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Original Research

Alternate-day fasting protects the livers of mice against high-fat diet–induced inflammation associated with the suppression of Toll-like receptor 4/nuclear factor κB signaling

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ABSTRACT

Because of unhealthy lifestyles, a large number of people are suffering from hepatic lipid accumulation and nonalcoholic steatohepatitis. Energy restriction (ER) is an effective nutritional intervention for preventing chronic disease. However, poor compliance with continuous ER limits its effectiveness. As an alternative to daily ER, alternate-day fasting (ADF) may be more effective. We hypothesized that ADF would improve obesity, hyperglycemia, and insulin resistance and protect the liver against high-fat diet (HFD)–induced steatosis and inflammation. In this study, we used C57BL/6 mice to test the beneficial effects of ADF. Thirty male 6-week-old C57BL/6 mice were divided into 3 groups (10 per group, total N = 30): 1 group was fed chow diet, the second was fed HFD ad libitum, and the third group was submitted to ADF. The mice in the third group were fed the HFD ad libitum every other day and fasted the following day. After 12 months, the mice submitted to ADF exhibited reduced body weights and fasting glucose levels and improved insulin resistance and hepatic steatosis compared with continuous HFD-fed mice. In addition, the serum transaminase levels in the mice of the ADF group were lower than those of the HFD group. Moreover, the ADF regimen suppressed the expression levels of Toll-like receptor 4 and nuclear factor κB protein in the liver and suppressed the inflammatory pathway genes interleukin 1β, tumor necrosis factor α, and serum amyloid A. These finding indicate that long-term ADF protects mouse livers against HFD-induced hepatic steatosis and hepatocellular damage associated with the suppression of Toll-like receptor 4/nuclear factor κB signaling.

Abbreviations: ADF, alternate-day fasting; ANOVA, analysis of variance; CHD, chow diet; CRP, C-reactive protein; ER, energy restriction; H&E, hematoxylin and eosin; HFD, high-fat diet; IL, interleukin; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor κB; PCR, polymerase chain reaction; SAA, serum amyloid A; TLR-4, Toll-like receptor 4; TNF-α, tumor necrosis factor α.

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1. Introduction

Energy restriction (ER) refers to a dietary regimen that is low in calories but does not induce undernutrition. Work in widely diverse species ranging from model organisms to rodents and human primates has demonstrated that ER is an effective nutritional intervention for slowing aging and preventing chronic disease [1-3]. However, ER regimens are difficult to apply due to poor compliance. We sought to find a therapy method to replace continuous ER. Alternate-day fasting (ADF) regimens were created to increase adherence to dietary restriction protocols because these regimens only require ER every other day rather than every day as with ER. Alternate-day fasting regimens consist of a "feed day" (ad libitum food intake for 24 hours) alternated with a "fast day" (complete fast for 24 hours), but the overall energy intake is not limited. Animal studies have found that ADF regimens have the same effects on reducing the risks of diabetes and cardiovascular disease as ER [4,5]. In humans, ADF leads to improved insulin sensitivity [6,7].

Because of unhealthy lifestyles that include high-fat diet (HFD) and high-carbohydrate diet, increasing numbers of people are suffering from nonalcoholic fatty liver disease (NAFLD), which is the most common chronic liver disease worldwide and a frequent sequela of obesity and insulin resistance that culminates in hepatic fibrosis, cirrhosis, and hepatocarcinoma [8]. The classical "2-hit" theory accounts for the pathogenesis of NAFLD. The "first hit" consists of the accumulation of excessive hepatic fat due to insulin resistance, which is associated with liver chronic inflammation, and the "second hit" entails enhanced lipid peroxidation and increased generation of reactive oxygen species. Hence, hepatic lipid accumulation and nonalcoholic steatohepatitis (NASH) are the pivotal pathogenic processes of NAFLD [9]. Because of the combination of the accumulation of macrophages that secret proinflammatory mediators and the altered function of hepatic cells [10,11], dietary surplus–induced obesity results in an insulin resistance state in the liver. Meanwhile, cytokine-mediated paracrine effects activate inflammatory pathways, such as the Jun N-terminal kinase and inhibitor of κB kinase pathways, which accordingly maintain a low-grade chronic inflammatory state [12,13].

Multiple animal models have demonstrated that HFDs increase liver fat and promote hepatocyte injury [14,15]. For these reasons, dietary fat may be an important modifiable factor in the development of NAFLD. However, the role of ADF in slowing the progress of NAFLD remains unknown. Moreover, few researchers have focused on the effects and mechanisms of nutritional interventions on hepatic inflammation, although it seems that ADF regimens are useful for improving steatosis based on the existing evidence [16]. Thus, it is worthwhile to determine whether ADF might exert beneficial effects in terms of protecting against HFD-induced liver inflammation. We hypothesized that long-term ADF would improve multiple risk factors for NAFLD by promoting positive effects on fasting blood glucose, glucose tolerance, lipid profiles, and inflammation. Because of the poor compliance of subjects in this type of long-term research and the scarcity of human specimens, we used a rodent mouse model to perform a 12-month study to explore the effects of ADF on the amelioration of hepatic lipid accumulation and NASH and the involved mechanisms.

2. Methods and materials

2.1. Animals

Thirty male 6-week-old C57BL/6 mice (SPF grade, certification no. SCXK 2012-0004) purchased from the Comparative Medicine Center of Yangzhou University were housed in cages with wood-chip bedding with 50% to 60% humidity and a 12-hour light/dark cycle and were provided free access to food and water. At 8 weeks of age, the mice were randomly divided into 3 groups of 10: one group was fed chow diet (CHD), the second was fed an HFD ad libitum, and the third group was submitted to ADF in which the mice were fed the HFD ad libitum every other day and fasted the following day for 12 months. After the 12-month experiment, the mice were fasted for 5 hours and then weighed and euthanized by a lethal injection of chloral hydrate (400 mg/kg body weight). Blood was obtained by cardiac puncture, and serum was collected by centrifuging the blood at 3000*g* for 10 minutes at 4°C and stored at –80°C until analysis. Meanwhile, the liver tissues were collected and divided into 3 parts: one part was snap frozen in liquid nitrogen and stored at –80°C for RNA and protein assays, the second part was embedded in paraffin solution for hematoxylin and eosin (H&E) staining, and the third part was embedded in optimal cutting temperature compound for Oil Red O staining. The compositions of the diets that were fed to mice are listed in Table 1. The care and treatment of these mice were conducted in accordance with National Institutes of Health publication number 85-23 (revised in 1996) on the "Principles of Laboratory Animal Care." The Institutional Animal Care and Use Committee approved the project. This study complied with the current Chinese laws pertaining to the conduct of scientific research.

Table 1 – Ingredient compositions of the diets fed to the mice

Ingredient (g/kg)	CHD	HFD
Casein	140.0	140.0
Cornstarch	465.7	54.8
Maltodextrin	155.0	76.8
Sucrose	100.0	251.4
Cellulose	50.0	118.7
Soybean oil	40.0	29.1
Lard	0.0	279.9
Mineral Mix S10026	10.0	10.0
Dicalcium phosphate	13.0	13.0
Calcium carbonate	5.5	5.5
Potassium citrate	16.5	16.5
Vitamin Mix V1001	2.3	2.3
Choline bitartrate	2.0	2.0
Total	1000.0	1000.0