Biochimica et Biophysica Acta 1841 (2014) 1596–1607

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Role of white adipose lipolysis in the development of NASH induced by methionine- and choline-deficient diet

Naoki Tanaka [a], Shogo Takahashi [a], Zhong-Ze Fang [a,b], Tsutomu Matsubara [a,c], Kristopher W. Krausz [a], Aijuan Qu [a], Frank J. Gonzalez [a,*]

[a] Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
[b] Department of Toxicology, School of Public Health, Tianjin Medical University, Tianjin, China
[c] Department of Anatomy and Regenerative Biology, Osaka City University, Osaka, Japan

ARTICLE INFO

Article history:
Received 31 March 2014
Received in revised form 16 August 2014
Accepted 21 August 2014
Available online 29 August 2014

Keywords:
Fasting response
Linoleic acid
Lipolysis
Metabolomics
Oleic acid
Choline deficiency

ABSTRACT

Methionine- and choline-deficient diet (MCD) is a model for nonalcoholic steatohepatitis (NASH) in rodents. However, the mechanism of NASH development by dietary methionine/choline deficiency remains undetermined. To elucidate the early metabolic changes associated with MCD-NASH, serum metabolomic analysis was performed using mice treated with MCD and control diet for 3 days and 1 week, revealing significant increases in oleic and linoleic acids after MCD treatment. These increases were correlated with reduced body weight and white adipose tissue (WAT) mass, increased phosphorylation of hormone-sensitive lipase, and up-regulation of genes encoding carboxylesterase 3 and β2-adrenergic receptor in WAT, indicating accelerated lipolysis in adipocytes. The changes in serum fatty acids and WAT by MCD treatment were reversed by methionine supplementation, and similar alterations were detected in mice fed a methionine-deficient diet (MD), thus demonstrating that dietary methionine deficiency enhances lipolysis in WAT. MD treatment decreased glucose and increased fibroblast growth factor 21 in serum, thus exhibiting a similar metabolic phenotype as the fasting response. Comparison between MCD and choline-deficient diet (CD) treatments suggested that the addition of MD-induced metabolic alterations, such as WAT lipolysis, to CD-induced hepatic steatosis promotes liver injury. Collectively, these results demonstrate an important role for dietary methionine deficiency and WAT lipolysis in the development of MCD-NASH.

Published by Elsevier B.V.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), defined by the presence of hepatic steatosis with no ethanol consumption, is a common chronic liver disease that is increasing worldwide [1]. NAFLD is classified into two categories, simple steatosis (SS) and nonalcoholic steatohepatitis (NASH), according to the liver histology [2]. The clinical course and outcome differ between SS and NASH, where SS has a benign clinical course, while NASH can develop into liver cirrhosis, hepatic failure, and hepatocellular carcinoma [3]. Several studies to clarify the pathogenesis of NASH using animal models and clinical trials regarding the treatment of NASH have been published [4–7]. The pathogenesis of NASH as a two-hit model was proposed in 1998 and has been widely accepted [8]. The first hit is singular triglyceride (TG) accumulation in hepatocytes resulting in hepatic steatosis and increasing the sensitivity of the liver to the second hits that causes hepatocyte damage, inflammation, and fibrosis. Considering clear differences in the clinical features between steatosis and steatohepatitis, it is of great value to clarify the mechanism of the progression from steatosis to steatohepatitis.

