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Original Article

Proteomic changes associated with metabolic syndrome in a fructose-fed rat model

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ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form
16 March 2016
Accepted 23 March 2016
Available online 24 June 2016

Keywords:
endoplasmic reticulum stress
fructose
insulin resistance
metabolic syndrome
oxidative stress

ABSTRACT

Metabolic syndrome (MetS), characterized by a constellation of disorders such as hyperglycemia, insulin resistance, and hypertension, is becoming a major global public health problem. Fructose consumption has increased dramatically over the past several decades and with it the incidence of MetS. However, its molecular mechanisms remain to be explored. In this study, we used male Sprague-Dawley (SD) rats to study the pathological mechanism of fructose induced MetS. The SD rats were fed a 60% high-fructose diet for 16 weeks to induce MetS. The induction of MetS was confirmed by blood biochemistry examination. Proteomics were used to investigate the differential hepatic protein expression patterns between the normal group and the MetS group. Proteomic results revealed that fructose-induced MetS induced changes in glucose and fatty acid metabolic pathways. In addition, oxidative stress and endoplasmic reticulum stress-related proteins were modulated by high-fructose feeding. In summary, our results identify many new targets for future investigation. Further characterization of these proteins and their involvement in the link between insulin resistance and metabolic dyslipidemia may bring new insights into MetS.

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http://dx.doi.org/10.1016/j.jfda.2016.03.005

1. Introduction

Metabolic syndrome (MetS), which involves obesity, insulin resistance, hypertension, and hyperlipidemia, is becoming a major global public health problem [1,2]. The modern lifestyle of an increased intake of a palatable high-fat diet in association with decreased energy expenditure contributes to the current rising prevalence of MetS [2,3]. Metabolic syndrome is complex and encompasses several interrelated disturbances of glucose and lipid homeostasis [4,5]. The major risk factors for MetS are abdominal obesity, elevated fasting plasma glucose, atherogenic dyslipidemia (i.e., increased levels of triacylglyerols, increased levels of low-density lipoprotein, and decreased levels of high-density lipoprotein), the presence of prothrombotic and proinflammatory states, and elevated blood pressure [6]. The most important interacting features of MetS have been proposed by Grundy [6] as obesity plus endogenous metabolic susceptibility, which is manifested by insulin resistance and other factors such as genetic factors, physical inactivity, advancing age, and endocrine dysfunction.

Fructose, which occurs naturally in honey and sweet fruits, is produced in crystalline and syrup forms for commercial use. The most commonly used form, corn syrup, contains approximately 55% free fructose. Its use as a sweetener in processed foods and soft drinks has increased by 20–30% over the past 20 years in the United States, similar to the dramatic rise in obesity over the same period [7]. The metabolic effects of fructose and its use by individuals with metabolic disorders have attracted much attention over the past two decades. Fructose has unique metabolic features because it is largely metabolized by splanchnic organs (i.e., gut and liver cells) through insulin-independent mechanisms. Fructose is involved in the progression to MetS through the dysregulation of many molecular signaling factors [7,8]. Animal model experiments have clearly demonstrated that fructose feeding in rats causes hypertension and hyperinsulinemia [9], and in hamsters causes insulin resistance, hypertriglyceridemia, hepatic very-low-density lipoprotein overproduction, obesity, and hyperglycemia [10,11].

Proteomics involves integrating several technologies with the aim of systematically analyzing the complement of proteins expressed in a biological system in response to specific stimuli and different physiological or pathological conditions. Examining changes in the proteome offers insights into cellular and molecular mechanisms that cannot always be obtained through genomic analysis. The information gap between the genome and cellular processes can be largely attributed to post-translational modifications such as phosphorylation and glycosylation. These modifications, which cannot be monitored by genomic analyses alone, modulate important regulatory processes such as protein turnover, protein activity, and protein localization within a cell.

In the current study, we employed the electrospray ionization-tandem mass spectrometry (ESI-MS/MS) proteomics approach to identify candidate molecules that link high-fructose consumption to the pathogenesis of MetS. Our results showed that high-fructose feeding was associated with significant alterations in the expression of hepatic enzymes in multiple pathways. In addition to the marked upregulation of hepatic functions that promote triglyceride synthesis and very-low-density lipoprotein-triglycerides production, high-fructose consumption also resulted in perturbations of antioxidant functions in protein folding.

2. Methods

2.1. Establishment of the high-fructose diet-induced MetS rat model

Male Sprague–Dawley (SD) rats weighing 200–250 g were housed two animals per cage in an air-conditioned room (22°C ± 2°C) on a 12 hour light cycle (7:00 AM to 7:00 PM). The animals were maintained in accordance with the guidelines established in the Taiwan Government Guide for the Care and Use of Laboratory Animals. In this study, we used a well-established rat model in which insulin resistance, hypertension, and dyslipidemia can be induced by feeding the animals a high-fructose diet [12,13]. Rats were randomly divided into two groups: Group I, which was fed the standard Purina chow (#5001, Purina, St. Louis, MO, USA; consisting of 23% protein, 56% carbohydrate, 4.5% fat, and 6% fiber), and Group II, which was fed a 60% high-fructose diet plus the supplement of 21% protein, 5% fat, and 8% fiber. The dietary manipulation lasted for 16 weeks. Blood pressure was measured every week, total cholesterol concentrations were measured every 2 weeks, and an oral glucose tolerance test was performed every 4 weeks. At the end of the experiment, blood pressure was measured, and the rats were decapitated after overnight fasting. Blood samples were collected in heparinized tubes, and the plasma was separated by centrifugation and stored at −20°C until assayed for glucose, insulin, triglyceride, cholesterol, and thiobarbituric acid-reactive substances. The livers were stored in liquid nitrogen and subjected to two-dimensional (2-D) gel-based proteomics.

2.2. Tissue harvest

Frozen livers were crushed in liquid nitrogen into a fine powder. The resulting powder was dissolved in a lysis buffer [7M urea, 2 M thiourea, 4% w/v 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 0.5% Triton X-100, and 10 mM dithiothreitol (DTT)] containing a cocktail of protease inhibitors (cOmplete™ Mini, Roche, Mannheim, Germany) and centrifuged at 4°C and 16,000 × *g* for 60 minutes. The supernatant was used as the tissue protein lysate.

2.3. Two-dimensional gel electrophoresis and protein labeling

The protein concentration was determined using the PlusOne 2-D Quant Kit (Amersham Biosciences, Piscataway, NJ, USA) in accordance with the manufacturer's manual. Approximately 150 μg of protein was dissolved in 350 μL of rehydration buffer (7M urea, 2 M thiourea, 1% w/v CHAPS, 0.5% Triton X-100, 100 mM DTT and 0.2% v/v immobilized pH gradient buffer, pH 3–11, nonlinear; Amersham Biosciences) and applied to an 18 cm, nonlinear pH 3–10 Immobiline DryStrip (Amersham