Plasma tyrosine and its interaction with low high-density lipoprotein cholesterol and the risk of type 2 diabetes mellitus in Chinese

Jing Li1†, Yun-Feng Cao2†, Xiao-Yu Sun2, Liang Han1, Sai-Nan Li3, Wen-Qing Gu3, Min Song3, Chang-tao Jiang4, Xilin Yang1*, Zhong-ze Fang3*

1Department of Epidemiology and Biostatistics, School of Public Health, Tianjin Medical University, Tianjin, 2Key Laboratory of Liaoning Tumor Clinical Metabolomics (KLLTCM), Jinzhou, Liaoning, 3Department of Toxicology, School of Public Health, Tianjin Medical University, Tianjin, and 4Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, Peking University, Beijing, China

Keywords
Amino acids, Lipoprotein, Type 2 diabetes

*Correspondence
Zhong-ze Fang
Tel: +86-22-8333-6637
Fax: +86-22-8333-6608
E-mail address: fangzhongze@tmu.edu.cn

Xilin Yang
Tel: +86-22-8333-6617
Fax: +86-22-8333-6608
E-mail address: yxl@hotmail.com or yangxilin@tmu.edu.cn

J Diabetes Investig 2019; 10: 491–498
doi: 10.1111/jdi.12898

ABSTRACT

Aims/Introduction: Metabolomic markers have the potential to improve the predicting accuracy of existing risk scores for type 2 diabetes mellitus. The present study aimed to test the associations between plasma tyrosine and type 2 diabetes mellitus with special attention to identifying possible cut-off points for type 2 diabetes mellitus, and its interactive effects with low high-density lipoprotein cholesterol (HDL-C) and/or high triglyceride for type 2 diabetes mellitus.

Methods: From 27 May 2015 to 3 August 2016, we retrieved the medical notes of 1,898 inpatients with type 2 diabetes mellitus as the cases, and 1,522 individuals without diabetes as the controls who attended annual medical checkups from the same tertiary care center in Jinzhou, China. Logistic regression analyses were carried out to obtain odds ratios (ORs) and 95% confidence intervals (CIs). Restricted cubic spline analysis nested in the logistic regression analysis was used to identify possible cut-off points of tyrosine for type 2 diabetes mellitus. The additive interaction was used to estimate interactions between high tyrosine and low HDL-C in type 2 diabetes mellitus patients.

Results: The OR of tyrosine for type 2 diabetes mellitus did not increase until 46 μmol/L and after that point, the OR rapidly rose with increasing tyrosine in a nearly linear manner. If 46 μmol/L was used to define high tyrosine, high tyrosine was associated with an increased OR of type 2 diabetes mellitus (adjusted OR 1.88, 95% CI 1.44–2.45). The presence of low HDL-C greatly enhanced the ORs of tyrosine for type 2 diabetes mellitus from 1.11 (95% CI 0.82–1.51) to 54.11 (95% CI 33.96–86.22) with significant additive interaction.

Conclusions: In Chinese adults, tyrosine >46 μmol/L was associated with increased odds of type 2 diabetes mellitus, which was contingent on low HDL-C.

INTRODUCTION

Type 2 diabetes mellitus has become a heavy burden on limited medical resources. In China, the prevalence of diabetes reached 11.6% in 2010, affecting approximately 113.9 million adults1. Type 2 diabetes mellitus stems from interactions between genetic predispositions and environmental factors. Among the environmental factors, overweight and obesity are believed to play a causal role in the increasing burden of type 2 diabetes mellitus2. Obesity, especially central obesity, often appears in clusters with insulin resistance, high triglyceride and low high-density lipoprotein cholesterol (HDL-C); that is, so-called metabolic syndrome3. Although type 2 diabetes mellitus is
preventable by lifestyle modifications\(^4\), it remains a challenge to accurately predict diabetes at individual levels\(^5\).

Previous animal experiments found that insulin resistance was connected with metabolism of tyrosine\(^6\), and elevated tyrosine levels might inhibit the insulin signaling pathway\(^2\), which is related to the development of type 2 diabetes mellitus. In addition, it is believed that there is an association between hyperglycemia and tyrosine nitration\(^7\), suggesting that altered levels of tyrosine might reflect the degree of oxidative stress or inflammation in people with diabetes or prediabetes conditions. Consistently, human studies also observed that increased plasma concentration of tyrosine is associated with hyperglycemia\(^9\), and might be one of the manifestations of subclinical inflammation and immune activation\(^10\). The relationship between tyrosine levels and the risk of type 2 diabetes mellitus was robust by ethnicity and study designs\(^11\)-\(^14\). It is interesting to note that although plasma levels of many amino acids have been repeatedly linked to type 2 diabetes mellitus, tyrosine has the strongest association with the occurrence of type 2 diabetes mellitus, independent of obesity\(^13\). To our knowledge, only a few studies carried out in Chinese populations tested the association between tyrosine and type 2 diabetes mellitus.

