Reduced Serum Cholesterol and Triglyceride Levels in a Choline-Deficient L-Amino Acid-Defined High-Fat Diet (CDAHFD)-Induced Mouse Model of Non-alcoholic Steatohepatitis (NASH)

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Received April 20, 2019; accepted January 27, 2020

Non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) is one of the major health problems worldwide, because of increased abdominal obesity. To date, specific and effective medications to treat or prevent NAFLD/NASH have not been established. To identify appropriate molecular targets for that purpose, suitable animal models of NAFLD/NASH have been explored. A choline-deficient amino acid-defined high fat diet (CDAHFD)-induced mouse model of NASH has been developed. However, its relevance to human NASH, including serum lipid profiles, have not been clearly defined. In this study, we have revealed that mice fed CDAHFD showed significantly lowered serum total cholesterol and triglyceride (TG) levels, in addition to reduced body weight (BW). Furthermore, hepatic microsomal triglyceride transfer protein (MTP) expression was significantly down-regulated in CDAHFD-fed mice. Thus, the current CDAHFD-fed mouse model has points that are distinct from human NAFLD/NASH, in general, which is based upon abdominal obesity.

Key words steatohepatitis; serum lipid; CD36; microsomal triglyceride transfer protein

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) appears to be a hepatic phenotype of metabolic syndrome or abdominal obesity, and its prevalence keeps increasing worldwide. Among NAFLD, non-alcoholic steatohepatitis (NASH) is a severe phenotype which eventually will develop into liver cirrhosis and hepatocellular carcinoma (HCC). To date, specific and reliable biomarkers for diagnosis of NASH have not been developed yet. With regard to molecular mechanisms of NASH, multiple parallel hit hypothesis has been proposed. According to this hypothesis, steatosis, inflammation and fibrosis appear to progress in parallel in response to their appropriate stimuli, such as lipids, bacterial endotoxin from the gut, oxidative stress, and inflammatory cytokines. Concerning hepatic lipid accumulation, increased influx of fatty acids (FA) from adipose tissue, enhanced de novo FA synthesis, decreased β-oxidation of FA, and reduced secretion of triglyceride-rich very low density lipoprotein (VLDL) into the blood appear to be involved. In fact, some patients with simple liver steatosis develop hepatic inflammation and fibrosis, and finally HCC; however, others remain to be simple steatosis but do not progress to NASH. Therefore, suitable experimental animal models of NASH are required to explore the molecular mechanisms and to find out the appropriate and specific molecular targets for the treatment and prevention of this disorder. Several mouse models of NASH have been established, including those induced by genetic and dietary manipulations. Among these models, genetic obese models, such as leptin-deficient (ob/ob) and leptin-resistant (db/db) mice showed abdominal obesity, hyperglycemia, insulin resistance and hepatic steatosis, based upon hyperphagia. On the other hand, a dietary model, which was induced by high fat diet (HFD), induced hepatic steatosis. However, none of these mice showed significant hepatic fibrosis. In contrast, feeding with a methionine- and choline-deficient diet (MCD) has been shown to exhibit hepatic steatosis, inflammation and fibrosis. However, this MCD model in mice showed remarkable body weight (BW) reduction, decreased serum lipid levels, and suppressed hepatic expression of microsomal triglyceride transfer protein (MTP) which is crucial for secretion of VLDL. Thus, this MCD model appeared to be distinct from the features of NASH based upon abdominal obesity in humans. To overcome these problems, a choline-deficient L-amino acid-defined (CDAA) dietary model has been developed. In mice, however, long-time feeding of 20 weeks or more was necessary to observe liver fibrosis. More recently, a choline-deficient L-amino acid-defined high fat diet (CDAHFD) model has been developed in mice, in order to improve above points. Mice fed CDAHFD showed hepatic fibrosis much earlier than CDAA and finally HCC. However, relevance of this CDAHFD model with human NASH disease phenotypes has not yet been fully elucidated. The aim of this study, therefore, is to explore changes in serum lipid profiles and expression levels of genes related to NAFLD/NASH.

MATERIALS AND METHODS

Animal Model Specific pathogen-free male C57BL/6J mice at the age of 8 weeks were purchased from Japan SLC (Shizuoka, Japan). They were adapted for a week under feeding standard chow diet (SD, MF; Oriental Yeast, Tokyo, Japan) and then randomly divided into two groups. One group was fed CDAHFD (A06071302; Research Diets, Inc., NJ, U.S.A.) containing choline-deficient, 0.1% methionine and 62% kcal fat, and the other group was kept feeding the SD. Contents of CDAHFD and SD are shown in Supplementary Table. Both groups were fed for 14 weeks, and accessing water was ad libitum. All the mice were controlled conditions of room temperature at 22°C and 12h light-dark cycles. After 14 weeks, their liver tissue specimens were collected and immediately frozen in liquid nitrogen.

Biochemical Analyses Blood samples were collected from orbital veins of anesthetized mice after overnight fasting to obtain serum. Total cholesterol (T-Chol) and triglycerides (TG) levels in the sera and hepatic TG level were measured by standard enzymatic assays.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR) qRT-PCR was performed by use of 7500 Fast Real-
Time PCR System (Applied Biosystems, MA, U.S.A.) and specific genes were amplified using THUNDERBIRD SYBR qPCR Mix (TOYOBO, Osaka, Japan). Target gene expression levels were normalized by comparison to β-actin expression levels. All reactions were performed in duplicate. Gene specific primers were indicated in Table 1.

