Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Response of genes involved in lipid metabolism in rat epididymal white adipose tissue to different fasting conditions after long-term fructose consumption

Jin-xiu Li [a], Da-zhi Ke [b], Ling Yao [a], Shang Wang [c], Peng Ma [c], Li Liu [c], Guo-wei Zuo [d], Li-rong Jiang [c], Jian-wei Wang [a, *]

[a] Chongqing Key Laboratory of Traditional Chinese Medicine for Prevention and Cure of Metabolic Diseases, Chongqing Medical University, 400016 China
[b] The Second Affiliated Hospital, Chongqing Medical University, Chongqing 400010 China
[c] Faculty of Basic Medical Sciences, Chongqing Medical University, Chongqing 400016 China
[d] College of Laboratory Medicine, Chongqing Medical University, Chongqing 400016 China

ARTICLE INFO

Article history:
Received 18 January 2017
Accepted 22 January 2017
Available online xxx

Keywords:
eWAT (epididymal white adipose tissue)
Genes
Lipid metabolism
Fructose
Fasting

ABSTRACT

There has been much concern regarding the dietary fructose contributes to the development of metabolic syndrome. High-fructose diet changes the expression of genes involved in lipid metabolism. Levels of a number of hepatic lipogenic enzymes are increased by a high-carbohydrate diet in fasted-refed model rats/mice. Both the white adipose tissue (WAT) and the liver play a key role in the maintenance of nutrient homeostasis. Here, the aim of this study was to analyze the expression of key genes related to lipid metabolism in epididymal WAT (eWAT) in response to different fasting condition after long-term chronic fructose consumption. Rats were fed standard chow supplemented with 10% w/v fructose solution for 5 weeks, and killed after chow-fasting and fructose withdrawal (fasting) or chow-fasting and continued fructose (fructose alone) for 14 h. Blood parameters and the expression of genes involved in fatty acid synthesis (*ChREBP*, *SREBP-1c*, *FAS*, *SCD1*), triglyceride biosynthesis (*DGAT-1*, *DGAT-2*) and lipid mobilization (*ATGL*, *HSL*) in eWAT were analyzed. In addition, mRNA levels of *PPAR-*γ, *CD36* and *LPL* were also detected. As expected, fructose alone increased the mRNA expression of *FAS*, *SCD1*, and correspondingly decreased *ATGL* and *HSL* mRNA levels. However, *ChREBP*, *DGAT-2*, *ATGL* and *HSL* mRNA levels restored near to normal while *FAS* and *SCD1* tend to basic level under fasting condition. The mRNA expression of *SREBP-1c*, *PPAR-*γ and *LPL* did not changed at any situations but *CD36* mRNA decreased remarkably in fructose alone group. In conclusion, these findings demonstrate that genes involved in lipid metabolism in rat eWAT are varied in response to different fasting conditions after long-term fructose consumption.

© 2017 Published by Elsevier Inc.

1. Introduction

Fructose consumption has increased significantly during the past decades. A moderate amount of fructose from fresh fruits and vegetables has been a component of human diets. However, abundant production of refined sweeteners has dramatically increased fructose intake. There has been much concern regarding the role of dietary fructose in the development of metabolic diseases. In humans, high fructose in diets reduces insulin sensitivity and elevates plasma triglycerides in both fed and fasting conditions [1]. In animals, diets high in fructose cause multiple symptoms of metabolic syndrome such as insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertension, and hypertriglyceridemia [2]. Thus, high consumption of dietary fructose has been implicated as a contributing factor to the development of obesity, insulin resistance, and fatty liver disease [3]. Obesity confers a high risk of developing numerous metabolic and cardiovascular complications [4]. The gradually increasing prevalence of obesity has caused adipose tissue to be a focus [5]. Adipose tissue, which has been divided into two main types, white adipose tissue (WAT) and brown adipose tissue (BAT), is a complex and highly active metabolic organ [6]. The WAT, best known for its role

* Corresponding author. Chongqing Key Laboratory of Traditional Chinese Medicine for Prevention and Cure of Metabolic Diseases, Chongqing Medical University, 1 Yixueyuan Road, Yuzhong District, Chongqing 400016, China.
E-mail address: wjwcq68@163.com (J.-w. Wang).

http://dx.doi.org/10.1016/j.bbrc.2017.01.119
0006-291X/© 2017 Published by Elsevier Inc.

in the storage of energy, is distributed mainly in visceral and subcutaneous sites. In research studies, consumption of fructose has been associated with increased visceral adiposity such as eWAT [7].

Fructose is a lipogenic monosaccharide which stimulates de novo fatty acid synthesis [8]. It was reported that the gene expression and induction of liver lipogenic enzymes were coordinately altered with changes of the hormonal and nutritional conditions, whereas the degree of response to the changes was different among the enzymes. The adipose tissue is another major tissue in which the lipogenic enzyme genes are expressed [9]. The maintenance of nutrient homeostasis under feeding/fasting conditions and the metabolic response to these situations involve hormonal and metabolic adaptations, which are accompanied by changes in gene expression. Both the WAT and the liver play a key role in the maintenance of nutrient homeostasis [10]. It was reported that fasting and then refeeding a high-carbohydrate diet promotes an increase in hepatic fatty acid synthase expression and activity, hepatic triacylglycerol (TG) accumulation and secretion, and plasma TG concentration [11]. However, it is unknown whether or not the fructose affects the expression of genes involved in lipid metabolism in WAT. Therefore, in the present study we investigate the expression of key genes related to lipid metabolism in eWAT in response to different fasting conditions after long-term chronic fructose consumption.

