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Fetuin-B links nonalcoholic fatty liver disease to type 2 diabetes via inducing insulin resistance: Association and path analyses

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ABSTRACT

Objective: Laboratory models suggested that Fetuin-B impaired insulin action in myotubes and hepatocytes and caused glucose intolerance in mice. We aimed to explore the independent associations and pathways among serum Fetuin-B, nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2D).

Methods: A cross-sectional study of 1318 obese adults who underwent serum Fetuin-B test and hepatic ultrasonography scanning was conducted in Xiamen, China. Multivariable logistic regression was used to calculate adjusted odds ratio (OR) and 95% confidence intervals (CI) of serum Fetuin-B level and NAFLD for T2D in different models with adjustment for potential confounders. Structural equation modeling (SEM) was used to examine the paths among NAFLD, serum Fetuin-B, metabolic/insulin resistance syndrome and T2D.

Results: Subjects with T2D or NAFLD showed significantly increased serum Fetuin-B levels compared to their controls (4.25 ± 1.35 vs. 4.08 ± 1.38 μg/ml for diabetes; and 4.26 ± 1.41 vs. 4.07 ± 1.33 μg/ml for NAFLD; both p-values < 0.05). NAFLD and higher serum Fetuin-B were significantly associated with higher risk of T2D with adjustment for sociodemographic and lifestyle habits; and the adjusted ORs (95%CIs) were 2.90 (2.17–3.87, $p < 0.001$) and 1.16 (1.01–1.32, $p = 0.032$), respectively. With further adjustment for metabolic/insulin resistance syndrome (BMI, systolic and diastolic BP, triglyceride, total cholesterol, HDL- and LDL-cholesterol, HOMA-IR and serum uric acid), NAFLD but not serum Fetuin-B was significantly associated with increased risk of T2D (ORs (95%CIs): 1.58 (1.12–2.21, $p = 0.009$) and 1.07 (0.92–1.23, $p = 0.384$), respectively). A one pathway model by using SEM fitted well ($\chi^2 = 497.92$, $p < 0.001$; CFI = 0.965; TLI = 0.926; and RMSEA = 0.097) and showed that NAFLD increased serum Fetuin-B and elevated Fetuin-B increased fasting insulin level, which in turn induced insulin resistance and T2D. Besides, NAFLD increased the risk of T2D directly in addition to its indirect effects of inducing metabolic/insulin resistance syndrome which in turn increased the risk of T2D.

Conclusions: Fetuin-B links NAFLD to T2D via inducing insulin resistance, and NAFLD contributes to the pathogenesis of T2D via multiple mechanisms.

1. Introduction

There has been growing evidence to show that nonalcoholic fatty liver disease (NAFLD) is not only a kind of chronic liver disease but also contributes to extra-hepatic diseases, such as type 2 diabetes (T2D) and cardiovascular disease (CVD) [1,2]. NAFLD is closely associated with metabolic/insulin resistance syndrome, which may therefore predict T2D incidence [3]. After systematic reviewing 20 publications on predicting effect of NAFLD, diagnosed by either ultrasonograpy (n = 6) or liver enzymes (n = 14), on incidence of T2D in longitudinal studies, Lallukka et al. found that NAFLD predicted T2D independently of confounders such as age and obesity [4]. However, due to the heterogeneity of study subjects and difference on diagnose methods of NAFLD, there are still some controversial reports about the relationship between

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NAFLD and incidence of T2D. After following up on 358 Australian adults during a 11-year period, NAFLD was not found to be associated with incidence of T2D independently of obesity and insulin resistance [5]. Since metabolic/insulin resistance syndrome has been consistently shown to play important roles in the pathogenesis of both T2D and NAFLD [3,6,7], further studies are warranted to test the association between NAFLD and T2D with adjustment for features of metabolic/insulin resistance syndrome.

Factors linking NAFLD to T2D are not fully understood, although some hepatokines have been found to be involved in liver steatosis and metabolic cross-talk [8,9]. Fetuin-B, secreted from the liver as a member of the cystatin superfamily of cysteine protease inhibitors, shares 22% homology with Fetuin-A which has been found to be associated with the risk of T2D incidence in a few large prospective, population-based studies [10,11]. Meex RC et al. reported that Fetuin-B was increased in humans with liver steatosis, impaired insulin action in myotubes and hepatocytes and caused glucose intolerance in mice [12]. However, there is no evidence currently available about association between Fetuin-B and diabetes in humans.

Zhu et al. reported that serum Fetuin-B increased in subjects with NAFLD [13]. Our unpublished data suggested that serum Fetuin-B level was positively correlated with intrahepatic triglyceride content, and elevated serum Fetuin-B was independently associated with increased risk of insulin resistance in Chinese adults. Whether Fetuin-B is associated with T2D independently of NAFLD and metabolic/insulin resistance syndrome or Fetuin-B mediates the association of NAFLD with diabetes by through metabolic/insulin resistance syndrome is currently unknown. Therefore, pathways about the relationships of Fetuin-B, NAFLD and metabolic/insulin resistance syndrome with T2D should be explored.