Methionine- and choline-deficient diet (MCD) is a conventional and useful model to induce NASH in rodents. In 1942, Gyorgy and Goldblatt

Abbreviations: Acaca, acetyl-coenzyme A carboxylase alpha; Acly, ATP citrate lyase; Adrb, β-adrenergic receptor; ALT, alanine aminotransferase; Apob, apolipoprotein B; ATGL, adipose triglyceride lipase; CD, choline-deficient diet; Ces3, carboxylesterase 3; Dgat, diacylglycerol O-acyltransferase; Emr1, EGF-like module containing, mucin-like, hormone receptor-like sequence 1; ER, endoplasmic reticulum; eWAT, epididymal white adipose tissue; FA, fatty acid; Fasn, fatty acid synthase; FGF, fibroblast growth factor; Glut4, glucose transporter type 4; GSH, glutathione; HETE, hydroxyeicosatetraenoic acid; HSL, hormone-sensitive lipase; IL, interleukin; Itgam, integrin alpha M; Lcat, lecithin cholesterol acyltransferase; Lpl, lipoprotein lipase; MCD, methionine- and choline-deficient diet; MCS, methionine- and choline-supplemented MCD diet; MD, methionine-deficient diet; Mttp, microsomal triglyceride transfer protein; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NEFA, non-esterified fatty acid; OPLS, orthogonal projection to latent structure; PCA, principal component analysis; Pnpla2, patatin-like phospholipase domain containing 2; PPAR, peroxisome proliferator-activated receptor; qPCR, quantitative polymerase chain reaction; ROS, reactive oxygen species; Scd1, stearoyl-coenzyme A desaturase 1; SS, simple steatosis; TG, triglyceride; TNF, tumor necrosis factor; UPLC-ESI-QTOFMS, ultra-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry; VLDL, very-low-density lipoprotein; WAT, white adipose tissue

* Corresponding author at: Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA. Tel.: +1 301 496 9067; fax: +1 301 496 8419.
E-mail address: gonzalef@mail.nih.gov (F.J. Gonzalez).

http://dx.doi.org/10.1016/j.bbalip.2014.08.015
1388-1981/Published by Elsevier B.V.

