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Targeted metabolomic analysis reveals the association between the postprandial change in palmitic acid, branched-chain amino acids and insulin resistance in young obese subjects

Liyan Liu [a], *Rennan Feng* [a], *Fuchuan Guo* [a], *Ying Li* [a], *Jundong Jiao* [b,*], *Changhao Sun* [a,*]

[a] *Department of Nutrition and Food Hygiene, Public Health College, Harbin Medical University, Harbin, PR China*
[b] *The Second Affiliated Hospital of Harbin Medical University, Harbin, PR China*

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ABSTRACT

Obesity is the result of a positive energy balance and often leads to difficulties in maintaining normal postprandial metabolism. The changes in postprandial metabolites after an oral glucose tolerance test (OGTT) in young obese Chinese men are unclear. In this work, the aim is to investigate the complex metabolic alterations in obesity provoked by an OGTT using targeted metabolomics. We used gas chromatography–mass spectrometry and ultra high performance liquid chromatography–triple quadrupole mass spectrometry to analyze serum fatty acids, amino acids and biogenic amines profiles from 15 control and 15 obese subjects at 0, 30, 60, 90 and 120 min during an OGTT. Metabolite profiles from 30 obese subjects as independent samples were detected in order to validate the change of metabolites. There were the decreased levels of fatty acid, amino acids and biogenic amines after OGTT in obesity. At 120 min, percent change of 20 metabolites in obesity has statistical significance when comparing with the controls. The obese parameters was positively associated with changes in arginine and histidine ($P < 0.05$) and the postprandial change in palmitic acid (PA), branched-chain amino acids (BCAAs) and phenylalanine between 1 and 120 min were positively associated with fasting insulin and HOMA-IR (all $P < 0.05$) in the obese group. The postprandial metabolite of PA and BCAAs may play important role in the development and onset of insulin resistance in obesity. Our findings offer new insights in the complex physiological regulation of the metabolism during an OGTT in obesity.

1. Introduction

Obesity, which has reached epidemic proportions, has become a priority in public health policies [1]. At present, 1.5 billion adults 20 years and older are overweight, and nearly 500 million of them are obese [2]. Obesity is closely associated with disorders of glucose and lipid metabolism which lead to insulin resistance and diabetes [3]. Therefore, better tools are needed to monitor the disease. With the development of many high-throughput measurement

* Corresponding author at: Department of Nutrition and Food Hygiene, Public Health College, Harbin Medical University, 157 Baojian Road, Nangang District, Harbin 150086, PR China. Tel.: +86 0451 87502801/+86 0451 87502725; fax: +86 0451 87502885/+86 0451 87502836.
E-mail addresses: Jiaojundong@163.com (J. Jiao), changhaosun2002@163.com (C. Sun).
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technologies, metabolomics have been used to investigate biomarkers of obesity.

Metabolomics, which is the study of complex metabolite profiles in biological samples, may provide a systems approach to understand the global metabolic regulation in an organism in relation to pathology [4]. Recently, there has been interest in applying metabolomics to examine alterations in the metabolic profile of obese subjects. These studies have provided strong evidence to suggest that alterations in the amino acid and lipid profiles are associated with obesity [5,6]. In those studies, glycero-phosphatidylcholine, fatty acids, glycine and glutamine were found to be important biomarkers in the diagnosis of obesity. Moreover, Huffman et al. reported that fatty acids and neutral amino acids were independently associated with insulin resistance (IR) in obesity [7]. These findings suggested that metabolic alterations occurred in obesity, and that fatty acids and amino acids are closely related to IR in obesity based on metabolomics. However, these studies were performed in the fasting state [8,9], and the postprandial changes in metabolism could also contribute to the physiological function of the body. Therefore, it is necessary to investigate the potential effects of postprandial metabolic changes in fatty acids and amino acids in obese subjects.

Time-dependent variations in metabolic responses to the postprandial state are of significant importance in human health. The oral glucose tolerance test (OGTT), consisting of a standardized meal of pure carbohydrates, has been used to investigate these time-dependent variations. Numerous human studies have used the OGTT to investigate metabolic responses to this carbohydrate challenge (based on metabolomics [10,11]. Thus, in order to investigate metabolic changes in the physiological response during an OGTT in young obese and non-obese subjects, we analyzed the serum fatty acids, amino acids and biogenic amines profiles using a gas chromatography–mass spectrometry (GC–MS) and ultra-high performance liquid chromatography–triple quadrupole mass spectrometry (UPLC–TQ–MS) targeted metabolomics approach. We aimed to determine the metabolic changes influenced by this metabolic carbohydrate challenge and explore the association between these profiles and IR, thereby opening new perspectives in the study of the physiological reaction of obese subjects to glucose ingestion.

2. Methods

2.1. Subjects

The study was approved by the Ethics Committee of Harbin Medical University. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant.

2.2. Metabolic change in college students (MCS)

Subjects were young college students aged 18–23 years. 15 Lean [BMI (kg/m^2) >18.5 and <23] and 15 obese (BMI > 27.5) healthy young men (Table S1) were recruited from Harbin Medical University in Harbin City via posters on campus, according to the criteria of the International Obesity Task Force [12] for Asians. The weight of all subjects was stable (<2.5 kg change over the past 3 months), and no medications likely to confound the study outcomes were taken by the subjects.

2.3. Metabolic change in validation study (MVS)

Thirty obese subjects aged 40–55 years (BMI, 33.87 ± 3.15) were recruited from the Hexing district in Harbin city of Heilongjiang in northern China via posters in the district. None of the subjects had diabetes mellitus, hyperlipidemia, hypertension or prior cardiovascular disease.

Anthropometric and biochemical measurements were shown in the Supplemental method (Method S1).

3. Serum fatty acids and amino acid profile analysis

Sample preparation for the serum free fatty acids, amino acids and biogenic amines was showed in the Supporting method (Method S1).

3.1. UPLC–TQ–MS analysis

UPLC–TQ–MS analysis was performed using a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) coupled to a Waters Xevo TQD Mass Spectrometer (Waters Corporation, Manchester, UK). A 2 μl aliquot of the sample solution was injected into an ACQUITY UPLC^{TM} HILIC column (100 mm × 2.1 mm i.d., 1.7 μm; Waters Corporation, Milford, MA, USA). The conditions of UPLC and MS were described in the supporting information (Method S1). In addition, the validation of this targeted method was also showed the result S1.

3.2. GC–MS analysis

GC–MS analysis was performed using gas chromatography coupled to an ion-trap mass spectrometer (TRACE GC/PolarisQ MS, Thermo Finnigan, USA) according to our previous work [13]. Separation was performed on a J&W DB-WAX capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness).

4. Statistical analysis

All data were presented as means ± SD. Multivariate statistical analysis was performed using SIMCA-P 12.0 software (Umetrics, Umeå, Sweden). Principal component analysis (PCA) was used first in all samples to observe the general separation. Partial least-squares-discriminant analysis (PLS-DA) was used to discriminate metabolite patterns between the OGTT time points.

Statistical analysis was performed using SPSS 16.0 (SPSS Chicago, IL Inc, UAS). The time course of postprandial glucose and insulin response was analyzed by 2-factor repeated-measures ANCOVA with group and time as main effects. Differences in the postprandial response between time periods and weight status were assessed via time × group

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