Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

# Abnormal circulating amino acid profiles in multiple metabolic disorders

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ARTICLE INFO

*Article history:*
Received 8 November 2016
Received in revised form
2 June 2017
Accepted 13 July 2017
Available online 21 July 2017

*Keywords:*
Amino acids
Obesity
Type 2 diabetes
Metabolic syndrome
Metabolomics
Meta-analysis

ABSTRACT

*Aim:* To evaluate circulating amino acids (AA) profiles in obesity, type 2 diabetes (T2D) and metabolic syndrome (MetS).

*Methods:* Serum AA were profiled among 200; healthy, obese, T2D and MetS subjects matched by sex, age and BMI using ultra-high performance liquid chromatography tandem quadruple mass spectrometry (UPLC-TQ-MS). A meta-analysis, including 47 case-control studies (including the current study) on serum AA in obesity, T2D and MetS searched through October 2016 was conducted to explore the AA differences in obesity, T2D and MetS.

*Results:* In comparison with healthy controls, 14 AA (10 increased and 4 decreased) were significantly altered ($P < 0.05$) in all non-healthy subjects. Also, mean differences of valine (obese: 34.13 [27.70, 40.56]μmol/L, $P < 0.001$, T2D: 19.49 [3.31, 35.68]μmol/L, $P < 0.05$, MetS: 29.18 [16.04, 42.33]μmol/L, $P < 0.001$), glutamic acid (obese: 18.62 [11.64, 25.61]μmol/L, $P < 0.001$, T2D: 19.94 [0.28, 39.61]μmol/L, $P < 0.05$, MetS: 12.45 [3.98, 20.91]μmol/L, $P < 0.001$), proline (obese: 16.72 [6.20, 27.24]μmol/L, $P < 0.001$, T2D: 20.72 [15.82, 25.61]μmol/L, $P < 0.001$, MetS: 29.95 [25.18, 34.71]μmol/L, $P < 0.001$) and isoleucine (obese: 11.39 [8.54, 14.24]μmol/L, $P < 0.001$, T2D: 7.37 [1.52, 13.22]μmol/L, $P < 0.05$, MetS: 10.40 [4.90, 15.89]μmol/L, $P < 0.001$) were significantly higher compared to healthy controls. Similarly, mean differences of glycine (obese: −30.99 [−39.69, −22.29]μmol/L, $P < 0.001$, T2D: −30.37 [−41.80, −18.94]μmol/L, $P < 0.001$ and MetS: −35.24 [−39.28, −31.21]μmol/L, $P < 0.001$) were significantly lower compared to healthy controls.

*Conclusion:* In both the case-control study and meta-analysis, obesity was related to the most circulating AA changes, followed by MetS and T2D. Valine, isoleucine, glutamic acid and proline increased, while Glycine decreased in all metabolic disorders.



## 1. Introduction

Amino acids (AA) are indispensable biological compounds [1,2], which play vital roles in nutrition and metabolism [3]. They act as metabolic regulators for cellular functions (such as glucose homoeostasis, protein synthesis, reproduction, immunity, and hormone secretion), and also maintain gut integrity [2,4,5]. Carbohydrates and lipids in multiple meta-

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http://dx.doi.org/10.1016/j.diabres.2017.07.023
0168-8227/

bolic disorders have been thoroughly investigated; however, little is known about the roles and importance of AA in metabolic disorders with some findings from many studies regarding AA profiles and obesity, T2D or MetS at variance. For example, Newgard et al. [6] and Lee et al. [7] reported significantly higher levels of proline (Pro) among obese subjects compared to healthy controls, which was in tandem with the report by Badoud et al. [8]. However, Felig et al. [9] and Niu et al. [10] reported insignificant differences in Pro. Similarly, Zhou et al. [11] and Yamakado et al. [12] reported significantly lower levels of serine (Ser) among T2D subjects in relation to healthy subjects, while Menge et al. [13] and Thalacker-Mercer et al. [14] reported insignificant differences in level of Ser in T2D patients compared to healthy subjects. Similarly, no consistent conclusions have ever been drawn with respect to the relationship between other AA and metabolic disorders, such as histidine (His), glutamine (Gln) and tyrosine (Tyr). Furthermore, many AA were closely associated and could interact with each other; it is of great significant to evaluate AA profiles change in order to clarify the link between AA and metabolic disorders. However, whether and how much circulating AA changed in metabolic disorders, whether there are differences in circulating AA profiles in obesity, T2D and MetS, were all not sure. It is difficult to draw clear conclusions from previous one or two population studies, because of the variations in participants' selections, study designs, detection methods, and others. In addition, information on the association between metabolic health status and circulation AA profiles are still limited making it impossible to clarify whether alterations in circulating AA profiles are sufficient to affect metabolic health status or these alterations are only a reflection of already compromised metabolic health status. To this effect, a comprehensive analysis, including previous studies across the population worldwide, is necessary.