In the present study based on 1318 community-living obese Chinese adults, we firstly aimed to explore the independent associations of Fetuin-B and NAFLD with T2D with adjustment for features of metabolic/insulin resistance syndrome. Secondly, we aimed to explore the possible pathways among Fetuin-B, NAFLD, metabolic/insulin resistance syndrome and T2D.

2. Methods

2.1. Subjects

Details on subjects evaluation have been described previously [14,15]. Briefly, 1523 community-living healthy adults aged 40 years or older living in Lianqian community, Xiamen, China with central obesity (waist circumference greater than 90 cm for men and 80 cm for women) were recruited in 2011. Of them, 92 had incomplete data on clinical or hepatic ultrasonography scanning and 113 did not undergo serum Fetuin B measurements, then 1318 (86.5%) subjects with the complete data on serum fetuin B levels and other examinations were left for the present analysis. The study was conducted in accordance with the Helsinki Declaration and the International Conference on Harmonization/Good Clinical Practice guidelines. The study protocol was approved by the human ethics committees of the First Affiliated Hospital of Xiamen University, Xiamen, China. Written informed consent was obtained from all patients.

For each subject, face-to-face interview was conducted to collect socio-demographic status, lifestyle habits, present and previous history of health and medications. Subjects were excluded if they drank regularly with alcohol consumption ≥140 g/week for men or ≥70 g/week for women, had cancer, or received current treatment with systemic corticosteroids, biliary obstructive diseases, acute or chronic virus hepatitis, drug-induced liver diseases, total parenteral nutrition, autoimmune hepatitis, Wilson's disease, known hyperthyroidism or hypothyroidism.

2.2. Measurements

Subjects underwent weight, height and waist circumference measurements by using a calibrated scale after removing shoes and heavy clothes. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. Body fat was quantified with the Hologic whole body DXA systems (Hologic Inc., Bedford, MA). Arterial blood pressure was measured with a mercury sphygmomanometer after sitting for at least 15 min. Three readings were taken at 5-min intervals and the mean of them was recorded.

Blood samples were obtained after 12-hour fasting and 75-g oral glucose tolerance test were conducted for each subject. All biochemical measurements were tested in the central laboratory of the First Affiliated Hospital, Xiamen University. Plasma glucose and serum lipid profiles, including triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined on a HITACHI 7450 analyzer (HITACHI, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula. Fasting plasma glucose (FPG) and 2-hour plasma glucose (2-h PG) concentrations were measured by the hexokinase method. Serum fasting insulin concentration was measured by electrochemiluminiscence immunoassay (Roche Elecsys Insulin Test, Roche Diagnostics, Mannheim, Germany). Homeostasis model assessment - insulin resistance (HOMA-IR) was calculated using the formula: fasting serum insulin (mU/L) * fasting plasma glucose (mmol/L)/22.5. And insulin resistance was defined as HOMA-IR $\geq 2.6 * 10^{-6}$ mol U/L^2 [16]. Serum Fetuin-B concentration was measured using the enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, UK). The sensitivity of the assay was 4 ng/ml and the linear range of the standard was 4–50 ng/ml. The intra-assay variation was < 10% and the inter-assay variation was < 12%.

Hepatic ultrasonography scanning was performed by an experienced radiologist using GE LOGIQ P5 scanner (GE Healthcare, Milwaukee, USA) with a 4-MHz probe, who was blinded to the subjects' health status. Hepatic steatosis was diagnosed on the basis of characteristic sonographic features, including hepatorenal echo contrast, liver parenchymal brightness, deep beam attenuation, and vessel blurring [17]. The definition of NAFLD was based on hepatic ultrasonography diagnosis of hepatic steatosis without excessive alcohol consumption, viral or autoimmune liver disease.

According to American Diabetes Association (ADA) 2017 criteria, diabetes was defined as (1) a self-reported history of diabetes previously diagnosed by health care professionals; (2) fasting plasma glucose (FGP) ≥126 mg/dL (7.0 mmol/L); (3) 2-hour plasma glucose (2-h PG, OGTT) ≥200 mg/dL (11.1 mmol/L); or (4) HbA1c ≥ 6.5% (48 mmol/mol). T2D was identified for diabetes cases with the age of 40 years or older who are overweight or obese and/or have a family history of diabetes [18].

2.3. Statistics

Data was presented as the mean ± standard deviation for continuous variable or number and percentage for categorical variable. Skewness and kurtosis test for normality of serum Fetuin-B level was conducted and found it followed approximation of normal distribution. Differences between subjects categorized by NAFLD and T2D were analyzed using one-way ANOVA for continuous variables and chi-square test for categorical variables.

Multivariable logistic regression was used to calculate adjusted odds ratio (OR) and 95% confidence intervals (CI) of serum Fetuin-B level and NAFLD for T2D in different models with adjustment for potential confounders. In model 1, age and sex were adjusted for; in model 2, educational level, smoking and drinking habits and regular physical exercise plus model 1 were adjusted for; in model 3, BMI, systolic and diastolic BP, triglyceride, total cholesterol, HDL- and LDL-cholesterol, HOMA-IR and serum uric acid plus model 2 were adjusted for. In each