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Down-regulation in muscle and liver lipogenic genes: EPA ethyl ester treatment in lean and overweight (high-fat-fed) rats

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Abstract

The precise mechanisms by which ω-3 fatty acids improve fat metabolism are not completely understood. This study was designed to determine the effects of eicosapentaenoic acid (EPA) ethyl ester administration on the expression levels of several muscle, liver and adipose tissue genes involved in lipogenesis and fatty acid oxidation pathways. Male Wistar rats fed a standard diet (control animals) or a high-fat diet were treated daily by oral gavage with EPA ethyl ester (1g/kg) for 5 weeks. The high-fat diet caused a very significant increase in plasma cholesterol ($P<.01$) levels, which was reverted by EPA ($P<.001$). A significant decrease in circulating triglyceride levels ($P<.05$) was also observed in EPA-treated groups. EPA administration induced a significant down-regulation in some lipogenic genes such as muscle acetyl CoA carboxylase β (*ACC β*) ($P<.05$) and liver fatty acid synthase (*FAS*) ($P<.05$). Furthermore, a decrease in glucokinase (*GK*) gene expression was observed in EPA-treated animals fed a control diet ($P<.01$), whereas a significant increase in *GK* mRNA levels was found in groups fed a high-fat diet. On the other hand, no alterations in genes involved in β-oxidation, such acetyl CoA synthase 4 (*ACS4*), acetyl CoA synthase 5 (*ACS5*) or acetyl CoA oxidase (*ACO*), were found in EPA-treated groups. Surprisingly and opposite to the expectations, a very significant decrease in the expression levels of liver *PPARα* ($P<.01$) was observed after EPA treatment. These findings show the ability of EPA ethyl ester treatment to down-regulate some genes involved in fatty acid synthesis without affecting the transcriptional activation of β-oxidation-related genes.

Keywords: High-fat diet; Eicosapentaenoic acid (EPA); Lipid metabolism; Muscle; Liver; Adipose tissue

1. Introduction

Over two decades, numerous research studies and clinical investigations have focused on the healthy beneficial effects of consuming marine polyunsaturated fatty acids (PUFAs) [1,2]. There is much evidence suggesting that the intake of these ω-3 fatty acids, especially eicosapentaenoic acid (EPA), produces some benefits on cardiovascular disease markers [3], insulin resistance [1], obesity features [4] and regulation of serum lipid level [5]. In fact, several studies have demonstrated that fish oil administration caused hypolipidemic effects by coordinately suppressing new fatty acid synthesis and by inducing fatty acid oxidation in different tissues such as liver, skeletal muscle and white adipose tissues [6–8]. However, the intimate mechanisms by which specific PUFAs improve lipid metabolism, particularly by reduction of blood lipids such as triglycerides (TGs), are not completely understood.

Some studies have suggested that ω-3 fatty acids are important mediators of gene expression acting through the peroxisome proliferator-activated receptor (PPAR) and sterol regulatory element binding protein (SREBP) pathways [9,10], which are two critical transcriptional factors involved in β-oxidation and lipogenesis, respectively. In this sense, Neschen et al. [11] have recently confirmed that ω-3 fatty acids act as PPARα ligands and thereby induce hepatic β-oxidation. It has been also reported that mRNA levels of acetyl CoA oxidase (*ACO*), the first and rate-limiting enzyme of peroxisomal fatty acid β-oxidation [12], are increased by fish oil rich in EPA and docosahexaenoic acid (DHA) [13,14]. However, it has been suggested that EPA in the form

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of ethyl ester could not mimic the physiological activity of fish oil, particularly regarding hepatic fatty acid oxidation in rats [15].

On the other hand, it was shown that lipogenic gene expression of several enzymes was decreased by ω-3 fatty acids such as EPA and DHA in triacylglycerol form [16]. Indeed, experimental studies showed that fish oils regulate *SREBP-1c* mRNA levels, a transcriptional factor that controls some lipogenic genes [10,17]. It was also observed that liver fatty acid synthase (*FAS*) is down-regulated by fish oils [13,18]. The same effects on this enzyme elicited by EPA ethyl ester were found in adipose tissue [19]. Moreover, the effect of EPA ethyl ester on acetyl CoA carboxylase β (ACCβ) — another important enzyme involved in muscle lipogenesis and, in an indirect manner, fatty acid oxidation [20] — apparently has not been determined, although controversial effects on this enzyme in the liver of EPA-fed mice have been described [21,22]. Furthermore, several authors indicated that dietary ω-3 fatty acids could inhibit glucokinase (GK), the enzyme responsible for glucose phosphorylation in the liver [1,23].

