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Association of serum metabolites with impaired fasting glucose/diabetes and traditional risk factors for metabolic disease in Chinese adults

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ABSTRACT

Background: Hyperglycemia has become a major health problem worldwide. We investigated the associations of serum metabolite levels with hyperglycemia (impaired fasting glucose/diabetes) and traditional risk factors for metabolic disease.

Methods: A total of 563 Chinese adults were categorized into hyperglycemia and control groups. Associations of serum metabolites, including branched-chain amino acids (BCAAs), aromatic amino acids (AAAs), glutamine (Gln), glutamic acid (Glu), Gln/Glu ratio, 25-hydroxyvitamin D, and lysophosphatidylcholine (LPC), with hyperglycemia and traditional risk factors of metabolic disease were investigated using our targeted metabolomics method.

Results: Participants with impaired fasting glucose or diabetes exhibited markedly lower levels of Gln/Glu and unsaturated LPC and higher levels of Glu and BCAAs. Gln/Glu ratio, unsaturated LPC, and 25-hydroxyvitamin D were positively correlated with protective factors, while saturated LPC, BCAAs, AAAs, and Glu revealed close correlations with traditional risk factors. In the logistic regression, low Gln/Glu ratio and high BCAA level were independent risk factors for hyperglycemia; the odds ratios (95% confidence interval) of the highest quartile compared with the lowest quartile were 0.499 (0.274–0.910) and 2.588 (1.313–5.102) ($P < 0.05$), respectively.

Conclusions: Gln/Glu ratio, BCAAs, and LPC were significantly related to hyperglycemia development and risk factors for metabolic disease.

1. Introduction

Hyperglycemia, as a major clinical symptom of diabetes and impaired fasting glucose, has attracted widespread attention due to its association with metabolic disorders [1–4]. Impaired fasting glucose and diabetes-related perturbations in metabolism are very complicated. It is important to understand how metabolite profiles are altered in impaired fasting glucose and diabetes. Earlier identification of such changes in metabolites is particularly important for effective evaluation of individuals at risk of developing metabolic disease and could add information beyond traditional risk factors.

Metabolomics has been used to investigate the relationships between phenotype and metabolism [5,6]. Recent advances in metabolomics, based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), have allowed for high-throughput targeted or untargeted exploration of the metabolite changes in body fluids and tissues. These metabolites represent intermediate and end products of metabolic pathways that reflect physiological dysfunction and that might mirror earlier stages of diseases. Therefore, metabolomics technology is particularly useful for identifying alterations in several specific metabolites and for understanding of disease-relevant metabolic processes [7,8].

Branched-chain amino acids (BCAAs) consist of leucine (Leu), isoleucine (Ile), and valine (Val), which play a vital role as building blocks for protein synthesis and biosynthesis of sterol, ketone bodies, and glucose [9]. Prior clinical studies showed significant associations of the plasma levels of specific BCAAs and aromatic amino acids (AAAs) with body mass index (BMI) or glucose regulation [10,11]. Recently, several studies have also documented that BCAAs could predict impaired glucose tolerance before the onset of diabetes mellitus [12]. Meanwhile, glutamine (Gln) and glutamic acid (Glu) as well as some molecular species of lysophosphatidylcholine (LPC), for example, LPC 16:0 (palmitoyl), 18:0 (stearoyl), 18:1 (oleoyl), and 18:2 (linoleoyl), were found to be differentially altered in people with abnormal metabolic conditions such as diabetes, insulin resistance, and obesity [13,14]. These observations reveal that altered metabolite levels are significantly associated with hyperglycemia and its related metabolic syndrome.

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Monitoring these alterations allows predicting the development of impaired fasting glucose and diabetes, and therefore could aid in identifying at-risk individuals. However, there are different epidemiological results between dietary intake and risk of metabolic disease. In a study regarding insulin resistance, BCAA supplementation was found to attenuate insulin resistance and improve health-related quality of life in patients with chronic hepatitis [15]. Similarly, some large-scale population studies showed that high intake of BCAAs was significantly correlated with a lower prevalence of obesity and a decreased risk of diabetes, which thereby distinguishes the findings on the relationships between plasma BCAA levels and hyperglycemia [16]. Additionally, Barber et al. [17] suggested that insulin resistance or diabetes was not crucial for the alterations in plasma LPC profile. Overall, the associations of these serum metabolites with impaired fasting glucose/diabetes and traditional risk factors for metabolic disease are still controversial. Moreover, most of the related studies mainly involved North American and European people. Thus, the relationships need to be further validated in external independent populations, especially in Asian people.

2. Materials and methods

2.1. Study design and participants

This cross-sectional study was conducted in 563 Chinese urban residents (311 men and 252 women aged 19–97 y) who underwent an annual physical examination from October to December 2013. The participants were categorized into hyperglycemia (impaired fasting glucose/diabetes) and normal groups according to the Chinese Guideline for the Prevention and Treatment of Diabetes Mellitus [18]. The hyperglycemia group consisted of 134 participants with diabetes (FPG ≥ 7.0 mmol/l, measured twice) or impaired fasting glucose (6.1 ≤ FPG < 7.0 mmol/l), while the normal-FPG group comprised 429 participants (3.9 < FPG < 6.1 mmol/l without glucose-lowering therapy). Dyslipidemia was defined as serum total cholesterol (TC) > 6.21 mmol/l, low-density lipoprotein cholesterol (LDL-C) > 4.14 mmol/l, triglyceride (TG) > 1.70 mmol/l, or high-density lipoprotein cholesterol (HDL-C) < 1.04 mmol/l. Hypertension was defined as systolic blood pressure (SBP) > 140 mmHg or diastolic blood pressure (DBP) > 90 mmHg. Exclusion criteria comprised pregnancy, immune system disease, and chronic renal or liver insufficiency. Fasting venous blood samples of all subjects were collected using vacuum tubes and stored at −80 °C until analysis. Regular smoking and drinking statuses and physical parameters such as height, weight, and sitting blood pressure were also recorded. This study was reviewed and approved by the Ethics Committee of Beijing Hospital. All individuals were informed in writing of the intended use of their sample and provided written consent.

