the study. The fructose-containing beverage challenge was administered to all participants. For each participant, a baseline blood sample was obtained after at least a 10-h overnight fast. SSB (330 mL can of cola) was drunk by each participant within a 10-min period in the morning after the overnight fast. A can of SSB (330 mL) contains a total of 10.6 g sugar, of which 10.3 g is high-fructose corn syrup (55% fructose and 43% glucose) and 0.3 g sucrose. Participants refrained from eating and drinking until the final blood sample was collected. Serial venous blood samples were drawn at 0, 0.5, 1, 1.5, 2, 3, 4, 5, and

6 h. Blood was centrifuged at  $3,000g$  for 10 min, and serum was collected and stored at  $-80^{\circ}\text{C}$  until assay.

### Statistical Analysis

Normally distributed continuous variables are presented as means with SDs, whereas skewed distributed continuous variables are presented as geometrical median and interquartile range. Continuous variables were compared by Student *t* test and one-way ANOVA, whereas skewed distribution variables were compared by Mann-Whitney *U* and Kruskal-Wallis tests. Categorical variables were expressed as proportions and compared across groups using  $\chi^2$  tests.

To minimize the potential confounding factors, covariates were selected on the basis of biologic interest, well-established risk factors for diabetes, or associated exposures and outcomes. Age- and sex-adjusted models and multivariable-adjusted models were used to explore the independent effect of fructose on risk of diabetes. Cox proportional hazards regressions were used to estimate hazard ratios (HRs) and 95% CIs of incident type 2 diabetes according to quartiles of serum fructose concentration, with the lowest quartile as the reference. In addition, the HR for per-SD increase of fructose levels was also tested. Potential effect modification was analyzed for age, sex, glucose tolerance status, obesity status, and family history of diabetes by assessing the significance of multiplicative interaction terms using Wald tests. Multivariable linear regression analysis was performed to build a composite predictor score for incident diabetes. Log-likelihood ratios (LHRs) were calculated to identify the predictive model fit, and the C-statistic was used to evaluate model discrimination.

Statistical analyses were performed on SAS 9.3 software (SAS Institute, Cary, NC). A two-sided  $P < 0.05$  was considered statistically significant.

## RESULTS

### Baseline Characteristics of the Participants

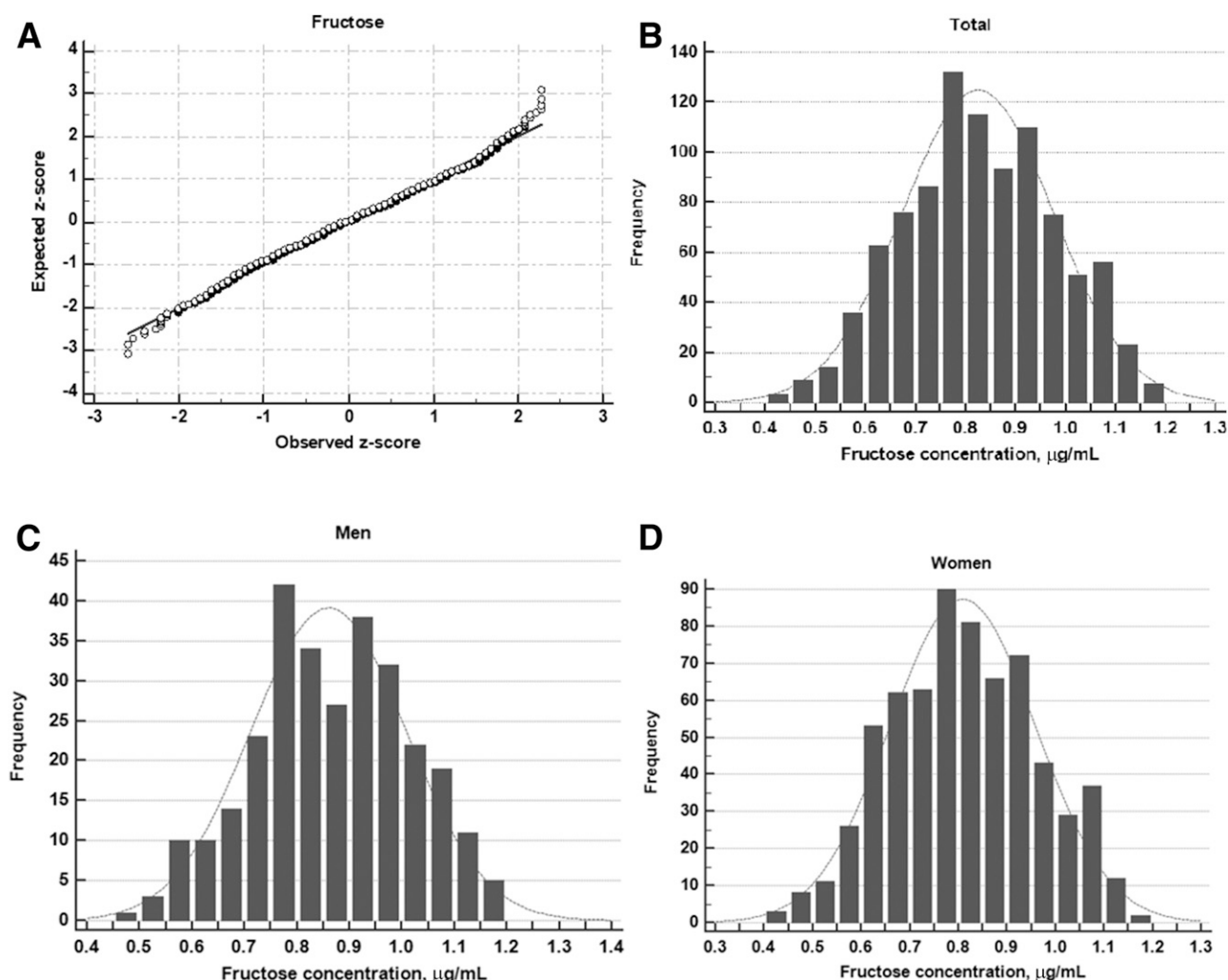
In the prospective cohort, 949 individuals without diabetes were assessed. The mean age was 56 years (range 40–71 years), and 291 (30.7%) individuals were male. Fasting serum fructose concentrations were approximately normally distributed with a mean value of  $0.85 \pm 0.21 \mu\text{g/mL}$  (Fig. 1).