Furthermore, the importance of these circulating TGs and cholesterol levels in the development of fatty liver and in the accumulation of lipids in skeletal muscle, which are both involved in obesity and insulin resistance, is well known. Thus, fish oil induces improvement in nonalcoholic steatohepatitis in rats [24,25]. However, the effects of EPA ethyl ester on fatty liver development in overweight rats have not been determined, although it has been reported that this pure ω-3 fatty acid induces TG and cholesterol accumulation in skeletal muscle despite its healthy effects [26].

In order to clarify the mechanisms by which EPA ethyl ester improves lipid metabolism, the aim of the present work was to determine the effects of EPA ethyl ester administration on the expression levels of several liver, muscle and adipose tissue target genes involved in lipogenesis and β-oxidation pathways in rats fed a standard diet (control animals) or a high-fat diet (overweight rats).

2. Materials and methods

2.1. Animals and treatment

Twenty-nine male Wistar rats (6 weeks old) supplied by the Center of Applied Pharmacology (CIFA, Pamplona, Spain) were housed in a temperature-controlled room (22±2°C) with a 12-h light–dark cycle. All experimental procedures were performed in accordance with the National and Institutional Guidelines for Animal Care and Use at the University of Navarra, Spain. Animals were distributed into four experimental groups: control, control+EPA (CEPA), overweight and overweight+EPA (OEPA). All animals were maintained for an adaptation period of 4 days, fed chow diet (Rodent Toxicology Diet; B&K Universal) and given deionized water ad libitum. After this period of time, the control and CEPA groups were fed a standard pelleted diet (Rodent Toxicology Diet; B&K Universal) containing 76% carbohydrates, 6% lipids and 18% proteins (362 kcal/100 g). On the other hand, the overweight and OEPA groups were fed a cafeteria diet composed of the following items: paté, chips, bacon, chocolate, biscuits and pelleted diet (relative ratio, 2:1:1:1:1:1) [27]. The composition of this diet was as follows: 9% energy as protein, 29% energy as carbohydrate and 62% energy as lipid, by dry weight. All animals had ad libitum access to water and food for 5 weeks. The fatty acid composition of both control and high-fat diets was analyzed as previously reported [4], with the finding that both control and cafeteria diets have no EPA. Thus, the rats' only source of this fatty acid is oral gavage. Thus, the CEPA and OEPA groups were treated, simultaneously with the maintenance of diets for 35 days, with 1g/kg animal weight of highly purified EPA ethyl ester (Brudy S.L., Spain). This dose of EPA ethyl ester has been previously reported by Nobukata et al. [28] to have a beneficial effect on diabetes prevention. The same volume of water was orally administrated to the control and overweight groups, as previously described in other studies [26,29], for 35 days. These control and overweight groups without treatment with any other type of fatty acid (such as saturated fatty acids with equal chain length as EPA or oleic acid) are more likely to be considered as control groups in our study design [4]. This is because it has been demonstrated that supplementation with some other fatty acids is able to modify adiposity and circulating levels of biochemical and hormonal markers planned to be determined in the present study [29].

Body weight and food intake were measured daily. After 35 days, animals were submitted to overnight fasting and euthanized by decapitation. Gastrocnemius muscle, liver and epididymal adipose tissues were collected and frozen in liquid nitrogen before being stored at −80°C [4].

2.2. Gene expression analysis

Gene RNA levels were analyzed by Northern blot analysis or RT-PCR. Thus, liver *SREBP-1c* was determined by Northern blot analysis. Total RNA was extracted from the liver in accordance with Gibco Life Technologies procedure using Trizol (Life Technologies, Inc., Grand Island, NY). For each tissue sample, 15 μg of total RNA was fractionated by electrophoresis on a denaturing 1% agarose gel containing 2.2 M formaldehyde and 1× MOPS running buffer. One microliter of 50 μg/ml ethidium bromide (Gibco BRL, Gaithersburg, MD) stock solution was added in order to check RNA integrity and even loading. After electrophoresis, RNA was transferred to a nylon membrane (Duralon-UV; Stratagene, La Jolla, CA) by overnight capillary transfer and UV cross-linked (Stratalinker 1800; Stratagene). Blots were then hybridized for 1 h at 68°C in the presence of the previously labeled cDNA probe (2×10^6 cpm/ml Express Hyb solution; Clontech, Palo Alto, CA). After the blots had been washed at high stringency, they were exposed to X-ray film with an intensifying screen for enough time at −80°C. To allow loading of an equal mass of RNA in each well, after the