Therefore, we investigated the differences in serum AA profiling through a case-control study among healthy controls, obesity controls, T2D and MetS patients. A meta-analysis was also conducted to determine the differences in circulating AA profiles among obesity, T2D and MetS.

## 2. Materials and methods

### 2.1. Case-control study

The study population was selected from 4715 eligible adults who attended physical examination at the Second Affiliated Hospital of Harbin Medical University, in Harbin, China, between February and September 2013, in a cross-sectional study described previously [15]. Four groups, healthy control (normal weight and without any metabolic disorders; n = 50), obese control (obesity without any metabolic disorders; n = 50), T2D patients (T2D without any metabolic disorders; n = 50) and MetS patients (≥ 2 metabolic disorders in blood pressure, glucose and lipid; n = 50), were matched for age, sex and BMI. A flowchart of the participants' selection is shown in Fig.1A. The Harbin Medical University Ethics Committee approved the study and the recruitment of participants was executed in line with the Helsinki declaration. Lifestyle characteristics, anthropometric data (height, weight, waist circumference (WC), etc.), dietary intakes and blood pressure measurements were obtained using standard procedures as described elsewhere [10,15].

#### 2.1.1. Classification of subjects

I. Healthy controls: BMI = 18–24 kg/m$^2$, WC < 102 cm (men) or <88 cm (women), triglyceride (TG) < 1.70 mmol/L, high density lipoprotein cholesterol (HDL-c) > 1.04 mmol/L (men) or >1.29 mmol/L (women), systolic blood pressure (SBP) < 130 mmHg, diastolic blood pressure (DBP) < 85 mmHg, fasting blood glucose (FBG) < 6.1 mmol/L, and 2 h post-oral glucose tolerance test (OGTT) blood glucose (PBG) < 11.1 mmol/L;
II. Obese controls: BMI ≥ 28 kg/m$^2$, FBG < 7 mmol/L, PBG < 11.1 mmol/L, WC > 102 cm (men) or >88 cm (women), TG < 1.70 mmol/L, HDL-c > 1.04 mmol/L (men) or >1.29 mmol/L (women), SBP < 130 and DBP < 85 mmHg;
III. T2D: BMI ≥ 28 kg/m$^2$, FBG ≥ 7 mmol/L, PBG ≥ 11.1 mmol/L, WC ≥ 102 cm (men) or ≥88 cm (women), TG < 1.70 mmol/L, HDL-c > 1.04 mmol/L (men) or >1.29 mmol/L (women), SBP < 130 and DBP < 85 mmHg;
IV. MetS: BMI ≥ 28 kg/m$^2$, FBG ≥ 6.1 mmol/L, WC ≥ 102 cm (men) or ≥ 88 cm (women), TG ≥ 1.70 mmol/L, HDL-c ≤ 1.04 mmol/L (men) or ≤ 1.29 mmol/L (women), SBP ≥ 130 and DBP ≥ 85 mmHg.

#### 2.1.2. Laboratory methods

Blood samples were collected before 9:00 am (after ≥ 10-h overnight fast), and in 2hours after all subjects drunk water containing 75 g glucose. All blood samples were centrifuged at 2500*g* for 15 min at room temperature in 30 min after collection, then the supernatant was stored at −80 °C until biochemical and serum AA measurements. Determination of fasting insulin (F-insulin), total cholesterol (TC), TG, HDL-c, low-density lipoprotein cholesterol (LDL-c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), C-reactive protein (CRP), tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), human glutathione peroxidases (GSH-Px), super oxide dismutase (SOD), malondialdehyde (MDA) and adiponectin, has been described elsewhere [10,15]. Serum AA determination using UPLC-TQ-MS has been previously described [16]. In brief, a 50 μL serum sample and 250 μL acetonitrile/methanol/formic acid (74.9:24.9:0.2 v/v/v) with two additional stable isotope-labelled internal standards for Val-d8 and Phe-d8 were vortex mixed (1 min). This was kept at ambient temperature for 10 min, then centrifuged at 14,000*g* for 10 min at 4 °C and the supernatant was filtered using a pump filter (0.22 μm) and transferred into the sampling vial for UPLC-TQ-MS measurement. Waters ACQUITY UPLC equipment (Waters Corporation, Milford, MA, USA) coupled to a Waters Xevo TQD mass spectrometer (Waters Corporation) was used for the UPLC-TQ-MS investigation [17]. All samples