Both high triglyceride and low HDL-C are components of metabolic syndrome and markers of insulin resistance\(^3\), but triglyceride and HDL-C might link to insulin resistance through different mechanisms or pathways. In this regard, HDL-C might upregulate phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and acetyl-CoA carboxylase to increase glucose uptake in the muscle and insulin sensitivity\(^15\), whereas high insulin levels might increase levels of triglyceride through selectively activating key enzymes involved in the synthesis of free fatty acids\(^16\). It is unknown whether there are interactions of high tyrosine and low HDL-C or high triglyceride for type 2 diabetes mellitus.

In the present cross-sectional study, we aimed to test the association between plasma levels of tyrosine and type 2 diabetes mellitus. We also explored possible cut-off points of tyrosine for type 2 diabetes mellitus and if possible, further tested any additive interactions between higher tyrosine levels and lower HDL-C and/or higher triglyceride for type 2 diabetes mellitus in Chinese patients with type 2 diabetes mellitus.

**METHODS**

**Study population and settings**

Liaoning Medical University First Affiliated Hospital, located in Jinzhou, Liaoning Province, China, is a comprehensive tertiary care center serving a population of 3.1 million. In 2013, the metabolomic laboratory was established, which offered metabolomic assays to all patients including outpatients or inpatients, or those individuals at their health examinations who agreed to pay the fee. A total of 71,020 patients having a metabolomic profile were measured from 27 May 2015 to 3 August 2016, in Liaoning Medical University First Affiliated Hospital. Among them, 1,898 patients were diagnosed with type 2 diabetes mellitus, and their electronic medical records were retrieved. Patients aged <18 years, and lacking information on height, weight and blood pressure were not included. Based on these exclusion criteria, 1,032 diabetes patients diagnosed by the 1999 World Health Organization’s criteria\(^17\) or treated with antidiabetic drugs were remaining and were designated to the case group. During this period, a total of 10,648 individuals without diabetes from the hospital’s catchment areas participated in a health examination, and 4,488 of them without information on height, weight and blood pressure were excluded. Of the remaining 6,160 individuals, 1,522 individuals with metabolomic profiles measured using the same method (aged >18 years) were retrieved and used as the control group. Finally, we organized a hospital-based non-matched case–control study with 2,554 individuals (1,032 cases and 1,522 controls) to address our research questions. The Ethics Committee for Clinical Research of FAHLMU approved the ethics of the study, and informed consent was waivered due to the retrospective nature of the study, which is consistent with the Declaration of Helsinki.

**Data collection and definitions**

The retrieved data in the cases included demographic and anthropometric information, and current clinical factors, drugs and diabetes complications. The clinical parameters included glycated hemoglobin, blood pressure, lipid profile, plasma creatinine, urinary creatinine and albumin. Diabetes complications included coronary heart disease, cerebrovascular disease, diabetic retinopathy and diabetic nephropathy. The details use of medications were documented, including oral antidiabetic drugs (OADs) and insulin, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and other antihypertensive drugs, statins, and other lipiodowering drugs.

The retrieved data in the control group included demographic information, anthropometric information and laboratory assays. In this hospital, standardized procedures were used to measure anthropometric indices. Participants wore light clothing and no shoes. Height and bodyweight were measured to the nearest 0.5 cm and 0.1 kg, respectively. Blood pressure was measured using standard mercury sphygmomanometers and appropriate sizes of adult cuffs on the right arm, after a 10-min rest in a sitting position. Age was calculated as the period in years from the date of birth to the date of inpatient hospitalization or health examination. Body mass index (BMI) was calculated to estimate adiposity as the ratio of weight in kilograms to height squared in meters, and categorized for overweight and obesity according to Chinese adults’ criteria\(^18\). The definition of metabolic syndrome was used to define low HDL-C and high triglyceride\(^19\); that is, low HDL-C defined as <1 mmol/L in men and 1.3 mmol/L in women, whereas high triglyceride was defined as >1.7 mmol/L.
Laboratory assays

LC-MS/MS analysis
Details of the metabolomics assessment method were published previously\textsuperscript{20}. Briefly, capillary whole blood was taken after at least 8-h fasting, which was stored as dried blood spot and used in the assay of metabolomics. Metabolites in dried blood spot were measured by direct infusion mass spectrometry technology equipped with the AB Sciex 4000 QTrap system (AB Sciex, Framingham, MA, USA). High-purity water and acetonitrile from Thermo Fisher (Waltham, MA, USA) were used as the diluting agent and mobile phase. 1-Butanol and acetyl chloride from Sigma-Aldrich (St Louis, MO, USA) were used to derive samples. Isotope-labeled internal standard samples of 12 amino acids (NSK-A) were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA), while standard samples of the amino acids were purchased from Chrom Systems (Grafelfing, Germany).

