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# Omega-3 polyunsaturated fatty acids ameliorate ethanol-induced adipose hyperlipolysis: A mechanism for hepatoprotective effect against alcoholic liver disease

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ABSTRACT

Alcohol exposure induces adipose hyperlipolysis and causes excess fatty acid influx into the liver, leading to alcoholic steatosis. The impacts of omega-3 polyunsaturated fatty acids (n-3 PUFA) on ethanol-induced fatty liver are well documented. However, the role of n-3 PUFA in ethanol-induced adipose lipolysis has not been sufficiently addressed. In this study, the *fat-1* transgenic mice that synthesizes endogenous n-3 from n-6 PUFA and their wild type littermates with an exogenous n-3 PUFA enriched diet were subjected to a chronic ethanol feeding plus a single binge as model to induce liver injury with adipose lipolysis. Additionally, the differentiated adipocytes from 3T3-L1 cells were treated with docosahexaenoic acid or eicosapentaenoic acid for mechanism studies. Our results demonstrated that endogenous and exogenous n-3 PUFA enrichment ameliorates ethanol-stimulated adipose lipolysis by increasing PDE3B activity and reducing cAMP accumulation in adipocyte, which was associated with activation of GPR120 and regulation of $Ca^{2+}$/CaMKKβ/AMPK signaling, resultantly blocking fatty acid trafficking from adipose tissue to the liver, which contributing to ameliorating ethanol-induced adipose dysfunction and liver injury. Our findings identify that endogenous and exogenous n-3 PUFA enrichment ameliorated alcoholic liver injury by activation of GPR120 to suppress ethanol-stimulated adipose lipolysis, which provides the new insight to the hepatoprotective effect of n-3 PUFA against alcoholic liver disease.

## 1. Introduction

Consuming excess amount of alcohol leads to alcoholic liver disease (ALD), encompassing from hepatic steatosis to hepatitis, cirrhosis, and hepatocellular carcinoma [1]. As a reversible pathological condition, alcoholic hepatic steatosis is characterized by lipid accumulation in hepatocytes and considered as an important cause for the progression of ALD [2]. The reduced hepatic fatty acid β-oxidation and defective export of fat from the liver can increase hepatic lipid accumulation, and the recent studies demonstrate that the increased mobilization of depot fat is the main source of hepatic lipid deposition [3,4]. As a major organ for energy storage, adipose tissue plays the critical role in maintaining whole-body lipid homeostasis. Disordered function of adipose tissue may increase adipose lipolysis and cause excess fatty acid influx into non-adipose tissues, such as the heart, skeletal muscle, pancreas and liver, leading to ectopic fat deposition [5,6]. Recent studies demonstrate that alcohol exposure increases adipose lipolysis and promotes lipid accumulation in the liver [2,7]. These findings suggest that inhibition of adipose lipolysis and free fatty acid entry into the liver might be an efficient strategy to prevent alcohol-induced hepatic injury.

*Abbreviations:* ALD, alcoholic liver disease; ALP, alkaline phosphatase; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATGL, adipose triglyceride lipase; cAMP, cyclic adenosine 3′,5′-monophosphate; CD36, fatty acid translocase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FABP4, fatty acid binding protein 4; FATP2, fatty acid transporter protein 2; FFAs, free fatty acids; GC–MS, gas chromatography–mass spectrometry; GPR120, G protein-coupled receptor 120; H & E, hematoxylin and eosin; HSL, hormone-sensitive lipase; IL-1β, interleukin-1β; ISO, isoproterenol; Lpl, lipoprotein lipase; MCP-1, monocyte chemoattractant protein-1; MUFA, monounsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated fatty acids; PDEs, phosphodiesterases; PPARγ, peroxisome proliferator-activated receptor-γ; SFA, saturated fatty acids; siRNA, small interfering RNA; TC, total cholesterol; TG, triacylglycerol; TNF-α, tumor necrosis factor-alpha