During a median of 3.5 years follow-up, 179 of 949 (18.9%) individuals developed diabetes. Individuals who developed diabetes tended to have higher age, BMI, fasting plasma glucose, 2-h postload glucose,  $\text{HbA}_{1c}$ , systolic blood pressure, diastolic blood pressure, TC, TG, ALT, and AST at baseline (Table 1). Fasting serum fructose concentrations were elevated in individuals who developed diabetes compared with those who did not ( $0.85 \pm 0.15$  vs.  $0.82 \pm 0.15 \mu\text{g/mL}$ ,  $P = 0.0053$ ) (Table 1).

### Serum Fructose Levels Were Associated With an Increased Risk of Incident Diabetes

The participants were categorized according to quartiles of fasting serum fructose concentrations (Table 2). Fasting plasma glucose, 2-h postload glucose,  $\text{HbA}_{1c}$ , HOMA-IR, serum TG, ALT, AST, and uric acid were increased by increasing serum fructose quartiles (all  $P < 0.05$ ), whereas HOMA-IS decreased by increasing serum fructose quartiles ( $P < 0.0001$ ). Pearson correlation analyses revealed that sex, current smoker status, current drinker status, fasting plasma glucose, 2-h postload glucose,  $\text{HbA}_{1c}$ , serum TG, ALT, AST, and uric acid levels were significantly correlated with fasting serum fructose concentrations (Supplementary Table 1).

A graded increase of incident diabetes rate was observed with an increase of fasting serum fructose quartiles. Furthermore, Cox regression models were performed to investigate the effect of fasting serum fructose concentrations on developing diabetes in a series of adjusted models (Table 3). In the age- and sex-adjusted model, compared with the lowest quartile, participants in the highest quartile had a 79% (HR 1.79 [95% CI 1.17–2.76]) higher risk of incident diabetes, and each SD increase in fasting serum fructose levels was associated with a 39% ( $1.39$  [ $1.13$ – $1.69$ ],  $P = 0.0015$ ) increased risk of incident diabetes. The association was slightly attenuated but still remained after additional adjustment for BMI, smoking and drinking status, family history of diabetes, systolic and diastolic blood pressure, serum TC, TG, LDL-C, HDL-C, ALT, AST, and baseline glucose level. In addition, we adjusted the potential mediators and factors influencing serum fructose, including SSB consumption, uric acid, and eGFR. In the final model, each SD increase in fasting fructose



**Figure 1**—Distribution of fasting serum fructose concentrations in all participants and in men and women separately.

concentrations was associated with a 33% (1.33 [1.07–1.65],  $P = 0.010$ ) increased risk of incident diabetes. Furthermore, a subgroup analysis was performed in participants with exclusion of frequent SSB consumption ( $\geq 1$  serving per day), and the association of fasting serum fructose levels with incident diabetes remained, with a 30% increased risk of incident diabetes by 1-SD increase of fructose concentrations (1.30 [1.04–1.62],  $P = 0.023$ ) (Supplementary Table 2).

Next, we addressed the differences in the association between fasting serum fructose levels and incident diabetes among the subgroups according to age, sex, glucose tolerance status, BMI, and family history of diabetes (Fig. 2). The association was similar in individuals aged  $< 60$  or  $\geq 60$  years, with or without prediabetes, with BMI  $< 25 \text{ kg/m}^2$  or  $\geq 25 \text{ kg/m}^2$ , and with or without family history

of diabetes (all  $P$  for heterogeneity  $\geq 0.10$ ). However, the association was stronger in men than in women ( $P$  for heterogeneity = 0.037). In men, each SD increase in fasting serum fructose levels was associated with a 78% (HR 1.78 [95% CI 1.20–2.65]) increased risk for incident diabetes in the final adjusted model, whereas the association was not detected in women (1.13 [0.86–1.49]).

