ELSEVIER

Archives of Medical Research 45 (2014) 116–124

Archives of Medical Research

ORIGINAL ARTICLE

Time-dependent Changes and Association Between Liver Free Fatty Acids, Serum Lipid Profile and Histological Features in Mice Model of Nonalcoholic Fatty Liver Disease

Milena N. Stanković,[a] Dušan R. Mladenović,[a] Ivana Đuričić,[b] Slađana S. Šobajić,[b] Jasmina Timić,[b] Bojan Jorgačević,[a] Vuk Aleksić,[a] Danijela B. Vučević,[a] Rada Ješić-Vukićević,[c] and Tatjana S. Radosavljević[a]

[a]*Faculty of Medicine, Institute of Pathophysiology "Ljubodrag Buba Mihailović", University of Belgrade, Belgrade, Serbia*
[b]*Department of Bromatology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia*
[c]*Institute of Digestive Diseases, Clinical Centre of Serbia, Belgrade, Serbia*

Received for publication July 23, 2013; accepted December 24, 2013 (ARCMED-D-13-00402).

Background and Aims. Methionine-choline deficient (MCD) diet duration necessary for development of non-alcoholic fatty liver disease (NAFLD) and the dynamic of lipid profile and fatty acids are not completely established. The study examined dynamics and association between liver free fatty acids (FFA), serum lipid profile and liver morphological changes on MCD diet-induced NAFLD in mice.

Methods. Male C57BL/6 mice ($n = 28$) were divided into four groups ($n = 7$ per group): control: fed with standard chow, MCD diet-fed groups: 2, 4 or 6 weeks. After treatment, liver and blood samples were taken for histopathology, serum lipid profile, and liver FFA composition.

Results. Hepatic FFA profile showed a decrease in saturated acids, arachidonic and docosahexaenoic acid, whereas proportions of docosapentaenoic, oleic and linoleic acid were increased. Total cholesterol, HDL and triglycerides progressively decreased, whereas LDL level progressively increased. Focal fatty change in the liver appeared after 2 weeks, whereas diffuse fatty change with severe inflammation and ballooned hepatocytes were evident after 6 weeks.

Conclusions. Six-week diet model may be appropriate for investigation of the role of lipotoxicity in the progression of NAFLD. Therefore, supplementation with n-3 polyunsaturated acid like DHA, rather than DPA, especially in the initial stage of fatty liver disease, may potentially have preventive effects and alleviate development of NAFLD/NASH and may also potentially reduce cardiovascular risk by moderating dyslipidemia. © 2014 IMSS. Published by Elsevier Inc.

Key Words: Free fatty acids, Lipid status, Methionine-choline deficient diet, NAFLD, Mice.

Introduction

Nonalcoholic fatty liver disease (NAFLD) includes a wide spectrum of liver diseases ranging from simple steatosis through steatohepatitis to irreversible cirrhosis and in some cases hepatocellular carcinoma. Simple hepatic steatosis is the earliest stage of NAFLD and is characterized by deposition of triglycerides in >5% of hepatocytes. In some patients, hepatic steatosis can progress to nonalcoholic steatohepatitis (NASH), which is characterized by the presence of hepatocyte ballooning and an inflammatory infiltrate. Within 10 years, 10–30% of patients with NASH develop cirrhosis, whereas ~28% of patients with NASH-induced cirrhosis develop hepatocellular carcinoma (HCC). However, altered lipid and glucose metabolism in NAFLD

Address reprint requests to: Tatjana S. Radosavljević, MD, PhD, Institute of Pathophysiology, Faculty of Medicine, University of Belgrade, Dr. Subotica 9, 11000 Belgrade, Serbia; Phone/Fax: +381 11 2685 340; E-mail: tanjamm@med.bg.ac.rs

http://dx.doi.org/10.1016/j.arcmed.2013.12.010

and metabolic syndrome may directly affect tumor suppressor genes and contribute to HCC development directly from NASH. Accordingly, recent study has shown that 41.7% of individuals with NAFLD/NASH-HCC had no evidence of cirrhosis (1). Prevalence of NAFLD increases nowadays parallel with obesity and diabetes and is rapidly becoming a global health problem (2). The pathogenesis of NAFLD and mechanisms involved in the progression of steatosis to NASH are complex and not completely understood. However, increased visceral adiposity and insulin resistance with increased free fatty acids (FFA) release play a major role in the development of liver steatosis (3). Further events that contribute to liver injury include oxidative stress and lipid peroxidation, decreased antioxidant defense, early mitochondrial dysfunction, iron accumulation, imbalance of adipokines, adipose-derived cytokines with chronic inflammation, and gut-derived microbial adducts (4).