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Adipose lipolysis is defined that triacylglycerol stored in adipocytes is catabolized into glycerol and free fatty acids (FFAs) by lipolytic enzymes, including adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) [8]. Lipolysis is initiated by cAMP/PKA signaling. Cyclic adenosine 3′,5′-monophosphate (cAMP) accumulation activates PKA, a cAMP dependent protein kinase, further phosphorylates HSL and perilipin, resulting in the catalysis of triglycerides breakdown [9]. As a second messenger, cAMP is synthesized by adenylate cyclase, while its degradation is mediated through phosphodiesterases (PDEs). Lipolysis is associated with adipose dysfunction, and endoplasmic reticulum (ER) stress and inflammation response could increase cAMP accumulation by impairing PDEs activity, leading to enhanced lipolysis [10,11]. Alcohol consumption induces oxidative stress and inflammation in adipose tissue, contributing to alcohol liver disease [12,13]. Although chronic alcohol exposure stimulates lipolysis in adipose tissue [7], the implication in liver diseases is little known.

Omega-3 polyunsaturated fatty acids (n-3 PUFA) have beneficial effects on metabolic disorders, including atherosclerosis, coronary heart disease, diabetes and Alzheimer's disease, by modulating cell proliferation and suppressing inflammatory response and oxidative stress [14–16]. N-3 PUFA, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are well documented to ameliorate ethanol-induced fatty liver by regulating fatty acid metabolism, inhibiting lipogenesis and suppressing inflammation and oxidative stress [17–20]. Adipose dysfunction is associated with alcoholic liver disease [21] and alcohol could promote lipid deposit in the liver *via* enhancing lipolysis in adipose tissue [2,7]. N-3 PUFA exert the ability to ameliorate adipose function by inhibition of inflammation [22], therefore, it is tempting to know if n-3 PUFA could prevent ethanol-induced fatty liver by ameliorating adipose dysfunction.

In addition to providing energy sources, free fatty acids act as signaling molecules to regulate cellular responses. G protein-coupled receptor 120 (GPR120) is a receptor for unsaturated long-chain free fatty acids and is highly expressed in adipose tissue [23,24]. It is demonstrated that GPR120 is the functional n-3 PUFA receptor mediating potent anti-inflammatory and insulin-sensitizing effects in obese mice [25]. GPR120 deficient mice fed with high-fat diet develop obesity with adipose inflammation and liver fattiness, indicating the potential role of n-3 PUFA in the improvement of adipose function [26]. The *fat-1* transgenic mice are engineered to encode n-3 PUFA desaturase that catalyses conversion of n-6 PUFA to n-3 PUFA in mammals [14,27]. In the present study, by using *fat-1* transgenic mice, we investigated the effects of endogenous and exogenous n-3 PUFA enrichment on ethanol-induced adipose dysfunction with focus on the regulation of GPR120, aiming to test the hypothesis that amelioration of ethanol-induced adipose dysfunction contributes to attenuating fat accumulation in the liver. Our work showed that by activation of GPR120, n-3 PUFA reduced cAMP accumulation and inhibited lipolysis in a manner dependent of AMPK activation, contributing to preventing liver injury. These findings established a previously unrecognized role of n-3 PUFA in the regulation of adipose function.