Besides, we investigated the incremental predictive value of fasting fructose levels when added to the models derived from traditional diabetes risk factors. For the noninvasive predictor model that was based on age, sex, BMI, family history of diabetes, and smoking and alcohol consumption, each SD increment in the basic clinical model was associated with a 31% increased risk of future diabetes ( $P = 0.0027$ ). For combined noninvasive variables and fasting fructose concentration, each SD increment was associated with a

44% increased risk of future diabetes. We assessed the predictive performance of clinical models with and without fasting fructose concentration. The basic clinical model had an LHR of 9. Addition of fasting fructose concentration to the noninvasive predictor improved model fit with an LHR of 18, whereas adding fasting blood glucose, postprandial blood glucose, and HbA<sub>1c</sub> to noninvasive predictors yielded LHRs of 21, 27, and 10, respectively. Similar patterns were observed with changes in the C-statistic across different models (Supplementary Table 3).

#### Oral SSB Challenge Test

The characteristics of the 11 participants in the SSB challenge test are presented in Supplementary Table 4. The time course of serum fructose concentration is shown in Supplementary Fig. 1. A time-dependent increase in serum fructose

**Table 1—Baseline characteristics of participants with and without incident type 2 diabetes: demographics and laboratory values**

	No diabetes ( <i>n</i> = 770)	Incident diabetes ( <i>n</i> = 179)	<i>P</i> value
Age (years)	55 ± 8	57 ± 8	0.044
Male sex	233 (30)	58 (32)	0.58
BMI (kg/m <sup>2</sup> )	24.0 ± 3.2	24.8 ± 3.3	0.0057
Glucose metabolism			
Fasting plasma glucose (mmol/L)	5.66 ± 0.52	5.83 ± 0.48	<0.0001
2-h postload glucose (mmol/L)	7.00 ± 1.59	7.69 ± 1.75	<0.0001
HbA <sub>1c</sub> (%)	5.6 ± 0.4	5.7 ± 0.4	0.034
HbA <sub>1c</sub> (mmol/mol)	38 ± 3.2	39 ± 3.2	—
Fasting serum fructose (μg/mL)	0.82 ± 0.15	0.85 ± 0.15	0.0053
Smoking status			
Smoker	96 (13.2)	17 (10.0)	0.26
Nonsmoker	634 (86.8)	153 (90.0)	
Drinking status			
Drinker	59 (8.0)	17 (9.9)	0.43
Nondrinker	676 (92.0)	155 (90.1)	
Blood pressure (mmHg)			
Systolic	127 ± 18	134 ± 19	<0.0001
Diastolic	79 ± 10	82 ± 11	0.0010
Lipid profile			
TC (mmol/L)	4.55 ± 1.00	4.73 ± 1.01	0.025
TG (mmol/L)	1.53 ± 1.15	1.78 ± 1.15	0.0079
LDL-C (mmol/L)	2.56 ± 0.75	2.65 ± 0.75	0.15
HDL-C (mmol/L)	1.24 ± 0.33	1.23 ± 0.36	0.91
Liver function			
ALT (units/L)	14.8 ± 10.2	17.7 ± 11.6	0.0007
AST (units/L)	19.1 ± 8.4	21.8 ± 10.3	0.0002
Family history of diabetes	51 (6.80)	15 (8.47)	0.44
SSBs (servings/week)	0.31 ± 2.13	0.19 ± 0.92	0.43
Uric acid (μmol/L)	323.8 ± 92.6	336.6 ± 95.3	0.096
eGFR (mL/min/1.73 m <sup>2</sup> )	96.4 ± 12.8	95.4 ± 12.4	0.34

Data are mean ± SD or *n* (%).

levels was observed after an oral SSB load. A peak concentration of serum fructose was achieved at 0.5 h and returned to baseline at 6.0 h.

## CONCLUSIONS

In the current study, using a targeted liquid chromatography–tandem mass spectrometry method, we quantified fasting fructose concentration in the circulation. We discovered that elevated fasting serum fructose levels were significantly associated with increased risk of incident diabetes in middle-aged and older Chinese. This association was independent of established diabetes risk factors. The association appeared to be dose-responsive, and each SD (0.2 μg/mL) increase in fasting serum fructose levels was associated with a 33% increase in incident diabetes risk. To our knowledge, this study evaluated for the first time the association between serum fructose concentration and the risk of incident diabetes.

