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Highly purified eicosapentaenoic acid prevents the progression of hepatic steatosis by repressing monounsaturated fatty acid synthesis in high-fat/high-sucrose diet-fed mice

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ABSTRACT

Eicosapentaenoic acid (EPA) is a member of the family of n-3 polyunsaturated fatty acids (PUFAs) that are clinically used to treat hypertriglyceridemia. The triglyceride (TG) lowering effect is likely due to an alteration in lipid metabolism in the liver, but details have not been fully elucidated. To assess the effects of EPA on hepatic TG metabolism, mice were fed a high-fat and high-sucrose diet (HFHSD) for 2 weeks and were given highly purified EPA ethyl ester (EPA-E) daily by gavage. The HFHSD diet increased the hepatic TG content and the composition of monounsaturated fatty acids (MUFAs). EPA significantly suppressed the hepatic TG content that was increased by the HFHSD diet. EPA also altered the composition of fatty acids by lowering the MUFAs C16:1 and C18:1 and increasing n-3 PUFAs, including EPA and docosahexaenoic acid (DHA). Linear regression analysis revealed that hepatic TG content was significantly correlated with the ratios of C16:1/C16:0, C18:1/C18:0, and MUFA/n-3 PUFA, but was not correlated with the n-6/n-3 PUFA ratio. EPA also decreased the hepatic mRNA expression and nuclear protein level of sterol regulatory element binding protein-1c (SREBP-1c). This was reflected in the levels of lipogenic genes, such as acetyl-CoA carboxylase α (ACCα), fatty acid synthase, stearoyl-CoA desaturase 1 (SCD1), and glycerol-3-phosphate acyltransferase (GPAT), which are regulated by SREBP-1c. In conclusion, oral administration of EPA-E ameliorates hepatic fat accumulation by suppressing TG synthesis enzymes regulated by SREBP-1 and decreases hepatic MUFAs accumulation by SCD1.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognized cause of liver-related morbidity and mortality, which is frequently associated with metabolic syndromes such as obesity, insulin resistance (IR), and hyperlipidemia [1–3]. IR is believed to be a central mechanism in the progression of NAFLD [4], because the resulting hyperinsulinemia increases glucose levels in the blood and free fatty acids in adipose tissue, which then causes excessive accumulation of triglyceride (TG) in the liver [2]. Hyperlipidemia, especially hypertriglyceridemia, also increases lipoproteins and free fatty acids taken into liver resulting enhanced hepatic synthesis of TG [5,6]. Reduction of hepatic TG accumulation may prevent the progression from steatosis to NAFLD, because an aberrant accumulation of TG in the liver is an underlying cause of these diseases [7].

It is well established that TG is the most substantial fat component in the fatty liver. A molecule of TG is composed of a backbone of glycerol with three fatty acids bound to it. Palmitic acid (C16:0), palmitoleic acid (C16:1), and oleic acid (C18:1) are the predominant fatty acids in the liver in patients diagnosed with NAFLD [8]. It has been reported that increased plasma concentrations of monounsaturated fatty acids (MUFAs), mainly C16:1 and C18:1, were observed along with progression of hepatic damage [9]. High proportions of C16:0 in serum and adipose tissue have been consistently related to IR and arteriosclerosis [10,11]. Changes in hepatic fatty acid composition and content may be important in the pathogenesis of NAFLD.

The effects of n-3 long-chain polyunsaturated fatty acids (PUFAs) on coronary heart disease, obesity, dyslipidemia, and IR have been extensively investigated [12–14]. n-3 long-chain

Abbreviations: ACCα, acetyl-CoA carboxylase α; 36B4, acidic ribosomal phosphoprotein P0; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EPA-E, EPA ethyl ester; FAMEs, fatty acid methyl esters; FAS, fatty acid synthase; GPAT, glycerol-3-phosphate acyltransferase; HFHSD, high-fat and high-sucrose diet; IR, insulin resistance; MUFAs, monounsaturated fatty acids; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PUFAs, polyunsaturated fatty acids; PC, polyenephosphatidylcholine; PIO, pioglitazone hydrochloride; PPARs, peroxisome proliferator-activated receptors; RT-PCR, reverse transcription-polymerase chain reaction; SFAs, saturated fatty acids; SCD1, stearoyl-CoA desaturase 1; SREBP-1, sterol regulatory element binding protein-1; STD, standard pellet diet; TZD, thiazolidinedione; TG, triglyceride

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PUFAs are decreased in the hepatic tissue of patients with NAFLD, and are negative regulators of hepatic lipogenesis and inflammatory response [15,16]. It has been reported that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) down-regulate hepatic TG accumulation by decreasing sterol regulatory element binding protein-1 (SREBP-1) transcriptional activity and inducing fatty acid catabolism by activating the peroxisome proliferator-activated receptors (PPARs)-mediated pathway [17–20]. SREBP-1c, which is the predominant isoform of SREBP-1 in the liver, controls the transcription and expression of lipogenic enzymes such as stearoyl-CoA desaturase 1 (SCD1), fatty acid synthase (FAS), and glycerol-3-phosphate acyltransferase (GPAT) [21,22]. SCD1 converts saturated fatty acids (SFAs) into MUFAs, and is the rate-limiting enzyme in the biosynthesis of C16:1 and C18:1 [23]. A deficiency in the SCD1 gene leads to resistance to diet-induced obesity, decreased hepatic TG accumulation, and increased insulin sensitivity and metabolism [24,25].