Biochemical assays
After at least 8-h of fasting, 8.5 mL of venous blood was drawn from each of the participants in the morning between 08.00 and 09.30 hours. Laboratory assays were carried out at a special diagnostic laboratory. Lipid profiles were analyzed by an automatic biochemistry analyzer (Hitachi 7150, Tokyo, Japan). The level of HDL-C and low-density lipoprotein cholesterol (LDL-C) was analyzed by the selective solubilization method (Determiner I. HDL, LDL test kit; Kyowa Medex, Tokyo, Japan).

Statistical analysis
Data with normal distribution were expressed as the mean ± standard deviation (SD) or median (interquartile range). Student’s \( t \)-test or the Mann–Whitney \( U \)-test were carried out to determine significant differences in the continuous data, or the \( \chi^2 \)-test (or Fisher’s exact test where appropriate) was used to compare differences in categorical variables between the type 2 diabetes mellitus group and the healthy control group. Binary logistic regressions were carried out to obtain odds ratios (OR) and 95% confidence intervals (CI) of tyrosine for type 2 diabetes mellitus. A structured adjustment scheme was used to adjust for traditional risk factors for type 2 diabetes mellitus. First, we obtained the unadjusted OR. Second, we adjusted ORs for age, sex, BMI, systolic blood pressure, LDL-C, HDL-C and triglyceride to obtain the adjusted OR of tyrosine for type 2 diabetes mellitus.

Restricted cubic splines are piecewise cubic polynomials connected across different intervals of a continuous variable, which can fit sharply curving shapes\textsuperscript{21}. To capture the full-range association between tyrosine and type 2 diabetes mellitus, and to identify possible cut-off points of tyrosine for type 2 diabetes mellitus, we used restricted cubic splines in logistic regression. We used this method in a number of our previous studies to identify cut-off points of lipids for cancer in type 2 diabetes mellitus\textsuperscript{22}. Briefly, we chose four knots at quantiles 0.05, 0.35, 0.65 and 0.95, as suggested by Harrell\textsuperscript{23}. ORs between two points of height can be estimated by EXP (the exponential functions with base e and denoted by \( e^l \); \( Y_2 - Y_1 \)), where \( Y_2 \) and \( Y_1 \) were the values of restricted cubic spline functions at tyrosine levels 2 and 1. As before, a cut-off point was selected if the odds of type 2 diabetes mellitus rapidly increased by visual checking of the curve. Further confirmation analysis was carried out by stratifying tyrosine into a categorical variable at the selected cut-off points in logistic regression analysis.

Interactions between high tyrosine and low HDL-C (and high triglyceride) were estimated using additive interaction\textsuperscript{23}. Three measures; that is, relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP) and synergy index (S), were used to estimate additive interactions. A significant RERI >0, additive interaction >0 or S >1 indicates an additive interaction or synergistic effect between high tyrosine and low HDL-C (or high triglyceride) for type 2 diabetes mellitus. A calculator is available at http://www.epinet.se.22.

Sensitivity analysis
Use of non-incident type 2 diabetes mellitus was a potential source of bias. We carried out a sensitivity analysis with exclusion of 631 patients with duration of diabetes >2 years to check changes in the effect sizes of high tyrosine for type 2 diabetes mellitus.

All the analyses were carried out using the Statistical Analysis System (release 9.2; SAS Institute Inc., Cary, North Carolina, USA), and a two-tailed \( P \)-value <0.05 was considered statistically significant.

RESULTS

Characteristics of the study population
The 2,554 participants had a mean age of 50.7 years (SD 14.7 years), mean height of 168.4 cm (SD 8.2 cm), mean bodyweight of 72.3 kg (SD 13.4 kg) and mean BMI of 25.4 kg/m\(^2\) (SD 3.6 kg/m\(^2\)). Compared with their counterparts without diabetes, the cases had an older age, shorter height, higher systolic blood pressure and diastolic blood pressure. They were also more likely to have lower levels of HDL-C and LDL-C, but higher levels of triglyceride and tyrosine. Patients with type 2 diabetes mellitus had a median of 5 years (25th to 75th: 0–10) of duration of diabetes. Furthermore, they had a mean glycated hemoglobin of 9.60% (SD 2.38%), and the prevalence of macrovascular and microvascular disease is shown in Table 1.