Various animal models of NAFLD/NASH were used in previous studies and they may be classified into genetic models (sterol regulatory element binding protein (SREBP)-1c transgenic mice, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) null mice; methionine adenosyltransferase-1A (MAT1A) null mice, ob/ob, db/db, etc.), nutritional models (methionine- and choline-deficient (MCD) diet, a high-fat diet) and combined models that include genetic and nutritional modifications. Although numerous animal models of NAFLD/NASH are used, none completely reflects pathophysiology and liver histopathology of human NAFLD/NASH (5). An ideal model should demonstrate steatosis, intralobular inflammation (especially in acinar zone 3) and hepatocellular ballooning. Furthermore, animals should develop metabolic abnormalities such as dyslipidemia, increased FFA level, elevated liver enzymes, obesity, insulin resistance and altered adipokine profile (6). Although MCD diet was used for induction of NAFLD, the precise time course of liver injury in this model was not elucidated. Understanding the dynamics of liver injury, especially hepatic FFA profile and lipid status is important because of their possible role in progression of NAFLD.

Accordingly, the objective of this study was to examine the effects of MCD diet on liver FFA composition, serum lipid profile, and liver morphology in order to evaluate the time dependency of these parameters changes, and potential association between hepatic FFAs and serum lipid profile in NAFLD/NASH animal model.

Materials and Methods

Animals

The experiment was performed on male C57BL/6 mice weighing on average 23 ± 3 g and reared at the Military Medical Academy in Belgrade. Animals were housed in controlled laboratory environment (temperature 22 ± 2°C, relative humidity 50 ± 10%, 12/12 h light-dark cycle with lights turned on at 9:00 a.m.) and had free access to water and standard chow before the experiment. All experimental procedures were in full compliance with Directive of European Parliament and of the Council (2010/63EU) and approved by the Ethics Committee of University of Belgrade (No. 695/2). This study was performed in September 2012.

All animals (n = 28) at the age of 8 weeks were randomly divided into the following groups: 1. control (C; n = 7)—continuously fed with standard chow; 2. groups fed with MCD diet for 2 (MCD2; n = 7), 4 (MCD4; n = 7) or 6 weeks (MCD6; n = 7). MCD (MP Biomedicals, Santa Ana, CA) diet composition is shown in Table 1. Control diet has the same formulation as MCD diet, except it contained 2 g/kg choline chloride and 3 g/kg methionine. Body weight was measured weekly during the experiment and compared to liver weight after sacrifice.

On the day prior to sacrifice mice were fasted overnight. After the treatment, animals were sacrificed by exsanguinations in ketamine (100 m g/kg i.p.) anesthesia. Blood samples were taken from the right side of the heart by cardiac puncture for determination of lipid status and liver enzyme (aminotransferases, alkaline phosphatase-ALP) activities. Liver samples were taken for histopathology and FFA level measurement.

Histopathological Analysis

Liver tissue was sectioned and incubated in 10% formalin solution at room temperature. After fixation, liver samples were processed by the standard method. Tissues were

Table 1. Methionine/choline deficient diet composition

Nutrient	g/kg
Alphacel non-nutritive bulk	10
Calcium phosphate dibasic	250
Corn oil	50
Corn starch	100
Cupric carbonate	1.15
Di-calcium phosphate	1.5
Ferric citrate	3
L-amino acids	85.2
Magnesium oxide	12
Manganese carbonate	1.7
Mineral mix	14.45
Potassium citrate monohydrate	110
Potassium sulfate	26
Sodium chloride	37
Sucrose	225
Sucrose, finely powdered	55
Vitamin mix	10
Zinc carbonate	8
Total	1000

Source: MP Biomedical, CA.