Fructose is a monosaccharide that is found naturally in its free form in honey, fruits, and other plants and in a combined form as half of the disaccharide sucrose. Fructose is also present in added sugars, mainly from high-fructose corn syrup. Epidemiologic studies have shown that sugar consumption, particularly in the form of SSBs, contributes to the increased risk of obesity, fatty liver, type 2 diabetes, cardiovascular disease, and mortality (6,7). The administration of beverages containing 25% fructose to overweight subjects caused more visceral obesity and insulin resistance compared with subjects administered 25% glucose (20). Mice fed a high-fat diet supplemented with fructose developed more pronounced obesity, glucose intolerance, and fatty liver compared with mice fed a glucose-supplemented high-fat diet, despite similar caloric intake (21). Thus, fructose intake possibly increases the risk of development of obesity, fatty liver, and type 2 diabetes. In addition to diet, fructose can be produced

endogenously in the body by aldose reductase and the polyol pathway. Physiologically, endogenously synthesized fructose is the primary energy source for sperm and may be important for fertility (22). Of note, overproduction of hepatic endogenous fructose could cause systemic metabolic changes (10). However, the association of fasting serum fructose concentrations with incident diabetes has not been evaluated previously. Our data presented here demonstrate a longitudinal link between serum fructose levels and incident diabetes in humans.

When ingested by mouth, fructose is initially absorbed by the small intestine and metabolized mostly in the liver. As a consequence of the high rate of extraction of fructose by liver, corresponding low-fructose concentrations are found in the circulation. Estimated serum concentrations of fructose in humans range from 0.008 to 0.12 mmol/L (23). Although fructose circulates in micromolar concentrations (only ~1/1,000 of glucose levels in

**Table 2—Baseline characteristics of participants according to quartiles of fasting serum fructose levels**

	Fasting serum fructose levels				P for trend
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Fasting serum fructose ( $\mu\text{g/mL}$ )	<0.71	0.71–0.82	0.82–0.93	$\geq 0.93$	
Participants, <i>n</i>	222	240	238	249	
Age (years)	54.9 $\pm$ 7.9	55.6 $\pm$ 8.0	55.3 $\pm$ 7.2	56.3 $\pm$ 7.5	0.074
Sex, <i>n</i> male/ <i>n</i> female (%)	43/179 (23.4)	73/167 (25.3)	76/162 (25.1)	99/150 (26.2)	<0.0001
BMI, $\text{kg/m}^2$	24.1 $\pm$ 3.6	23.8 $\pm$ 3.0	24.3 $\pm$ 3.2	24.5 $\pm$ 3.2	0.088
Glucose metabolism					
Fasting plasma glucose (mmol/L)	5.46 $\pm$ 0.51	5.67 $\pm$ 0.50	5.75 $\pm$ 0.50	5.86 $\pm$ 0.50	<0.0001
2-h postload glucose (mmol/L)	6.64 $\pm$ 1.51	7.04 $\pm$ 1.56	7.29 $\pm$ 1.66	7.49 $\pm$ 1.72	<0.0001
HbA <sub>1c</sub> (%)	5.6 $\pm$ 0.4	5.7 $\pm$ 0.4	5.7 $\pm$ 0.4	5.7 $\pm$ 0.4	0.0027
HbA <sub>1c</sub> (mmol/mol)	38 $\pm$ 3.2	39 $\pm$ 3.2	39 $\pm$ 3.2	39 $\pm$ 3.2	—
HOMA-IR	1.58 $\pm$ 0.83	1.65 $\pm$ 0.85	1.84 $\pm$ 0.90	1.92 $\pm$ 0.97	<0.0001
HOMA-IS	0.80 $\pm$ 0.43	0.78 $\pm$ 0.45	0.69 $\pm$ 0.42	0.66 $\pm$ 0.35	<0.0001
Smoking status					
Current smoker	22 (10.6)	27 (12.0)	32 (14.0)	32 (13.4)	0.30
Nonsmoker	186 (89.4)	198 (88.0)	196 (86.0)	207 (86.6)	
Drinking status					
Current drinker	9 (4.3)	17 (7.5)	19 (8.3)	31 (12.9)	0.0012
Nondrinker	202 (95.7)	220 (92.5)	209 (91.7)	210 (87.1)	
Blood pressure (mmHg)					
Systolic	129 $\pm$ 19	128 $\pm$ 19	127 $\pm$ 17	128 $\pm$ 18	0.45
Diastolic	79 $\pm$ 10	79 $\pm$ 10	80 $\pm$ 10	80 $\pm$ 10	0.11
Lipid profile					
TC (mmol/L)	4.42 $\pm$ 0.95	4.74 $\pm$ 1.07	4.55 $\pm$ 0.98	4.61 $\pm$ 0.99	0.25
TG (mmol/L)	1.31 $\pm$ 0.82	1.60 $\pm$ 1.14	1.73 $\pm$ 1.54	1.64 $\pm$ 0.96	0.0013
LDL-C (mmol/L)	2.49 $\pm$ 0.71	2.66 $\pm$ 0.81	2.51 $\pm$ 0.71	2.63 $\pm$ 0.76	0.22
HDL-C (mmol/L)	1.21 $\pm$ 0.34	1.29 $\pm$ 0.35	1.22 $\pm$ 0.32	1.22 $\pm$ 0.31	0.60
Liver function					
ALT (units/L)	14.6 $\pm$ 11.4	14.6 $\pm$ 8.5	15.8 $\pm$ 10.5	16.3 $\pm$ 11.5	0.045
AST (units/L)	18.7 $\pm$ 9.3	19.6 $\pm$ 7.2	19.6 $\pm$ 7.8	20.7 $\pm$ 10.5	0.020
Family history of diabetes	19 (8.8)	12 (5.2)	18 (7.7)	17 (7.0)	0.74
SSBs (servings/week)	0.44 $\pm$ 3.43	0.22 $\pm$ 1.14	0.28 $\pm$ 1.12	0.23 $\pm$ 1.34	0.33
Uric acid ( $\mu\text{mol/L}$ )	307.5 $\pm$ 85.5	324.2 $\pm$ 94.4	328.6 $\pm$ 93.0	342.5 $\pm$ 96.0	<0.0001
eGFR ( $\text{mL/min/1.73 m}^2$ )	97.3 $\pm$ 16.6	96.3 $\pm$ 10.3	96.2 $\pm$ 10.5	95.3 $\pm$ 12.8	0.10