Evidence from in vitro and in vivo studies suggests differential effects of EPA and DHA [26,27]. Several studies have suggested that EPA may be primarily responsible for the hypotriglyceridemic effect of n-3 long-chain PUFAs [28–31]. Rambjor et al. [32] concluded that EPA is responsible for the TG-lowering effect of fish oils in humans. In contrast, a hypolipidemic effect of DHA was also shown in patients with hypercholesterolemia and combined hyperlipidemia [33,34]. However, EPA is the only member in the class of n-3 long-chain PUFAs that is used clinically as a single-agent treatment for hypertriglyceridemia.

In this study, we assessed the pharmacological effects of highly purified EPA ethyl ester (EPA-E) on fatty liver induced by a Western-style diet, and investigated the relationship between the hepatic fatty acids and TG accumulation.

2. Materials and methods

2.1. Drugs

EPA-E (98% purity; Mochida Pharmaceutical, Tokyo, Japan) or polyenephosphatidylcholine (PC, Taiyo Pharmaceutical Industry, Nagoya, Japan) was suspended in 5% arabic gum (Wako Pure Chemical Industries, Osaka, Japan) solution using a homogenizer (Physcotron NS-56S; Microtec, Funabashi, Japan), and was administered orally. Pioglitazone hydrochloride (PIO, ChemPacific, Baltimore, MD, USA) was homogenized in 5% arabic gum solution using a Teflon homogenizer.

2.2. Animals

Male C57BL/6J mice (8 weeks of age) were purchased from Charles River Laboratories Japan (Yokohama, Japan). Mice were housed individually in a room under controlled temperature (21–25 °C), humidity (40–70%), and 12-h light/dark cycle with free access to sterilized water and a standard pellet diet (STD) containing no fish products (F1; Funabashi Farms, Funabashi). All animal experiments were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals at the Pharmaceutical Research Center of Mochida Pharmaceuticals.

2.3. Experimental protocols

After a 1-week acclimation, mice were randomly divided into five groups of eight mice each. They were fed STD (per weight basis; 4.4% crude fat, 61.4% carbohydrate, 22.3% crude protein, and 2.7% crude fiber) or a high-fat and high-sucrose diet (HFHSD) (TD.88137; Harlan Teklad, Madison, WI, USA) (per weight basis; 21% anhydrous milkfat, 34.1% sucrose, 15% corn starch, 19.5% casein, 0.15% cholesterol, and 5% cellulose) in pellet form for 2 weeks. The fatty acid composition of each of the two diets is shown in Table 1. The detailed combination of treatment in each group was as follows: group 1, STD-fed (control I); group 2, HFHSD-fed (control II); groups 3–5, HFHSD-fed and administered with EPA-E, PIO, or PC (1, 0.03, or 1 mg/g body weight, respectively) by gavage daily for 2 weeks. Groups 1 and 2 received 5% arabic gum solution as vehicle in the same way. Heparinized blood was collected from the inferior vena cava under anesthesia, and the liver was removed just after euthanasia. For measurement of hepatic TG content a portion of liver was immediately homogenized in saline using a Teflon homogenizer, and subjected to the assays described below. Another portion of the liver was fixed with 10% buffered formalin for histology, and the other was frozen in liquid nitrogen for analysis of fatty acids. To assess the dose-related effects of EPA-E (0.1, 0.3 and 1 mg/g), another set of mice (five groups of eight mice) was treated as above. Six out of eight mice were randomly assigned for analysis of nuclear SREBP-1 level before the experiment. A portion of liver was homogenized as mentioned above, and subjected to analyses of hepatic TG content and nuclear SREBP-1 level described below. For lipogenic gene analysis in the liver, an additional portion was immersed in RNAlater (Ambion, Austin, TX, USA) and stored in liquid nitrogen until RNA isolation.

Table 1
Fatty acid composition of STD and HFHSD diets.

		STD	HFHSD
		% (w/w) of total fatty acids	
Caproic acid	C6:0	ND	1.2
Caprylic acid	C8:0	ND	1.0
Capric acid	C10:0	ND	2.5
Lauric acid	C12:0	ND	3.2
Myristic acid	C14:0	0.2	11.1
Pentadecanoic acid	C15:0	ND	1.3
Palmitic acid	C16:0	17.4	31.1
Heptadecanoic acid	C17:0	0.1	0.9
Stearic acid	C18:0	5.6	12.0
Arachidic acid	C20:0	0.3	0.2
Behenic acid	C22:0	0.3	ND
Lignoceric acid	C24:0	0.2	ND
Total SFAs		24.1	64.7
Myristoleic acid	C14:1n-5	ND	0.8
Palmitoleic acid	C16:1n-7	0.1	1.8
Oleic acid	C18:1n-9	21.6	18.6
Eicosenoic acid	C20:1n-9	0.5	ND
Docosenoic acid	C22:1n-11, n-9	0.4	ND
Total MUFAs		22.6	26.1
Linoleic acid	C18:2n-6	48.7	1.3
α-Linolenic acid	C18:3n-3	3.9	0.8
Eicosapentaenoic acid	C20:5n-3	ND	0.1
Docosapentaenoic acid	C22:5n-3	ND	0.1
Docosahexaenoic acid	C22:6n-3	ND	ND
Total PUFAs		52.6	3.5

ND: Not detected.

2.4. Histology

Lipid accumulation was assessed by oil red-O staining of 4–5 μm frozen sections of fixed liver specimen. Briefly, OCT-embedded cryosections were pre-treated with 60% isopropanol, stained with 0.18% oil red-O in 60% isopropanol for 15–30 min, and then washed with 60% isopropanol. Sections were counterstained with Mayer's hematoxylin and mounted with aqueous solution.