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Metabolic profiling of urine in young obese men using ultra performance liquid chromatography and Q-TOF mass spectrometry (UPLC/Q-TOF MS)

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ABSTRACT

Obesity is currently epidemic in many countries worldwide. In the young adult, obesity often accompanies hyperlipemia, which is strongly related to the occurrence and development of obesity-related chronic diseases such as diabetes mellitus, hypertension and cardiovascular disease. This study investigated the differences in metabolomic profiling between obese (with hyperlipemia, $n = 30$) and normal-weight ($n = 30$) young men. Anthropometric parameters and conventional metabolites were measured. There were no significant differences between obese and normal-weight young men in age, height and fasting plasma glucose level. Obese young men showed increased weight, body mass index, fat mass, systolic blood pressure, and triglyceride, total cholesterol and insulin levels, and lower levels of testosterone. The endogenous metabolite profile of urine was investigated by UPLC/Q-TOF MS (ultra performance liquid chromatography and Q-TOF mass spectrometry) with electrospray ionization (ESI). Partial least squares (PLS) enabled clusters to be visualized. Eight urine principal metabolites contributing to the clusters were identified; these included increased L-prolyl-L-proline, leucyl-phenylalanine, and decanoylcarnitine in positive ESI mode (m/z 213.1267, 279.1715 and 316.2459, respectively) and N-acetylornithine, 17-hydroxypregnenolone sulfate, 11β-hydroxyprogesterone, 5a-dihydrotestosterone sulfate and glucosylgalactosyl hydroxylysine in negative ESI mode (m/z 173.0931, 411.1883, 331.185, 369.1751 and 485.1875, respectively). These metabolite changes in obese men suggested early changes of metabolism in young-male obesity with hyperlipemia. The study may further aid the clinical prevention and treatment of obesity and related chronic disease.

1. Introduction

The past couple of decades have witnessed a worldwide epidemic of obesity, which poses a major threat to human health [1,2]. In young adults obesity, obesity often accompanies hyperlipemia, which is strongly related to the occurrence and development of obesity-related chronic diseases such as diabetes mellitus, hypertension and cardiovascular disease [3,4]. The fundamental cause of obesity-related chronic diseases is the metabolic disorders in organism caused by obesity [5]. Many previous comparisons of obese and lean or normal-weight subjects focused on a small group of experimental variables, whereas the organism has tens of thousands of metabolites. Therefore, obesity with hyperlipemia-induced perturbations in metabolism has not been fully characterized. Especially in obese young adults with hyperlipemia, the metabolic changes are more interesting to the occurrence and development of obesity-related chronic diseases in the future. Complex etiologies highlight the need to understand how metabolite profiles are altered at this stage.

Metabolomics is a sensitive and unbiased analytical method that assesses all metabolites in biological samples [6]. The technique can generate substantial amounts of metabolic data that can give surprisingly detailed insights into the changes in metabolic processes in whole organisms [7,8]. Metabolomics can determine the relationships between phenotype and metabolism, and can be used to identify the key metabolites associated with a particular phenotype and investigate the biological function and metabolic changes in the organism. This approach has been used to identify urinary principal metabolites involved in dietary intake [9,10], diabetes [11,12] and coronary artery disease [13] in humans and animal models. Recently, ultra-performance liquid chromatography/mass spectrometry (UPLC/MS) has been applied widely in metabolomic studies owing to its high sensitivity and reproducibility [14,15]. In this field, ultra-performance liquid chromatography and Q-TOF mass spectrometry (UPLC/Q-TOF MS) adds a new dimension to

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metabolism studies, enabling attainment of better detection limits, better throughput, and increased chromatographic resolution, which in turn will improve data quality from the mass spectrometer.

We used metabolomics analyses based on UPLC-Q-TOF MS to gain a broader understanding of metabolic differences between obese individuals with hyperlipemia and normal-weight young men, and found the metabolic changes in young obese males with hyperlipemia that may be important for future clinical prevention and treatment of obesity and related chronic diseases.

2. Materials and methods

2.1. Subjects

Sixty young men, aged 18–26 years, were recruited at Harbin Medical University. The study cohort included both obese hyperlipemic subjects whose body mass index (BMI) was >28.0 kg/m^2 and triglyceride level (TG) ≥1.7 mmol/L (n = 30) and normal weight subjects (n = 30, 18.5 kg/m^2 < BMI < 24 kg/m^2). The inclusion criteria were: (1) stable body weight during the 6 months before the study; (2) not receiving prescribed medication and not having an oral or urinary tract infection within 1 month of commencing the study; (3) no smoking and drinking; (4) not having cardiopulmonary, renal, or liver disease. Informed consent was obtained from all subjects, and the study protocol was approved by the Ethics Committee of Harbin Medical University.

2.2. Anthropometry parameters, blood pressure, blood, and urine collection, and the control of dietary intake

Before commencing the standard diet, height and weight were measured twice, to an accuracy of ±0.1 cm and to ±0.1 kg, respectively, while fasting overnight and wearing only underwear. BMI (weight in kilograms divided by the square of height in meters) was used as a measure of overall adiposity. Fat mass (FM) was measured using the electric impedance method and a body fat mass analyzer (TANITA TBF-300, Tanita Corporation, Tokyo, Japan). Blood pressure was measured using a standard mercury sphygmomanometer on the right arm after at least 10 min of rest. Mean values were determined from two independent measurements (by the same researcher) at 2-min intervals.