Data are mean  $\pm$  SD, *n* (%), or median (interquartile range) unless otherwise indicated.

the circulation), fructose is much more reactive in terms of mediating pathology processes in vivo. Therefore, we used a targeted approach that coupled liquid chromatography with a triple quadrupole tandem mass spectrometer to quantify serum fructose concentration.

Systemic fructose concentrations were greatly influenced by diet fructose intake. Our SSB challenge test showed that circulating fructose concentrations were increased by  $\sim 20$ -fold at 0.5–1 h and returned to baseline at 6 h. Herein, we measured circulating fructose concentration on fasting, which is relatively stable. Patel et al. (24) also reported that baseline fructose concentration during fasting was not affected by high- or low-fructose diet intake. We might speculate that postprandial fructose concentrations largely reflect dietary fructose intake and

that fasting fructose concentrations largely represent endogenous fructose production. In multivariable-adjusted models, after adjustment for SSB consumption, the association of fasting fructose levels and the risk of incident diabetes did not change. Furthermore, a subgroup analysis in participants excluding frequent SSB intake showed that the association of fasting fructose levels with risk of incident diabetes still remained, with a 30% increase in risk by 1-SD increase of fasting serum fructose levels (HR 1.30 [95% CI 1.04–1.62]). Those results indicate that the association between fasting serum fructose concentration and incident diabetes is not influenced by SSB consumption.

Fructose intake also raises serum uric acid concentrations. Previous studies showed that phosphorylated fructose

stimulates AMP deaminase to convert AMP to inosine monophosphate and then inosine monophosphate is converted to uric acid (25). Our results showed that fasting serum fructose and uric acid concentrations were correlated, whereas adjustment for uric acid partially attenuated the association of fasting serum fructose with diabetes, indicating that a mechanism other than uric acid may likely also contribute to the link between fructose and diabetes. Since fructose is excreted through the kidney, we further explored the effect of kidney function on the association of serum fructose and diabetes. In our study, eGFR was  $>60$  mL/min/1.73 m<sup>2</sup> in almost all participants, with a mean eGFR of  $96.1 \pm 12.8$  mL/min/1.73 m<sup>2</sup>. Fasting serum fructose levels were not correlated with eGFR in our study participants, and yet further



**Table 3—HR of baseline fasting serum fructose levels on risk of incident diabetes**

	Fasting serum fructose levels				P for trend	HR (95% CI) for 1-SD increase
	Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Median, $\mu\text{g/mL}$	0.62	0.76	0.87	1.02		
Cases, <i>n</i>	34	42	46	57		
Incidence rate per 100 PYs	15.1	17.2	19.3	22.9		
HR (95% CI) for:						
Age- and sex-adjusted model	Ref.	1.47 (0.93–2.32)	1.57 (1.00–2.46)	1.79 (1.17–2.76)	0.0015	1.39 (1.13–1.69)
Multivariable model 1	Ref.	1.57 (0.98–2.54)	1.61 (1.01–2.56)	1.89 (1.20–2.98)	0.0012	1.41 (1.15–1.74)
Multivariable model 2	Ref.	1.42 (0.86–2.34)	1.67 (1.02–2.72)	1.81 (1.13–2.91)	0.0039	1.37 (1.11–1.70)
Multivariable model 3	Ref.	1.38 (0.83–2.27)	1.59 (0.98–2.60)	1.73 (1.07–2.79)	0.0069	1.35 (1.09–1.67)
Multivariable model 4	Ref.	1.36 (0.82–2.25)	1.61 (0.98–2.62)	1.72 (1.07–2.78)	0.0071	1.35 (1.08–1.67)
Multivariable model 5	Ref.	1.30 (0.79–2.14)	1.52 (0.93–2.48)	1.65 (1.02–2.65)	0.011	1.33 (1.07–1.65)
Multivariable model 6	Ref.	1.30 (0.79–2.15)	1.52 (0.93–2.48)	1.65 (1.02–2.67)	0.010	1.33 (1.07–1.65)