Associations of Tyrosine with Type 2 Diabetes Mellitus
In multivariable analysis, tyrosine was associated with type 2 diabetes mellitus in a V-shaped relationship. Obviously, at levels <30 \( \mu \)mol/L, tyrosine was inversely associated with type 2 diabetes mellitus in a roughly linear manner, while at >30 \( \mu \)mol/L, the odds ratio of tyrosine for type 2 diabetes mellitus started to decline gradually, reaching a nadir at 38 \( \mu \)mol/L and then rapidly increasing up to 46 \( \mu \)mol/L. From that point onwards, tyrosine was associated with type 2 diabetes mellitus nearly in a
linear manner (Figure 1). In the present study, 43.5% \((n = 1,113)\) of participants were categorized into the high level of tyrosine \((> 46 \text{ lmol/L})\) and 45.5% \((n = 506)\) of the patients with a high tyrosine level had type 2 diabetes mellitus. In contrast, 10.6% \((n = 272)\) of participants had low tyrosine \((< 30 \text{ lmol/L})\) and 37.5% \((n = 102)\) of the participants who had a low tyrosine level had type 2 diabetes mellitus. If the middle tyrosine levels, that is, \(\geq 30 \text{ but } \leq 46 \text{ lmol/L}\) used as the reference, the OR of the high tyrosine for type 2 diabetes mellitus was 1.47 (95% CI 1.24–1.73) in univariable analysis and 1.88 (95% CI 1.44–2.45) in multivariable analysis (Table 2). However, the association between low tyrosine levels and type 2 diabetes mellitus was not statistically significant.

**Additive interactions between high/low tyrosine and low HDL-C for type 2 diabetes mellitus**

If tyrosine \(\leq 46 \text{ lmol/L}\) and high HDL-C \(\geq 1.0 \text{ mmol/L} \) in men or \(\geq 1.3 \text{ mmol/L} \) in women were used as the reference, low HDL-C alone, but not high tyrosine alone, was associated with increased OR for type 2 diabetes mellitus in multivariable analysis. The co-presence of both associated factors greatly increased the OR to 54.11 (95% CI 33.96–86.22), with a

### Table 1 | Clinical and biochemical characteristics of participants according to the occurrence of type 2 diabetes mellitus

| Variables | Non-type 2 diabetes mellitus \((1,522)\) | Type 2 diabetes mellitus \((1,032)\) | \(P\)-value |
|-----------|--------------------------------------|--------------------------------------|----------------|
| Age (years) | 46.3 ± 13.7 | 57.2 ± 13.8 | <0.001 |
| Duration of diabetes (years) | 5 (0–10) | 401 (38.9%) | |
| Duration of diabetes ≤2 years | 401 (38.9%) | |
| Male sex | 1,131 (74.3%) | 549 (53.2%) | <0.001 |
| Height (cm) | 169.7 ± 8.0 | 166.5 ± 8.2 | <0.001 |
| Weight (kg) | 25.4 ± 3.5 | 25.3 ± 3.9 | 0.334 |
| BMI (kg/m²) | 23 (1.5%) | 27 (2.6%) | |
| BMI < 18.5 | 2 (0.1%) | 5 (0.5%) | |
| BMI ≥18.5 and <24 | 504 (33.1%) | 354 (34.3%) | |
| BMI ≥24 and <28 | 653 (42.9%) | 430 (41.7%) | |
| BMI ≥28 | 342 (22.5%) | 221 (21.4%) | |
| SBP (mmHg) | 130.9 ± 17.2 | 140.4 ± 24.0 | <0.001 |
| DBP (mmHg) | 81.0 ± 11.6 | 82.5 ± 13.5 | 0.005 |
| HDL-C (mmol/L) | 1.55 ± 0.35 | 1.08 ± 0.35 | <0.001 |
| Male (HDL-C <1.0 mmol/L) | 224 (17.9%) | 224 (21.7%) | <0.001 |
| Female (HDL-C <1.3 mmol/L) | 40 (2.5%) | 262 (25.4%) | |
| LDL-C (mmol/L) | 3.06 ± 0.70 | 2.89 ± 1.01 | <0.001 |
| Triglyceride (mmol/L) | 1.51 (1.02–2.35) | 1.67 (1.11–2.38) | 0.016 |
| Tyrosine (μmol/L) | 42.59 (34.74–52.00) | 45.78 (36.70–56.27) | <0.001 |
| <30 μmol/L | 170 (11.2%) | 102 (9.9%) | <0.001 |
| ≥30 to ≤46 μmol/L | 745 (48.9%) | 424 (41.1%) | |
| >46 μmol/L | 607 (39.9%) | 506 (49.0%) | |
| HbA1c (%) | 9.6 ± 2.4 | |
| Macrovascular complications | | |
| Prior CHD | 210 (20.4%) | 199 (19.3%) | |
| Prior stroke | 162 (15.7%) | 188 (18.2%) | |
| Microvascular complications | | |
| Diabetic retinopathy | 135 (13.1%) | |
| Diabetic nephropathy | 134 (13.0%) | |
| Diabetes medications | | |
| Oral antidiabetic drugs | 569 (55.1%) | 772 (74.8%) | |
| Insulin | 23 (2.2%) | 370 (35.9%) | |
| Statins | 135 (13.1%) | |
| Other lipid-lowering drugs | 134 (13.0%) | |
| ACEIs | 23 (2.2%) | 309 (29.9%) | |
| ARBs | | |
| Other antihypertensive drugs | | |
| Data are mean (standard deviation), median (interquartile range) or n (%). ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor antagonists BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure. |
We found that high plasma tyrosine was associated with type 2 diabetes mellitus in Chinese patients with type 2 diabetes mellitus, and tyrosine levels at ≥46 μmol/L were associated with a markedly increased OR of type 2 diabetes mellitus. However, its association with type 2 diabetes mellitus was contingent upon the presence of low HDL-C.

