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Plasma lipidomics reveal profound perturbation of glycerophospholipids, fatty acids, and sphingolipids in diet-induced hyperlipidemia

Hua Miao [a,b], Hua Chen [a], Songwen Pei [d], Xu Bai [c], Nosratola D. Vaziri [b,*], Ying-Yong Zhao [a,b,*]

[a] Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Northwest University, No. 229 Taibai North Road, Xi'an, Shaanxi 710069, China
[b] Division of Nephrology and Hypertension, School of Medicine, University of California, Irvine, Med Sci I, C352, UCI Campus, Irvine, CA 92897, USA
[c] Solution Centre, Waters Technologies (Shanghai) Ltd., No. 1000 Jinhai Road, Shanghai 201203, China
[d] Department of Computer Science and Engineering, University of Shanghai for Science and Technology, No. 516 Jungong Road, Shanghai 200093, China

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ABSTRACT

Hyperlipidemia is a major risk factor for coronary heart disease and has emerged as an important public health problem. Lipidomics is a powerful technology for assessment of global lipid metabolites in a biological system and for biomarker discovery. In the present study, hyperlipidemia was induced by feeding rats a high fat diet. A sensitive ultra-performance liquid chromatography coupled with quadrupole time-of-flight synapt high-definition mass spectrometry method was used for the analysis of plasma lipids. Orthogonal partial least squares-discriminant analysis, correlation analysis and heatmap analysis were performed to investigate the metabolic changes in rats with diet-induced hyperlipidemia. Potential biomarkers were detected using S-plot and were identified by accurate mass data, isotopic pattern and MS^E fragments information. Significantly increased total cholesterol, triglycerides and low-density lipoprotein cholesterol as well as decreased high-density lipoprotein cholesterol were observed in diet-induced hyperlipidemic rats. Combined with standard serum biochemical results, significant differences in plasma lipid compounds including eleven glycerophospholipids, six fatty acids, two sphingolipids, one eicosanoid, one sterol lipid and one glycerolipid were observed, highlighting the perturbation of lipid metabolism in diet-induced hyperlipidemia. These findings provide further insights into the lipid profile across a wide range of biochemical pathways in diet-induced hyperlipidemia.

1. Introduction

Hyperlipidemia is variably defined by increased serum levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) as well as decreased levels of high-density lipoprotein cholesterol (HDL-C) [1]. Hyperlipidemia is a major risk factor of coronary heart disease and has become one of the most important public health problems, with high incidence and prevalence [2].

Plasma TC, TG, HDL-C, LDL-C and lipoproteins are routinely used as biomarkers for the diagnosis of hyperlipidemia in clinical practice [3]. However these parameters do not address the mechanisms and nature of altered lipid metabolism. Lipidomics is defined as "the full characterization of lipid molecular species and of their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation" [4]. Lipidomics is a powerful technology to assess global lipids in a biological system, which has great strength in biomarker discovery. Analysis of the key lipid species in the body fluids has become an important part of the disease diagnosis, prognosis, and drug discovery and toxicity evaluation [5–7]. A variety of metabolic profiles in different animal models have been investigated and partial biomarkers were summarized in our published review and other published papers [7–10]. Our previous results showed urinary metabolic profiles of diet-induced hyperlipidemia in rats [11]. Metabolites including fatty acids, amino acids, nucleosides and bile acids were identified as biomarkers, indicating the perturbations of metabolism of fatty acids, amino acids, nucleosides and bile acids in diet-induced hyperlipidemic rats.

* Corresponding authors at: Division of Nephrology and Hypertension, School of Medicine, University of California, Irvine, Med Sci I, C352, UCI Campus, Irvine, CA 92897, USA. Tel.: +1 714 4565142; fax: +1 714 4566034 (N.D. Vaziri), Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Northwest University, No. 229 Taibai North Road, Xi'an, Shaanxi 710069, China. Tel.: +86 29 88304569; fax: +86 29 88304368 (Y.Y. Zhao).

E-mail addresses: ndvaziri@uci.edu (N.D. Vaziri), zyy@nwu.edu.cn, zhaoyybr@163.com (Y.-Y. Zhao).

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Use of the lipidomics to gain greater insight in the changes in lipid metabolism caused by diseases, specific dietary or drug regimens, has helped to unravel biochemical mechanisms of certain diseases. A number of publications have reported lipidomics using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry [12]. Among those analytical techniques, liquid chromatography–mass spectrometry is regarded as one of the best analytical techniques considering its sensitivity, selectivity and reproducibility [12]. Furthermore, among different liquid chromatography–mass spectrometry platforms, ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) is most suitable for metabolomics and lipidomics, especially for untargeted metabolic profiles due to its enhanced reproducibility of retention time [13–16]. In 2005, Wrona et al. were the first to introduce MS^E technique and MS^E for application to metabolomics and lipidomics [17]. MS^E can provide parallel alternating scans for acquisition at both low collision energy to obtain precursor ion and ramping of high collision energy to obtain full-scan mass fragment, precursor ion and neutral loss [18]. In the present study, hyperlipidemia model was induced in Sprague–Dawley rats with high fat diets. A sensitive ultra-performance liquid chromatography coupled with quadrupole time-of-flight synapt high-definition mass spectrometry (UPLC-Q-TOF/HDMS) method was used for analysis of the endogenous lipids in rat plasma. Orthogonal partial least squares discriminant analysis (OPLS-DA), correlation analysis and heat map analysis were performed to investigate the changes in lipid metabolites and to illuminate biochemical mechanism of hyperlipidemia in this model.