Multivariable model 1 adjusted for age, sex, BMI, family history of diabetes, and smoking and drinking status. Multivariable model 2 adjusted for age, sex, BMI, family history of diabetes, smoking and drinking status, systolic blood pressure, diastolic blood pressure, TC, TG, LDL-C, HDL-C, ALT, and AST. Multivariable model 3 adjusted for age, sex, BMI, family history of diabetes, smoking and drinking status, systolic blood pressure, diastolic blood pressure, TC, TG, LDL-C, HDL-C, ALT, AST, and basal glucose. Multivariable model 4 adjusted for age, sex, BMI, family history of diabetes, smoking and drinking status, systolic blood pressure, diastolic blood pressure, TC, TG, LDL-C, HDL-C, ALT, AST, basal glucose, and SSB consumption. Multivariable model 5 adjusted for age, sex, BMI, family history of diabetes, smoking and drinking status, systolic blood pressure, diastolic blood pressure, TC, TG, LDL-C, HDL-C, ALT, AST, basal glucose, SSB consumption, and uric acid. Multivariable model 6 adjusted for age, sex, BMI, family history of diabetes, smoking and drinking status, systolic blood pressure, diastolic blood pressure, TC, TG, LDL-C, HDL-C, ALT, AST, basal glucose, SSB consumption, uric acid, and eGFR. PYs, person-years; Ref., referent.

adjustment for eGFR did not change the association. Intriguingly, fasting serum fructose concentrations are higher in men than in women. Meanwhile, a stronger association of serum fructose concentration with developing diabetes was also observed in men than in women. One explanation could be the impact of sex hormones on fructose metabolism.

Accumulating evidence has demonstrated that fructose is more lipogenic than glucose and that administration of fructose increases hepatic hexose-phosphate levels, activates ChREBP, and causes glucose intolerance, hyperglycemia, hypertriglyceridemia, and hepatic steatosis in mice (26). Lanaspá et al. (10) reported that mice deficient in KHK, which is unable to metabolize fructose, were protected from an increased in energy intake and body weight, visceral obesity, fatty liver, and elevated insulin levels, even though blood and urinary fructose levels were markedly increased. Thus, elevated blood fructose itself is not deleterious; rather, fructose metabolism is essential for fructose-induced metabolic disease. Importantly, fructose-1-phosphate, the fructose-specific metabolite converted by KHK, can promote glucokinase release from regulatory protein (GCKR) and enhance glycogen synthesis (27).

The polyol pathway, which is active in a wide range of tissues, is responsible for endogenous fructose formation. Therefore, serum fasting fructose may be a marker of

polyol pathway activity. In this way, glucose is first reduced to sorbitol by aldose reductase. Sorbitol is then oxidized to fructose by sorbitol dehydrogenase. Previous studies showed that fructose produced through the polyol pathway could also be upregulated by high-fat diet and salt intake (28,29). Accordingly, we found that fasting fructose concentration was correlated not only with fasting glucose but also with other features of the metabolic syndrome. These findings suggested that fasting fructose produced endogenously could be promoted in individuals with metabolic dysfunction. All these metabolic disorders eventually contribute to a high risk of incident diabetes. We speculated that an active polyol pathway and endogenous fructose production might exist in patients with diabetes and even in individuals with prediabetes. We added fasting serum fructose to the traditional models to predict incident diabetes and found that the predictive value was improved. However, the slight difference in mean value of serum fructose limited its diagnostic value. Therefore, our findings demonstrate that fasting serum fructose could serve as a potential biomarker or contributor to incident diabetes.