A positive association between tyrosine and the risk of type 2 diabetes mellitus had been repeatedly reported in several studies9,11,14,24. A small cross-sectional study of 73 participants who were obese or at high risk for type 2 diabetes mellitus showed that elevated serum tyrosine levels were associated with increased insulin resistance24. A large study in 9,000 Finnish men reported that plasma tyrosine was positively associated with glycemiaa. The Framingham Offspring Studies also found that tyrosine, combined with two other amino acids, was able to predict incident type 2 diabetes mellitus11. Consistent with these findings, we observed a positive association between high tyrosine and the increased OR of type 2 diabetes mellitus in

**DISCUSSION**

We found that high plasma tyrosine was associated with type 2 diabetes mellitus in Chinese patients with type 2 diabetes mellitus, and tyrosine levels at ≥46 μmol/L were associated with a markedly increased OR of type 2 diabetes mellitus. However, its association with type 2 diabetes mellitus was contingent upon the presence of low HDL-C.

A positive association between tyrosine and the risk of type 2 diabetes mellitus had been repeatedly reported in several studies9,11,14,24. A small cross-sectional study of 73 participants who were obese or at high risk for type 2 diabetes mellitus showed that elevated serum tyrosine levels were associated with increased insulin resistance24. A large study in 9,000 Finnish men reported that plasma tyrosine was positively associated with glycemiaa. The Framingham Offspring Studies also found that tyrosine, combined with two other amino acids, was able to predict incident type 2 diabetes mellitus11. Consistent with these findings, we observed a positive association between high tyrosine and the increased OR of type 2 diabetes mellitus in

**Sensitivity analysis**

After exclusion of participants with >2 years of diagnosed diabetes, the co-presence of high tyrosine and low HDL-C led to a larger effect size; that is, the multivariable OR being increased to 60.34 (95% CI 35.17–103.59). Similarly, all the three interaction measures also increased in multivariable analysis (AP 0.72, 95% CI 0.57–0.88; RERI 43.69, 95% CI 13.36–74.02; and S 3.78, 95% CI 2.10–6.83; Table 3).

**Additive interaction between a high level of tyrosine and high triglyceride for type 2 diabetes mellitus**

If tyrosine ≤46 μmol/L and low triglyceride were used as the reference, the co-presence of both high triglyceride and high tyrosine was associated with an increased OR for type 2 diabetes mellitus in univariable analysis and multivariable analysis. The additive interaction was not significant (Table S2).

**Table 2 | Odds ratio of tyrosine and additive interaction with lower high-density lipoprotein cholesterol for type 2 diabetes mellitus**

| Odds ratio curves of tyrosine (Tyr) for type 2 diabetes mellitus in Chinese patients. The black curve was derived from univariable analysis, and the blue curve derived from multivariate analysis that adjusted for age, gender, body mass index, systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride. The red curve stands for the reference level (i.e., the odds ratio for type 2 diabetes mellitus was 1).