2. Materials and methods

2.1. Animals and sample collection

Male Sprague–Dawley rats were obtained from Fourth Military Medical University (Xi'an, China). The rats underwent an adaptation period of several days, during which they were fed a regular diet. After that, they were randomly assigned into a control and diet-induced hyperlipidemic groups ($n = 8$/group) The control group was maintained on regular diet throughout the experimental period, whereas the diet-induced hyperlipidemic group was fed a high fat diets containing 10% egg yolk powder, 7.5% lards, 0.3% sodium cholate, 0.2% methylthiouracil and 1% cholesterol for 6 weeks. After 6 weeks, rats were anesthetized with 10% urethane, and blood samples were obtained by carotid artery cannulation. Blood was centrifuged at 3000 rpm for 10 min, and the supernatant was collected and stored at −80 °C.

2.2. Plasma biochemistry test

Serum TC, TG, HDL-C and LDL-C concentrations were measured with an Olympus AU640 automatic analyser.

2.3. Sample preparation

Lipids extraction was performed in Ostro 96-well plate using a single-step in-well extraction. 100 μL of plasma was loaded into each well of a 2 mL Ostro sample preparation plate fitted onto a vacuum manifold. 300 μL of elution solvent (1:1, chloroform/methanol) was added to each well and mixed thoroughly by aspirating the mixture 10× using a micropipette. A vacuum of approximately 15″ Hg was applied to the plate until the solvent was completely drained. This step was repeated with another 300 μL of chloroform and methanol with the total fraction three times reaching a total fraction volume of approximately 900 μL. The eluate fraction was dried under nitrogen, reconstituted with 200 μL 1:1 (v/v) chloroform/methanol, and then injected into the UPLC/MS system.

2.4. Chromatography

The UPLC analysis was performed on a Waters Acquity™ Ultra Performance LC system (Waters, USA) equipped with a Waters Xevo™ G2 QTof MS. Chromatographic separation was carried out at 45 °C on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm). A gradient of 10 mM ammonium formate in 2-propanol/acetonitrile (90/10) in 0.1% formic acid (A) and 10 mM ammonium formate in ACN/H_2O (60/40) in 0.1% formic acid (A) was used as follows: a linear gradient of 0–10 min, 40.0–99.0% A and 10.0–12.0 min, 99.0–40.0% A. The flow rate was 0.5 ml/min. The temperatures of autosampler and chromatographic column were maintained at 4 and 55 °C, respectively. Two microliter sample solution was injected for each run.

2.5. Mass spectrometry

Mass spectrometry was performed on a quadrupole and orthogonal acceleration time-of-flight tandem mass spectrometer. The scan range was from 50 to 1200 m/z in positive electrospray mode. The cone and capillary voltage were set at 45 V and 2.5 kV, respectively. The desolvation gas was set at 900 L/h at a temperature of 550 °C; the cone gas was set at 50 L/h and the source temperature was set at 120 °C. The data acquisition rate was set to 0.1 s, with a 0.1 s interscan delay. All analyses were acquired using the lockspray to ensure accuracy and reproducibility. Data were collected in continuum mode. All the acquisition and analysis of data were operated by Waters MassLynx v4.1 software.

2.6. Analytical method assessment and statistical analysis

The reproducibility and precision of UPLC–MS were determined for assessment of the developed method according to previously reported literature [19]. The acquired mass data were imported to MarkerLynx XS software for peak detection and alignment. All of the data were normalized to the summed total ion intensity per chromatogram, and the resultant data matrices were introduced to the EZinfo 2.0 software for OPLS-DA. Lipid peaks were assigned by MS^E analysis or interpreted with available biochemical databases including HMDB, Chemspider and KEGG. Potential biomarkers were extracted from loading plots constructed following analysis with OPLS-DA, and the biomarkers were chosen based on their contribution to the variation and correlation within the data set. Correlation analysis and heatmap of the differential lipids were analyzed by Metaboanalyst software. Other statistical analyses were performed using SPSS 11.0. Significant differences were considered when test p values were less than 0.05.

3. Results and discussion

3.1. Clinical chemistry results

The serum concentrations of TC, TG, LDL-C and HDL-C in the control rats were 2.61, 0.59, 1.71 and 0.96 mmol/L, respectively. Compared with the control group serum TC, TG and LDL-C were significantly ($p < 0.05$) increased whereas HDL-C level was significantly ($p < 0.05$) decreased (3.12 mmol/L) in the diet-induced hyperlipidemic rats.