Statistical Analyses Numerical data were expressed as mean ± standard error of the mean (S.E.M.). Statistical significance in the differences between mean values was evaluated by Student t-test. p Values less than 0.05 were considered statistically significant.

RESULTS

Changes in the BW BW of mice fed CDAHFD were significantly less than those fed SD from 1 to 14 weeks after the feeding of CDAHFD or SD (Fig. 1). At 14 week, BW of mice fed CDAHFD and SD were 24.8 ± 0.2 and 32.0 ± 0.6 g, respectively.

Hepatic Lipid Contents as Well as Serum Lipid Concentrations Histological analyses showed remarkable accumulation of lipid droplets and leukocytes, as well as fibrosis in livers of mice fed CDAHFD, but not SD, as previously described (data not shown).

Liver TG contents were significantly higher in mice fed CDAHFD (205.2 ± 9.2 mg/g) than those in mice fed SD (32.8 ± 24.3 mg/g, Fig. 2A). Serum ALT levels are significantly higher in mice fed CDAHFD than those fed SD (data not shown). With regard to serum lipid profiles, T-Chol levels also were significantly reduced in mice fed CDAHFD (29.3 ± 1.6 mg/dL) than in those fed SD (67.3 ± 3.5 mg/dL, Fig. 2B). Serum TG concentrations also were significantly lower in mice fed CDAHFD (28.0 ± 2.0 mg/dL) than those fed SD (55.1 ± 7.6 mg/dL, Fig. 2C).

Hepatic Expression of Genes Related to Inflammation and Lipid Accumulation To explore whether hepatic genes related to inflammation and TG uptake were altered, we have performed qRT-PCR analyses. Tumor necrosis factor (TNF)-α mRNA expression levels were significantly higher in mice fed CDAHFD (0.74 ± 0.12 a.u.) than those fed SD (0.10 ± 0.02 a.u., Fig. 3A). CD36 mRNA expression levels were significantly increased in mice fed CDAHFD (0.96 ± 0.15 a.u.) comparing to those fed SD (0.18 ± 0.03 a.u., Fig. 3B). In contrast, MTP mRNA expression levels were significantly lower in mice fed CDAHFD (1.8 ± 0.29 a.u.) than those fed SD (2.4 ± 0.38 a.u., Fig. 3C).

DISCUSSION

In order to establish specific and effective treatment and

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Table 1. The Primer Sequences Used in qRT-PCR

| Target | Primer sequence (5’→3’) |
|--------|-------------------------|
| CD36 Fwd | TCTTATGGTGTGCTAGAGCTTGGC |
| CD36 Rev | AGGTCTGAAACTCTGGAGCTTGCC |
| MTP Fwd | CCTACAGGCCCAACAGAC |
| MTP Rev | CGCTCAATTTTGCGATGTACC |
| TNF-α Fwd | CCCTCACACTCACTCATCTCT |
| TNF-α Rev | GCTACGCGCTGGCTACAG |
| β-Actin Fwd | CTGACTGACTACCTCATGAAGACT |
| β-Actin Rev | CTGAATGCTACGCAGATTC |

Fwd: forward, Rev: reverse.

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Fig. 1. Comparison of BW Changes in Mice Fed CDAHFD or SD
Weekly changes in BW in mice fed CDAHFD or SD for 14 weeks are indicated (n = 10). *: p < 0.0001.

Fig. 2. Intrahepatic TG Content and Serum Lipid Levels in Mice Fed CDAHFD or SD
Intrahepatic TG content (A), serum total cholesterol (T-Chol; B) and TG (C) levels in mice fed CDAHFD or SD for 14 weeks are shown (n = 10). *: p < 0.01, **: p < 0.0001.
prevention of NAFLD/NASH, appropriate NAFLD/NASH models in mice appear to be necessary. In the present study, recently established CDAHFD model mice showed significantly reduced serum T-Chol and TG levels, as well as significantly lower BW, when compared to mice fed SD, although less prominent reductions than those reported in the MCD model, which appeared to be distinct, in general, from the features of NASH in humans.

MTP is a protein essential for formation and release of VLDL in the liver. In humans, therefore, lomitapide, an inhibitor of MTP has been developed and utilized for the treatment of extremely severe hypercholesterolemia. An an important side effect of lomitapide appears to be accumulation of TG in the liver. Furthermore, it has been shown that single nucleotide polymorphisms of MTP affected susceptibility to NAFLD/NASH. Therefore, this models may be a mouse model of NASH induced by MTP inhibitors or genetic abnormalities of MTP.

CD36 may be involved in hepatic fatty acid uptake and NAFLD/NASH. In addition, expression of CD36 appears to be induced by TNF-α as previously shown in U937 cells. Thus, enhanced CD36 expression, as well as reduced MTP expression, in the liver may contribute to the reduced serum lipid levels in this CDAHFD model.

The present study has clearly demonstrated that the CDAHFD mouse model of NASH, which has been recently developed, also includes characteristics that are different from those of human NASH induced by abdominal obesity and sedative life styles. These differences may cause critical problems in case of exploring suitable biomarkers or therapeutic molecular targets for NASH in humans without genetic abnormalities or drugs. For that purpose, MCD fed db/db mice, which showed remarkable hepatic fibrosis after steatosis, may be more relevant to human NASH induced by abdominal obesity and insulin resistance; however, their serum lipid profiles have not been clarified yet. Thus, future studies may provide clues to develop specific and effective prevention and treatment of NASH in humans.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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