2.2. Targeted metabolomics and biochemical assays

The following metabolites were measured using our targeted metabolomics method: 3 BCAAs (Leu, Ile, and Val), 3 AAAs (phenylalanine [Phe], tryptophan [Trp], and tyrosine [Tyr]), 4 major LPCs (16:0, 18:0, 18:1, and 18:2), Gln, Glu, Gln/Glu ratio, and 25-hydroxyvitamin D [19–21]. Briefly, 10 μl aliquots of calibrators or serum samples were mixed with 10 μl of the isotopically labeled internal standard solution. The serum metabolites were extracted with 1 ml isopropanol containing 0.1% formic acid. The vials were shaken on a mechanical shaker for 20 min, and then centrifuged at 2342 ×*g* for 10 min. A 0.2 ml aliquot of the supernatant was transferred to another vial and evaporated under a stream of nitrogen. The residue was reconstituted with 0.2 ml of the mobile phase (75% acetonitrile containing 7.5 mmol/l ammonium formate and 0.5% formic acid). The LC separation was performed using an Agilent 1260 Series HPLC system. Tandem MS/MS detection was carried out on an AB Sciex QTRAP 5500 system with positive electronic spray ionization in multiple-reaction monitor mode. Quality control samples were collected to control variations of assays. Levels of FPG, TC, TG, HDL−C, LDL-C, apolipoprotein AI (apoAI), apolipoprotein B (apoB), high-sensitivity C-reactive protein (hsCRP), Crea, UA, and urea were measured using assay kits from Sekisui Medical Technologies on a Hitachi 7180 chemistry analyzer.

2.3. Statistical analysis

Categorical variables are expressed as frequencies, and continuous variables as means and SDs, or medians and percentiles (25th, 75th percentiles). Differences in continuous variables between the hypoglycemia and normal groups were tested using analysis of variance. The Mann-Whitney test was performed to assess significant differences when the data had a skewed distribution. Correlations were assessed using the Spearman correlation coefficient. Stepwise multiple linear regression analysis was used to evaluate the independent relationships of the measured variables with FPG, and collinearity testing was carried out to avoid including interdependent model variables. The significance levels for entering and removing an explanatory variable were set at $P < 0.05$ and 0.10, respectively. Moreover, logistic regression was applied to assess the association of serum metabolites with impaired fasting glucose and diabetes. Results are presented as odds ratios (ORs) and 95% confidence intervals (95% CIs). SPSS 22 software (IBM Corporation) was used for all statistical analyses. All statistical tests were 2-tailed, with $P < 0.05$ considered as significant.

3. Results

3.1. Characteristics of participants

The clinical and biochemical characteristics of the participants are presented in Table 1. Overall, levels of FPG ($P < 0.001$), UA ($P < 0.01$), urea ($P < 0.001$), and SBP ($P < 0.001$) were significantly higher in the hyperglycemia group than in normal people. Compared with the control group, the participants with impaired fasting glucose/diabetes had significantly higher serum concentrations of Glu ($P < 0.001$), Val ($P < 0.001$), Leu ($P < 0.001$), Ile ($P < 0.05$), Phe ($P < 0.05$), and total BCAAs ($P < 0.001$). Conversely, Gln/Glu ratio ($P < 0.001$) and unsaturated LPC level ($P < 0.01$) were significantly lower among participants with impaired fasting glucose/diabetes than among normal participants. A stratified analysis was further performed for men and women to estimate the differences in Gln/Glu, BCAA, and unsaturated LPC levels between the hyperglycemia and control groups. Table 2 shows that men with impaired fasting glucose/diabetes exhibited markedly higher BCAA level and lower Gln/Glu ratio and unsaturated LPC level than men in the normal-FPG group; similar results were found in women between both groups.

3.2. Correlations between serum metabolites and traditional risk factors for metabolic disease

Correlations between metabolites and other parameters in the total study population were investigated. Fig. 1 shows heatmaps of the relationships between metabolites and various parameters. Gln/Glu ratio and unsaturated LPC were significantly and positively correlated with HDL-C ($P < 0.001$) and apoAI ($P < 0.001$) and were negatively correlated with BMI ($P < 0.001$), UA ($P < 0.001$), TG ($P < 0.001$), and apoB ($P < 0.05$) after adjusting for age and sex. Gln/Glu ratio and LPC 18:1 also displayed negative correlations with FPG as well as hsCRP and SBP, respectively. Moreover, 25-hydroxyvitamin D had positive correlations with HDL-C ($P < 0.001$) and apoAI ($P < 0.001$) and negative correlation with hsCRP ($P < 0.05$). In contrast, Glu, BCAA, and AAA levels were positively associated with BMI, UA, TG, and apoB, while they showed significantly negative correlations with HDL-C and apoAI ($P < 0.001$). There were also significantly positive associations of most of the BCAA and Glu concentrations with FPG, SBP, DBP, urea, and