| Univariable independent model | OR (95% CI) | P-value |
|-------------------------------|-------------|---------|
| Tyr (per μmol/L)               | 1.02 (1.01–1.03) | <0.001 |
| Multivariable independent model | 1.03 (1.02–1.04) | <0.001 |
| Univariable independent model | 1.05 (0.80–1.39) | 0.704  |
| ≥46 μmol/L                   | Reference |
| Multivariable independent model | 1.47 (1.24–1.73) | <0.001 |
| <30 μmol/L                   | Reference |
| ≥30 to ≤46 μmol/L             | 1.35 (0.89–2.07) | 0.163  |
| >46 μmol/L                   | 1.88 (1.44–2.45) | <0.001 |
| Univariable independent model | 21.80 (15.68–30.29) | <0.001 |
| Tyr ≤46 μmol/L & high HDL-C  | Reference |
| Tyr >46 μmol/L & high HDL-C  | 1.28 (0.98–1.67) | 0.072  |
| RERI                           | 54.35 (35.56–83.07) | <0.001 |
| AP                            | 32.27 (9.84–54.71) |
| S                             | 0.59 (0.40–0.79) |
| Multivariable independent model | 2.63 (1.56–4.11) |
| Tyr ≤46 μmol/L & low HDL-C    | Reference |
| >46 μmol/L & low HDL-C        | 18.23 (12.57–26.43) | <0.001 |
| Tyr >46 μmol/L & high HDL-C   | 11.11 (0.82–1.51) | 0.503  |
| RERI                           | 54.11 (33.96–86.22) | <0.001 |
| AP                            | 35.78 (11.66–59.89) |
| S                             | 0.66 (0.49–0.83) |
| Multivariable independent model | 3.06 (1.82–5.17) |

‡Adjusted for age, sex, body mass index, systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride. †Adjusted for age, sex, body mass index, systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride and tyrosine (Tyr) ≤30 μmol/L. Significant relative excess risk due to interaction (RERI) >0, attributable proportion due to interaction (AP) >0 or synergy index (S) >1 indicates a significant additive interaction. HDL-C, high-density lipoprotein cholesterol.
Table 3 | Odds ratio of tyrosine and additive interaction with lower high-density lipoprotein cholesterol for type 2 diabetes mellitus excluding patients with long duration (>2 years)

| Model                                      | OR (95% CI) | P-value |
|--------------------------------------------|-------------|---------|
| Univariable independent model              |             |         |
| Tyr per μmol/L                             | 1.03 (1.02–1.04) | 0.001   |
| Multivariable independent model            |             |         |
| Tyr per μmol/L                             | 1.03 (1.02–1.05) | 0.001   |
| Univariable independent model              |             |         |
| <30 μmol/L                                 | 0.86 (0.56–1.30) | 0.463   |
| ≥30 to ≤46 μmol/L                          | Reference   |         |
| >46 μmol/L                                 | 1.63 (1.29–2.05) | 0.001   |
| Multivariable independent model†           |             |         |
| <30 μmol/L                                 | 0.76 (0.40–1.44) | 0.339   |
| ≥30 to ≤46 μmol/L                          | Reference   |         |
| >46 μmol/L                                 | 2.22 (1.53–3.16) | 0.001   |
| Univariable independent model              |             |         |
| Tyr ≤46 μmol/L & high HDL-C                | Reference   |         |
| Tyr ≤46 μmol/L & low HDL-C                 | 19.34 (12.44–30.07) | 0.001   |
| Tyr >46 μmol/L & high HDL-C                | 1.24 (0.81–1.91) | 0.319   |
| Tyr >46 μmol/L & low HDL-C                 | 66.52 (40.36–109.64) | 0.001   |
| RERI                                       | 46.93 (16.03–77.83) |          |
| AP                                         | 0.71 (0.55–0.86) |          |
| S                                          | 3.53 (2.05–6.06) |          |
| Multivariable independent model‡           |             |         |
| Tyr ≤46 μmol/L & Low HDL-C                 | Reference   |         |
| Tyr ≤46 μmol/L & Low HDL-C                 | 16.67 (10.34–26.88) | 0.001   |
| Tyr >46 μmol/L & High HDL-C                | 1.00 (0.63–1.58) | 0.989   |
| Tyr >46 μmol/L & Low HDL-C                 | 60.34 (35.17–103.59) | 0.001   |
| RERI                                       | 43.69 (13.36–74.02) |          |
| AP                                         | 0.72 (0.57–0.88) |          |
| S                                          | 3.78 (2.10–6.83) |          |

†Adjusted for age, sex, body mass index, systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride. ‡Adjusted for age, gender, body mass index, systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol (HDL-C), triglyceride and tyrosine (Tyr) <30 μmol/L. Significant relative excess risk due to interaction (RERI) >0, attributable proportion due to interaction (AP) >0 or synergy index (S) >1 indicates a significant additive interaction.

Tyrosine is involved in gluconeogenesis and glucose transport. The surplus of tyrosine is rapidly catabolized, which could weaken the clearance of blood glucose and increase gluconeogenesis, and 3-nitrotyrosine formed by the combination of free tyrosine with free radicals could damage pancreatic islet β-cells25. Several studies reported that tyrosine metabolism was associated with insulin resistance. Elevated tyrosine might exaggerate pre-existing insulin resistance and also could inhibit the insulin signaling pathway6,7. Additionally, tyrosine could be synthesized when the body has enough phenylalanine, which stimulates insulin secretion8. In this regard, we found that low tyrosine levels tended to increase the risk of type 2 diabetes mellitus, although not significant. Thus, further prospective cohort studies with large sample sizes are warranted.