Volunteers were asked to record their dietary intake over a7-day period, and a preliminary test was performed to determine the amounts and types of foods that comprised their normal daily diet. Finally, the volunteers were provided with a full range and prescribed amounts of foods to form a standard diet [16] and were asked to avoid any vigorous activity. This was intended to attenuate the inter-individual variation in metabolite profiles associated with consuming different amounts and types of foods. Before commencing the standard diet, antecubital venous blood samples were collected after overnight fasting of 12 h. After following the standard diet for 3 days, the urina sanguinis in the morning of the 4th day was collected and centrifuged at 14,000 rpm (17,968 × g) for 10 min to remove particle contaminants and stored at −80 °C until analysis.

2.3. Chemicals and reagents

Acetonitrile (chromatographic grade) was purchased from Honeywell Burdick & Jackson (Muskegon, MI, USA). Deionized water was purified using an ultra-clear system (SG Water conditioning and Regeneration, Barsbüttel, Germany). Leucine enkephalin was purchased from Sigma–Aldrich (St. Louis, MO, USA). Plasma glucose was measured using a Kyoto blood sugar test meter and test strip (Arkray, Inc. Kyoto, Japan). Serum total cholesterol (TC) and TG were assayed using standard enzymatic colorimetric techniques and commercial kits (Biosino Biotechnology Ltd, Beijing, China) with an auto-analyzer (AUTOLAB PM 4000, AMS Corporation, Rome, Italy). Serum insulin and testosterone levels were measured using commercial kits (Tosoh Corporation, Tokyo, Japan) with an auto-immunoassay analyzer (AIA-2000 ST, Tosoh Corporation, Tokyo, Japan).

2.4. UPLC/Q-TOF MS analysis

UPLC/Q-TOF MS analysis was performed using an ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) coupled to a Micromass Q-tof (Quatropde-Time of Flight) microTM Mass Spectrometer (Waters Corporation, Manchester, UK) with electrospray ionization (ESI) in positive and negative modes. Urine samples were centrifuged at 14,000 rpm for 10 min and the supernatant was transferred into an autosampler vial. A 2-μL aliquot of supernatant was injected into an ACQUITY UPLC HSS-T3C_{18} column (50 mm × 4.6 mm i.d., 1.8 μm; Waters Corporation, Milford, MA, USA). The flow rate of the mobile phase was 300 μL/min. Analytes were eluted from the column with a gradient, where A was water and B was acetonitrile. The initial composition of B was 2% and increased to 20% in 5 min, 35% in 2 min, 70% in 2 min, and to 100% in a further 1.5 min, followed by re-equilibration to the initial conditions in 10 min. Each run time was 20.5 min.

For MS analysis, the source temperature was set at 100 °C with a cone gas flow of 50 L/h. A desolvation gas temperature of 300 °C and a desolvation gas flow of 600 L/h were used. The capillary voltage was set at 3.0 kV in positive ESI mode and 2.6 kV in negative ESI mode, and the cone voltage to 35 V. All analyses were performed using the lock spray to ensure accuracy and reproducibility. A lock-mass of leucine enkephalin for positive ESI mode ($[M+H]^+$ = 556.2771) and negative ESI mode ($[M+H]^-$ = 554.2615) was used via a lock spray interface. The MS data were collected in centroid mode from m/z 80 to 1000 with a lockspray frequency of 0.40 s, and data averaging over 10 scans. Order effects in the statistical analysis were avoided due to the randomized crossover design used. In addition, the repeatability of the present method was evaluated using a mixture of 10 urine samples (quality control, QC) injected interval of 12 samples. The overlapped performance of each spectral peak was evaluated, and then six single ions with different m/z were randomly selected in accordance with the six equal portions in the retention time. The reproducibility of the QC sample was examined by analyzing the differences in the retention time and peak intensity of the six ions.

2.5. Data analysis

Statistical analysis was performed using SPSS (version 13.01S; Beijing Stats Data Mining Co. Ltd., Beijing, China). Data were presented as mean ± SD. Differences between groups were analyzed using the independent samples t-test. All P values were 2-tailed and a P value < 0.05 was considered significant.

The UPLC/Q-TOF MS data were analyzed using the MarkerLynx Application Manager 4.1 SCN 714 (Waters Corporation, Milford, MA, USA). Mass window was set at 0.02 Da, noise elimination level at 10.00, RT tolerance at 0.01 min and RT window at 0.2 min. The resulting 3D matrix that contained arbitrarily assigned peak indexes (retention time-m/z pairs), sample names (observations), and normalized ion intensities for each peak area, was exported to EZINFO 2.0 (an component of MarkerLynx) for multivariate statistical analysis using partial least-squares (PLS), which were used to visualize the score plot and obtain the principal metabolites. The goodness of the fit was quantified by R2Y, while the predictive ability was indicated by Q2Y. Models with Q2Y greater than or equal to 0.5 were considered to have good predictive