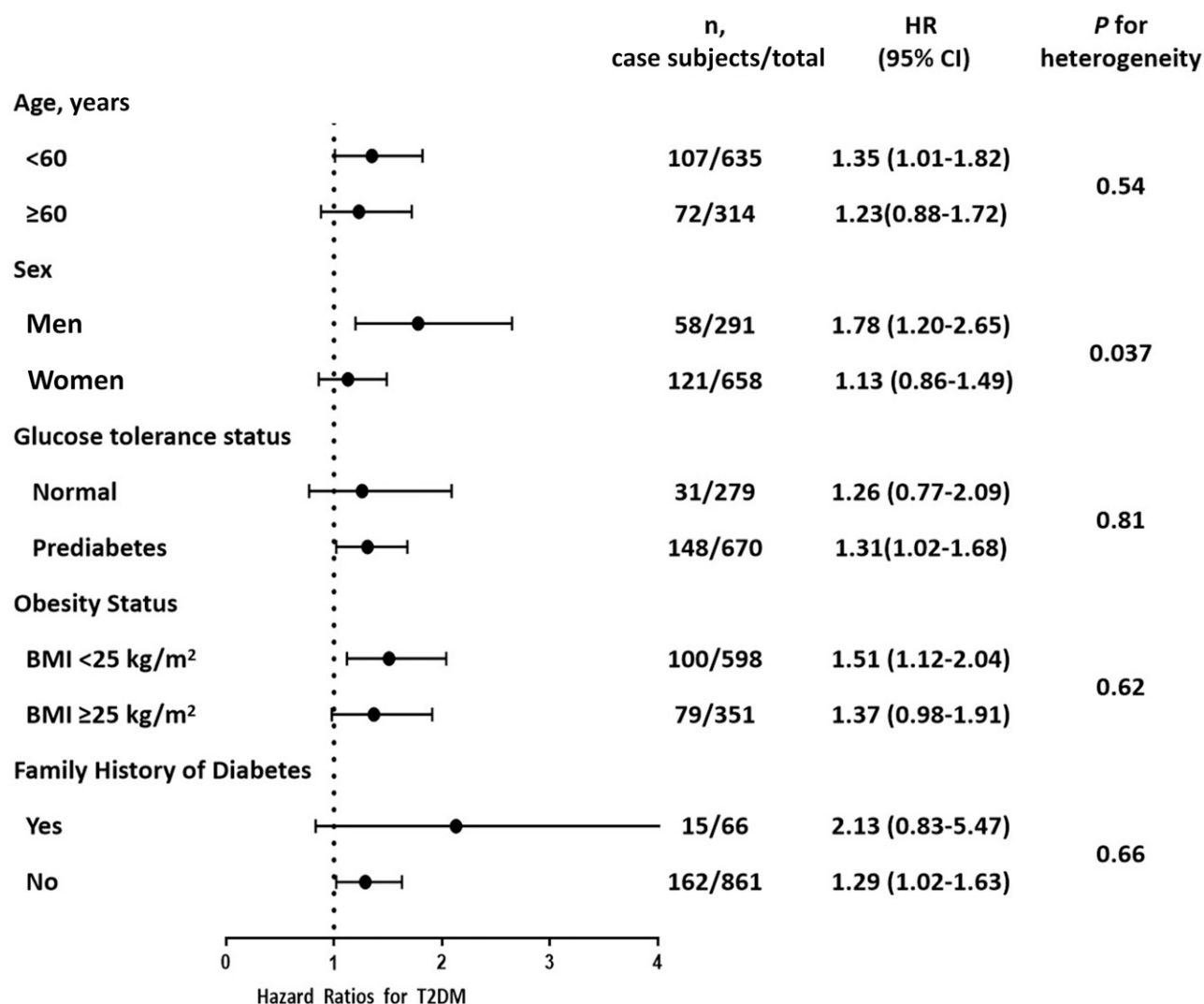
The strengths of our study are as follows. First, we conducted this study in a well-characterized prospective cohort. The prospective cohort design minimizes selection and recall bias. The details and standardized collection of demographic, lifestyle, and other covariates

allowed for adjustment of several relevant potential confounders. A large number of events provided appropriate statistical power. Second, we adopted a targeted liquid chromatography–tandem mass spectrometry method for quantification of serum fructose concentration at micromole levels, which was hardly differentiated from glucose. Our study also has several limitations. First, all participants in this study were middle-aged and older Chinese people, so the findings cannot be generalized to younger populations and other ethnicities. Second, we only measured serum fructose concentration at a single time point, which cannot reflect long-term exposure. The trajectory of serum fructose concentration may provide more information on diabetes risk than one time point.

In conclusion, elevated serum fructose level increases the risk of type 2 diabetes in middle-aged and older Chinese people. Although our population-based study cannot ascertain causality, our data suggest that higher fasting serum fructose levels might serve as a biomarker and/or a contributor to incident diabetes.

**Acknowledgments.** The authors are grateful to the field investigators for their work and the participants for their cooperation.

**Funding.** This work is supported by the National Key Research and Development Program (grants 2016YFC1304801, 2017YFC1310703), Shanghai Science and Technology Commission Scientific



**Figure 2**—Subgroup analyses of risk of incident diabetes according to 1-SD increase of fasting serum fructose. The final model adjusted for age, sex, BMI, family history of diabetes, smoking and drinking status, systolic blood pressure, diastolic blood pressure, TC, TG, LDL-C, HDL-C, ALT, AST, basal glucose, SSB consumption, uric acid, and eGFR, except the strata variable. Risk is estimated by 1-SD increased fasting fructose level. T2DM, type 2 diabetes mellitus.

Research Plan (18411951804, 17411962900), and Shanghai Municipal Science and Technology Major Project (grant 2017SHZDX01).

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

**Author Contributions.** Y.C. and H.L. analyzed and interpreted the data and wrote the manuscript. Y.C., H.L., L.Q., L.Z., M.X., J.J., Xiaom.L., and X.G. collected the data. Y.L. and C.Y. measured the samples. G.Z., Y.Z., and Xiaoy.L. interpreted the data and revised the manuscript. Q.S. and Xiaoy.L. designed the study. All authors approved the final version of the manuscript. Q.S. and Xiaoy.L. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

## References

1. Cho NH, Shaw JE, Karuranga S, et al. IDF Diabetes Atlas: global estimates of diabetes

prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* 2018;138:271–281

2. Rawshani A, Rawshani A, Franzén S, et al. Risk factors, mortality, and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2018;379:633–644