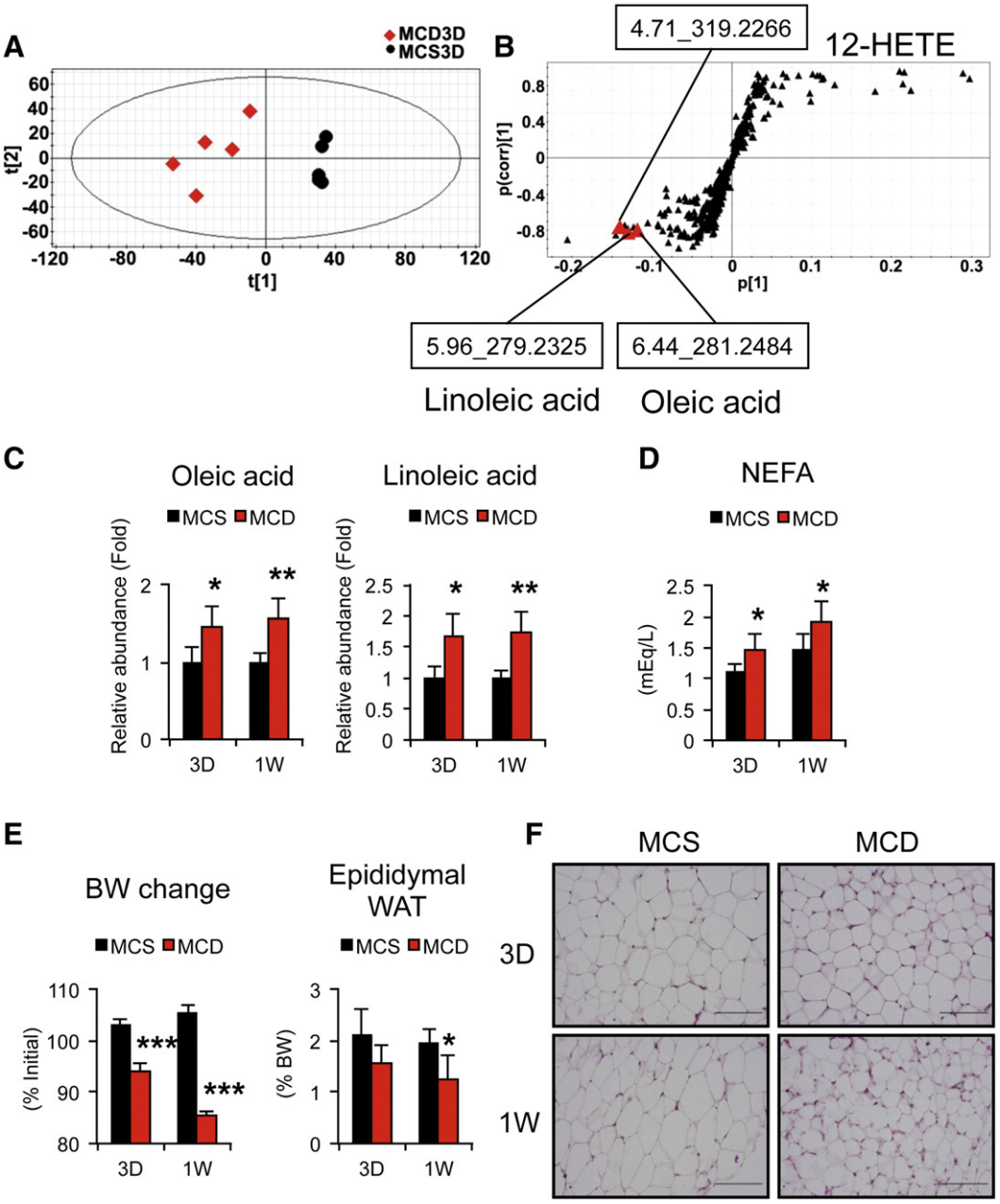

Fig. 1. Serum metabolomic analysis reveals significant increases in oleic and linoleic acids in early stage of MCD-NASH. Male C57BL/6NCr wild-type mice at 8–12 weeks of age were fed a methionine- and choline-deficient diet (MCD) or control methionine- and choline-supplemented MCD diet (MCS) for 3 days or 1 week ($n = 5$/group). (A) PCA of serum metabolites between mice treated with 3-day MCD (red diamond) and MCS (black circle). (B) S-plot of OPLA analysis using the same data as (A). Retention time and molecular mass are indicated. (C) Serum levels of oleic and linoleic acids. Values were normalized to those of MCS-treated mice in each time point and were expressed as relative abundance. (D) Serum levels of NEFA. (E) Body weight (BW) change and epididymal WAT (eWAT) weight. BW was measured just prior to killing and BW changes were expressed as the percentage relative to BW just before commencing the MCD or MCS treatment. (F) Histology of epididymal WAT. Hematoxylin and eosin staining. Bar = 100 μm. Statistical analysis was performed using the Student's *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. MCS-treated mice in the same time point.

reported that supplementation of choline or methionine reduced the incidence of rats having hepatic steatosis, hepatocyte necrosis, and cirrhosis induced by low casein diet, showing an important role for these nutrients in the pathogenesis of nutritional liver injury [9]. Mice treated with MCD show macrovesicular steatosis, hepatitis, hepatocyte ballooning, and enhancement of pro-inflammatory cytokines and oxidative stress in the liver within a few weeks, and the longer treatment results in hepatic fibrosis [10]. These pathologies are similar to those found in human NASH. Although this model is not accompanied by obesity and insulin resistance, such close similarities prompt the use of MCD for determining the mechanism of NASH. It is recognized that choline-deficient diet (CD) treatment causes hepatic steatosis in mice, mainly due to impaired secretion of very-low-density lipoprotein (VLDL) from the liver [11]. However, the mechanism by which dietary methionine/choline deficiency contributes to the NASH development is not fully understood.

Metabolomics using ultra-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOFMS) is a useful method to detect global metabolic alterations in the physiological/pathological conditions in an unbiased manner [12]. Previous studies revealed alterations in phospholipid and bile acid metabolism in mouse models of NASH as a result of enhanced inflammatory signaling [10]. However, the early metabolic changes related to the occurrence of MCD-NASH were not determined.