## 2. Materials and methods

### 2.1. Animals and treatments

The *fat-1* transgenic mice with C57BL/6 genetic background were generously provided by Dr. Jing X. Kang at Laboratory for Lipid Medicine and Technology, Massachusetts General Hospital and Harvard Medical School (Boston, MA, USA). Male heterozygous *fat-1* mice were bred with C57BL/6 female mice to obtain heterozygous *fat-1* and WT offsprings in specific pathogen-free room at Experimental Animal Center, Guangdong Pharmaceutical University (Guangzhou, China). A simple mouse model of alcoholic fatty liver was induced by chronic ethanol feeding plus a single binge ethanol feeding [28]. After phenotyping by the analysis of total lipids from mouse tail (**Fig. S1**) using gas chromatography–mass spectrometry (GC–MS), male heterozygous *fat-1* transgenic mice and their wild type littermates (n = 10) were fed the modified Lieber-DeCarli liquid diets (TROPHIC Animal Feed High-tech Co., Ltd. Nantong, China) containing isocaloric maltose dextrin as the control or ethanol for 10 days. An additional group of WT mice received the modified Lieber-DeCarli liquid ethanol diet containing EPA and DHA (n-3 PUFA, $n = 10$). EPA capsule (58% EPA, 15% DHA) and DHA capsule (11% EPA, 48% DHA) were provided by Haizhilai biotechnology limited company (Beijing, China). Diet composition was showed in Table 1. Liquid diets were freshly prepared daily from powder. Food intake of ethanol-fed mice were monitored, and average daily volume per mouse was calculated to adjust amount of control liquid diet. On day 11, the mice were gavaged with a single dose of 31.5% (*v/v*) ethanol (5 g/kg BW) or isocaloric maltose dextrin solution. After fasting for 9 h, the mice were euthanized, and their blood, entire liver and epididymal fat were collected for further analyses. All animal experiments were conducted according to the procedures approved by the Animal Ethics Committee (Approval No. AEC-13-002-1), Institute of Chinese Medical Sciences, University of Macau.

**Table 1**
Compositions of the modified Lieber-DeCarli liquid diets[a].

| Ingredients (g/L) | WT/CON | WT/ETH | *fat-1*/ CON | *fat-1*/ ETH | WT + n-3/ ETH |
|---|---|---|---|---|---|
| Casein | 41.4 | 41.4 | 41.4 | 41.4 | 41.4 |
| L-cystine | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| D, L-Methionine | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Cellulose | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Maltose dextrin | 115 | 44.8 | 115 | 44.8 | 44.8 |
| Corn oil | 39.6 | 39.6 | 39.6 | 39.6 | 18.8 |
| EPA (58% EPA, 15% DHA) | – | – | – | – | 9.9 |
| DHA (11% EPA, 48% DHA) | – | – | – | – | 9.9 |
| Mineral mix | 8.8 | 8.8 | 8.8 | 8.8 | 8.8 |
| Vitamin mix | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Choline bitartrate | 0.53 | 0.53 | 0.53 | 0.53 | 0.53 |
| Vitamin E acetate | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| 95% Ethanol (*v/v*), mL/L | – | 52.6 | – | 52.6 | 52.6 |

[a] Mice in alcohol-treated groups were fed a modified Lieber-DeCarli alcohol liquid diet with an energy composition of 18% protein, 19% carbohydrate, 35% fat and 28% ethanol (TROPHIC Animal Feed High-tech Co., Ltd. Nantong, China), whereas animals in the control groups were fed the Lieber-DeCarli control diet, in which maltose-dextrin (carbohydrate) isocalorically replaced ethanol.

### 2.2. Cell culture and differentiation

3T3-L1 cells (Cell Bank of Chinese Academy of Sciences, Shanghai, China) were cultured in DMEM supplemented with 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin. 3T3-L1 preadipocytes were differentiated by replacing the culture medium with DMEM containing 10% FBS, 0.5 mM IBMX, 1 mM dexamethasone (Dex), and 10 μg/mL insulin for 48 h, then change the medium with DMEM (10% FBS) containing 10 μg/mL insulin alone for 48 h. Thereafter, cells were cultured in DMEM with 10% FBS for 8–10 days, until most cells exhibited the phenotype of mature adipocytes.

### 2.3. Analysis of fatty acid composition in the liver

Hepatic fatty acid compositions were measured by GC–MS, as described previously [29]. The relative content of each fatty acid in the liver was expressed by the percentages of peak areas to total fatty acids in the GC–MS chromatogram.