3. Sattar N, Preiss D. Research digest: progress in microvascular complications. *Lancet Diabetes Endocrinol* 2018;6:93

4. Wang L, Gao P, Zhang M, et al. Prevalence and ethnic pattern of diabetes and prediabetes in china in 2013. *JAMA* 2017;317:2515–2523

5. Ezzati M, Riboli E. Behavioral and dietary risk factors for noncommunicable diseases. *N Engl J Med* 2013;369:954–964

6. Imamura F, O'Connor L, Ye Z, et al. Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. *BMJ* 2015;351:h3576

7. Choo VL, Vigiouliou E, Blanco Mejia S, et al. Food sources of fructose-containing sugars and glycaemic control: systematic review and meta-analysis of controlled intervention studies. *BMJ* 2018;363:k4644

8. Katzen HM, Schimke RT. Multiple forms of hexokinase in the rat: tissue distribution, age dependency, and properties. *Proc Natl Acad Sci U S A* 1965;54:1218–1225

9. Diggle CP, Shires M, Leitch D, et al. Ketohexokinase: expression and localization of the principal fructose-metabolizing enzyme. *J Histochem Cytochem* 2009;57:763–774

10. Lanaspá MA, Ishimoto T, Li N, et al. Endogenous fructose production and metabolism in the liver contributes to the development of metabolic syndrome [published correction appears in *Nat Commun* 2013;4:2929]. *Nat Commun* 2013;4:2434

11. Bjornstad P, Lanaspá MA, Ishimoto T, et al. Fructose and uric acid in diabetic nephropathy. *Diabetologia* 2015;58:1993–2002



12. Kawasaki T, Ogata N, Akanuma H, et al. Postprandial plasma fructose level is associated with retinopathy in patients with type 2 diabetes. *Metabolism* 2004;53:583–588
13. Dyck PJ, Zimmerman BR, Vilen TH, et al. Nerve glucose, fructose, sorbitol, myo-inositol, and fiber degeneration and regeneration in diabetic neuropathy. *N Engl J Med* 1988;319:542–548
14. Kawasaki T, Akanuma H, Yamanouchi T. Increased fructose concentrations in blood and urine in patients with diabetes. *Diabetes Care* 2002;25:353–357
15. Preston GM, Calle RA. Elevated serum sorbitol and not fructose in type 2 diabetic patients. *Biomark Insights* 2010;5:33–38
16. Yang Z, Zhang H, Niu Y, et al. Circulating periostin in relation to insulin resistance and nonalcoholic fatty liver disease among overweight and obese subjects. *Sci Rep* 2016;6:37886
17. 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
18. Malik VS, Hu FB. Fructose and cardiometabolic health: what the evidence from sugar-sweetened beverages tells us. *J Am Coll Cardiol* 2015;66:1615–1624
19. Han J, Lin K, Sequeira C, Yang J, Borchers CH. Quantitation of low molecular weight sugars by chemical derivatization-liquid chromatography/multiple reaction monitoring/mass spectrometry. *Electrophoresis* 2016;37:1851–1860
20. Schwarz JM, Noworolski SM, Wen MJ, et al. Effect of a high-fructose weight-maintaining diet on lipogenesis and liver fat. *J Clin Endocrinol Metab* 2015;100:2434–2442
21. Softic S, Gupta MK, Wang GX, et al. Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. *J Clin Invest* 2017;127:4059–4074
22. Frenette G, Thabet M, Sullivan R. Polyol pathway in human epididymis and semen. *J Androl* 2006;27:233–239
23. Wahjudi PN, Patterson ME, Lim S, Yee JK, Mao CS, Lee WN. Measurement of glucose and fructose in clinical samples using gas chromatography/mass spectrometry. *Clin Biochem* 2010;43:198–207
24. Patel C, Sugimoto K, Douard V, et al. Effect of dietary fructose on portal and systemic serum fructose levels in rats and in KHK<sup>-/-</sup> and GLUT5<sup>-/-</sup> mice. *Am J Physiol Gastrointest Liver Physiol* 2015;309:G779–G790
25. van den Berghe G, Bronfman M, Vanneste R, Hers HG. The mechanism of adenosine triphosphate depletion in the liver after a load of fructose. A kinetic study of liver adenylate deaminase. *Biochem J* 1977;162:601–609
26. Kim MS, Krawczyk SA, Doridot L, et al. ChREBP regulates fructose-induced glucose production independently of insulin signaling. *J Clin Invest* 2016;126:4372–4386
27. Thurston JH, Jones EM, Hauhart RE. Decrease and inhibition of liver glycogen phosphorylase after fructose. An experimental model for the study of hereditary fructose intolerance. *Diabetes* 1974;23:597–604
28. Obrosova IG, Ilnytska O, Lyzogubov VV, et al. High-fat diet induced neuropathy of pre-diabetes and obesity: effects of “healthy” diet and aldose reductase inhibition. *Diabetes* 2007;56:2598–2608
29. Lanaspá MA, Kuwabara M, Andres-Hernando A, et al. High salt intake causes leptin resistance and obesity in mice by stimulating endogenous fructose production and metabolism. *Proc Natl Acad Sci U S A* 2018;115:3138–3143