The present findings suggested that there was an interactive effect between high tyrosine (>46 μmol/L) and low HDL-C for type 2 diabetes mellitus. It is well established that AMPK plays an important role in energy homeostasis by balancing lipolysis and protein and glycogen storage, which can be triggered by many upstream signals7. A mechanistic study found that under the circumstance of hyperglycemia, apolipoprotein A-I gene transcription would be reduced. Apolipoprotein A-I is the major lipoprotein component of HDL, and would affect phosphorylation of AMPK and acetyl-CoA carboxylase15,28. It is plausible that the observed interaction might suggest that the association between high tyrosine and type 2 diabetes mellitus is mediated through the AMPK pathway.

The present study had several limitations. First, because of the nature of a retrospective cross-sectional survey, these findings are not evidence of causality between tyrosine and type 2 diabetes mellitus. However, based on consistent findings from previous population-based studies, the present study suggests a strong need to validate these findings in other cohort studies, especially for the selected cut-off points. Second, in our analysis, BMI was associated with type 2 diabetes mellitus in a non-linear manner, and we directly used the spline function of BMI to control its confounding effect in multivariable analysis. However, waist circumference was not available to the analysis and its confounding effect was not adjusted. Third, physical activity and diet in patients with type 2 diabetes mellitus might be different from individuals without diabetes. These data were not collected in this survey and their confounding effects, if any, were not removed. Nevertheless, physical activity and diet were associated with BMI, and careful adjustment for BMI might have partially removed the confounding effect of diet and physical activity. Fourth, inpatients with type 2 diabetes mellitus had more serious disease, and they did not represent general patients with type 2 diabetes mellitus. Our sensitivity analysis showed that exclusion of the patients diagnosed >2 years increased the ORs of the co-presence of both risk factors and the additive interaction measures. Thus, the reported effect sizes of the OR and the additive interaction between high tyrosine and low HDL-C might underestimate their true effect sizes.

The present study has public health importance. China had 113.9 million adults with type 2 diabetes mellitus in 2010, and an increasing number of people are expected to have the devastating disease in the future. It is critically important to accurately predict incident cases at individual levels some years before its onset. However, recent efforts failed to have developed risk scores that can accurately predict incident type 2 diabetes mellitus5, even inclusion of genetic factors in the predicting tools29,30. The present study suggests that high tyrosine, especially combined with low HDL-C, might be a candidate marker for inclusion in future risk scores for type 2 diabetes mellitus.
diabetes mellitus in Chinese individuals if these findings can be replicated in cohort studies, especially, in China. In conclusion, we found that plasma tyrosine levels of >46 μmol/L were associated with a markedly increased odds of type 2 diabetes mellitus in Chinese adults. The association between tyrosine >46 μmol/L and type 2 diabetes mellitus depended on the presence of low HDL-C. As the present findings came from a case–control study, a reverse relationship cannot be excluded. Further follow-up studies are warranted to confirm our novel findings in Chinese people and other populations. If replicated, high tyrosine or the co-presence of high tyrosine and low HDL-C might be included in future risk scores for predicting incident type 2 diabetes mellitus.

ACKNOWLEDGMENTS
All authors approved the final version of the manuscript and agreed to submit. XY, ZF (the corresponding authors) and JL (the first author) take full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript. The authors thank all doctors, nurses and research staff at the Liaoning Medical University (FAHLMU) in Jinzhou for their participation in this study. This work was supported by the project for the National Key Research and Development Program (2016YFC0903100, 2016YFC0903102), the 13th five-year plan and TMU talent project (11601501/2016J0313), National Natural Science Foundation of China (No. 81602826, 81672961), Individualized diagnosis and treatment of colorectal cancer (No. LNCCC-B05-2015), Foundation of Committee on Science and Technology of Tianjin (Grant No. 15JCYBJC54700), the China Postdoctoral Science Foundation (2016M590210), Tianjin Health Bureau Science Foundation Key Project (16KG154), Tianjin Project of Thousand Youth Talents, and Natural Science Foundation of Liaoning Province (No. L2015317).

DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. Xu Y, Wang L, He J, et al. Prevalence and control of diabetes in Chinese adults. JAMA 2013; 310: 948–959.
2. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014; 384: 766–781.
3. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005; 112: 2735–2752.
4. Pan XR, Li GW, Hu YH, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care 1997; 20: 537–544.
5. Gao WG, Dong YH, Pang ZC, et al. A simple Chinese risk score for undiagnosed diabetes. Diabet Med 2010; 27: 274–281.
6. Spencer CJ, Heaton JH, Gelehrter TD, et al. Insulin selectively slows the degradation rate of tyrosine aminotransferase. J Biol Chem 1978; 253: 7677–7682.
7. Ferguson AA, Roy S, Kornamik KN, et al. TATN-1 mutations reveal a novel role for tyrosine as a metabolic signal that influences developmental decisions and longevity in Caenorhabditis elegans. PLoS Genet 2013; 9: e1004020.
8. Koeck T, Corbett J, Crabb JW, et al. Glucose-modulated tyrosine nitration in beta cells: targets and consequences. Arch Biochem Biophys 2009; 484: 221–231.
9. Stancakova A, Civelek M, Saleem NK, et al. Hyperglycemia and a common variant of GCKR are associated with the levels of eight amino acids in 9,369 Finnish men. Diabetes 2012; 61: 1895–1902.
10. Murr C, Grammer TB, Meinitzer A, et al. Immune activation and inflammation in patients with cardiovascular disease are associated with higher phenylalanine to tyrosine ratios: the Ludwigshafen risk and cardiovascular health study. J Amino Acids 2014; 2014: 783730.
11. Wang TJ, Larson MG, Vasan RS, et al. Metabolite profiles and the risk of developing diabetes. Nat Med 2011; 17: 448–453.
12. Tillin T, Hughes AD, Godsland IF, et al. Insulin resistance and truncal obesity as important determinants of the greater incidence of diabetes in Indian Asians and African Caribbeans compared with Europeans: the Southall And Brent REvisited (SABRE) cohort. Diabetes Care 2013; 36: 383–393.
13. Tillin T, Hughes AD, Wang Q, et al. Diabetes risk and amino acid profiles: cross-sectional and prospective analyses of ethnicity, amino acids and diabetes in a South Asian and European cohort from the SABRE (Southall And Brent REvisited) Study. Diabetologia 2015; 58: 968–979.
14. Chen T, Ni Y, Ma X, et al. Branched-chain and aromatic amino acid profiles and diabetes risk in Chinese populations. Sci Rep 2016; 6: 20594.
15. Han R, Lai R, Ding Q, et al. Apolipoprotein A-I stimulates AMP-activated protein kinase and improves glucose metabolism. Diabetologia 2007; 50: 1960–1968.
16. Shimomura I, Bashmakov Y, Ikemoto S, et al. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci USA 1999; 96: 13656–13661.
17. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15: 539–553.
18. Chen C, Lu FC. Department of Disease Control Ministry of Health PRC. The guidelines for prevention and control of overweight and obesity in Chinese adults. Biomed Environ Sci 2004; 17(Suppl): 1–36.

19. Lam DW, LeRoith D. Metabolic Syndrome. Endotext, 2015.

20. Wang Q, Sun T, Cao Y, et al. A dried blood spot mass spectrometry metabolomic approach for rapid breast cancer detection. Onco Targets Ther 2016; 9: 1389–1398.

21. Harrell F. Regression Modelling Strategies. With Applications to Linear Models, Logistic Regression and Survival Analysis. New York, NY: Spinger-Varlag, 2001.

22. Yang X, Lee HM, Chan JC. Drug-subphenotype interactions for cancer in type 2 diabetes mellitus. Nat Rev Endocrinol 2015; 11: 372–379.

23. Andersson T, Alfredsson L, Kallberg H, et al. Calculating measures of biological interaction. Eur J Epidemiol 2005; 20: 575–579.

24. Huffman KM, Shah SH, Stevens RD, et al. Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. Diabetes Care 2009; 32: 1678–1683.

25. Chi Q, Wang T, Huang K. Effect of insulin nitration by peroxynitrite on its biological activity. Biochem Biophys Res Commun 2005; 330: 791–796.

26. van Loon LJ, Kruijshoop M, Menheere PP, et al. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. Diabetes Care 2003; 26: 625–630.

27. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 2007; 100: 328–341.

28. Murao K, Wada Y, Nakamura T, et al. Effects of glucose and insulin on rat apolipoprotein A-I gene expression. J Biol Chem 1998; 273: 18959–18965.

29. de Miguel-Yanes JM, Shrader P, Pencina MJ, et al. Genetic risk reclassification for type 2 diabetes by age below or above 50 years using 40 type 2 diabetes risk single nucleotide polymorphisms. Diabetes Care 2011; 34: 121–125.

30. Lyssenko V, Laakso M. Genetic screening for the risk of type 2 diabetes: worthless or valuable? Diabetes Care 2013; 36 (Suppl 2): S120–S126.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Additive interactions between tyrosine <30 µmol/L and with low high-density lipoprotein cholesterol for the risk of type 2 diabetes mellitus.

Table S2 | Additive interactions between tyrosine) >46 µmol/L and high triglyceride for the risk of type 2 diabetes mellitus.