2. Materials and methods

2.1. Animals, diet and experimental protocol

Male Sprague-Dawley rats weighing 210–230 g and the standard diet were supplied by the laboratory animal center, Chongqing Medical University, China. Rats were maintained in a temperature-controlled facility of 21 ± 1 °C with a 12/12-h light/dark cycle and a relative humidity of 55± 5%.

Sugar-sweetened nonalcoholic beverages, such as soft drinks, appear as the major source of fructose for all classes of age considered, except for children younger than 6 years and adults older than 50 years [12]. Thus, fructose used in solution could be accepted.

After 1 week of adaptable feeding, twenty rats were assigned into 2 groups: 1) a normal diet (Con, n = 6) with ad libitum feeding of the water, 2) a normal diet supplemented with 10% w/v fructose solution (Fru, n = 14). All rats had free access to the standard rodent chow. The consumed chow and fructose solution were measured daily for 5 weeks. The day before the experiment, all animals were deprived of chow starting at 8:00 p.m. During fasting 14 h: for half of the rats in the fructose group (Fru1, n = 7), the fructose solution was replaced with tap water (fasting); the other half of the rats (Fru2, n = 7) continued supplementation with the 10% fructose solution (fructose alone) until sacrifice the next morning. Blood samples were collected via retroorbital venous puncture for determination of triglyceride, total cholesterol and glucose. After sacrificed the animals by decapitation, eWAT were rapidly removed, weighed and immediately frozen in liquid nitrogen and stored at −80 °C until RNA analysis.

The animal protocol followed in this study was approved by the internationally accepted principles for laboratory animal use and care, in accordance with the Animal Ethics Committee, Chongqing Medical University, China.

2.2. Measurement of plasma triglycerides, cholesterol, glucose

Plasma concentrations of total cholesterol, triglyceride and glucose (kit from Kexin Institute of Biotechnology, Shanghai, China) were measured enzymatically according to the manufacture's instructions.

2.3. RNA extraction and reverse transcription

Total RNA was isolated from eWATs of individual rats using TRIzol (Takara, Dalian, China), quantified using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and reversely transcribed by M-MLV RTase cDNA Synthesis Kit (Takara, Dalian, China) according to the manufacturer's instructions.

2.4. Real-time PCR

Real-time PCR was performed with the CFX 96 Real Time PCR Detection System (Bio-rad Laboratories Inc, Hercules, CA, USA) using the SYBR Premix Ex Taq II (Takara, Dalian, China). To verify the purity of the products, a melting curve was produced after each run. The sequences of primers are presented in Table 1. Gene expression in individual samples was determined in duplicates and normalized against the reference gene *β-actin* due to the absence of significant variation in its expression in adipose tissue. Levels in control rats was arbitrarily assigned a value of 1.

2.5. Data analysis

All results are presented as means ± SEM. Data obtained from experiments with more than two groups of animals were analyzed by ANOVA using StatView software and followed by The Student Newman-Keuls test to locate the differences between groups. $P < 0.05$ was considered to be the threshold of significance.

3. Results

3.1. Fructose and chow intake, body weight and eWAT weight of rats

The groups showed no significant differences in body weight before the experiment commenced (data not shown) and at the end of the experiment (Fig. 1C). Both Fru1 and Fru2 groups had an equal level of fructose intake (Fig. 1A), and showed a similar decrease in chow at the same time compared with Con rats (Fig. 1B). A significant increase of eWAT weight was shown in Fru2 compared with the Con and Fru1 respectively (Fig. 1D). Correspondingly, the ratio of eWAT weight/body weight under fructose-remaining stage was significantly higher in Fru2 rats (Fig. 1E).

3.2. Blood biochemical parameters in rats

After 5-week supplementing with fructose, plasma

Table 1
Primer sequences for real-time PCR assays.

Gene	Forward primers	Reverse primers
β-actin	ACGGTCAGGTCATCACTATCG	GGCATAGAGGTCTTTACGGATG
ChREBP	GAAGACCCAAAGACCAAGATGC	TCTGACAACAAAGCAGGAGGTG
SREBP-1C	CTGTCGTCTACCATAAGCTGCAC	ATAGCATCTCCTGCACACTCAGC
ACC1	AACATCCCGCACCTTCTTCTAC	CTTCCACAAACCAGCGTCTC
SCD1	CAGTTCCTACACGACCACCACTA	GGACGGATGTCTTCTTCCAGAT
FAS	ACCTCATCACTAGAAGCCACCAG	GTGGTACTTGGCCTTGGGTTTA
PPARγ	CGGTTGATTTCTCCAGCATTTC	ATCGCACTTTGGTATTCTTGGAG
CD36	AACCCAGAGGAAGTGGCAAAG	GACAGTGAAGGCTCAAAGATGG
LPL	CACAGTGGCTGAAAGTGAGAACA	CCAGCGGAAGTAGGAGTCGTT
HSL	TTCGGGGAACACTACAAACGC	AGCACCTCGATCTCCGTGATATTC
ATGL	CTGATGACCACCCTTTCCAAC	AGATGCTACCTGTCTGCTCCTTC
DGAT-1	GGACAAAGACCGGCAGACCA	CAGCATCACCACGCACCAAT
DGAT-2	CCTGGCAAGAACGCAGTCAC	GAGCCCTCCTCAAAGATCACC

Sequences: 5′ to3′.

Please cite this article in press as: J.-x. Li, et al., Response of genes involved in lipid metabolism in rat epididymal white adipose tissue to different fasting conditions after long-term fructose consumption, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.01.119

Download English Version:

https://daneshyari.com/en/article/5505374

Download Persian Version:

https://daneshyari.com/article/5505374

Daneshyari.com