Liver and Pancreatic Sciences

open access text

Short Communication

Fatty liver and n-3 fatty acids ingestion: New mechanisms and perspectives from pre-clinical animal models

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of lipids caused by an imbalance among synthesis, ingestion, oxidation and exporting fatty acids. Animal models using diets with high levels of lipids or carbohydrates and excessive treatment with glucocorticoids have mimicked the alterations in hepatic steatosis. Nutritional interventions using n-3 polyunsaturated fatty acids (EPA-eicosapentaenoic acid and DHA-docosahexaenoic acid) have shown to be able to decrease hepatic steatosis, particularly by reducing the activity of lipogenic enzymes and an increasing β-oxidation flux. In such field, new mechanisms such as oxidative enzymes activity and changes in endocannabinoid system tone have been investigated to elucidate these effects. In addition, investigation of new EPA and DHA specific molecular targets have been discussed by new studies.

Non-alcoholic fatty liver disease (NAFLD) is one of the most common forms of chronic liver disease in developed countries, affecting 20 to 30% of the general population [1-3]. This disease is defined by the pathological accumulation of excessive fat in the liver without alcohol consumption, like the accumulation of hepatic triglycerides (TGs) resulting from unbalanced uptake, synthesis, exportation and oxidation of fatty acids [4,5]. Such state is characterized by hepatic steatosis, liver cell injury, and lobular hepatitis [4].

Obesity is an important risk factor for the development of NAFLD, mainly visceral fat accumulation, therefore, insulin resistance may also be responsible for the development of NAFLD even in non-obese and lean individuals [6-8]. However, the pathogenesis of NAFLD is not completely clear. Multiple mechanisms, such as aberrant lipid metabolism, dysregulated cytokine production, oxidative stress, and inflammation in hepatocytes, are believed to be involved [9-12].

Other factors that play a role in hepatic lipid content can include diet, *de novo* hepatic lipid synthesis, and genetics factors [13-16]. Following such line of thinking, to reproduce the etiology, development, progression and outcome of liver disease, animal-based experimental models are commonly used, such as high caloric diet intake (overfeeding), high fat intake (especially saturated fatty acids), high intake of simple sugars and cafeteria diet [17-21]. Furthermore, some pharmacological interventions can also cause NAFLD. Synthetic glucocorticoids (GCs) are substances mimicking endogenous steroid hormones secreted by the adrenal cortex upon activation of the hypothalamic-pituitary-adrenal (HPA)-axis. They can contribute to the development of metabolic syndrome. Synthetic GCs are commonly applied as anti-inflammatory drugs. However, the use for prolonged time or in high doses can cause side effects, such as weight gain, insulin resistance, hypertriglyceridemia, hyperphagia and central obesity [22-26].

Although the pathogenic mechanisms involved in hepatic lipid accumulation caused by diet or synthetic GC use are not completely understood, some studies have been conducted to find adjuvant strategies to attenuate the changes evidenced in this metabolic disorder, as the ingestion of polyunsaturated fatty acids (PUFAs) [27-30].

Fatty acids can influence many cell properties, resulting in altered metabolism, gene expression, modified responsiveness to hormones, and production patterns of biologically active substances. Therefore, fatty acids can modulate physiological functions and be beneficial to promote health and well-being [31].

In addition, some evidence suggests that PUFAs omega-3 (n-3), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can contribute to improve several metabolic dysfunctions (e.g. increase glucose tolerance, insulin sensitivity, and reducing the risk factors for chronic non-communicable diseases and metabolic syndrome) [32-39].

The effects of polyunsaturated fatty acids ingestion reducing accumulation of liver lipids in pre-clinical models have been shown by different research groups [40-44]. However, the molecular pathways explaining such effects are still under investigation. Among different pathways and molecular targets, the activation of the transcription factor peroxisome proliferator activated receptor-α (PPAR-α) is the most studied and most concise result [32,46-49]. These studies show that the activation of the PPAR leads to a lower expression and activity of lipogenic genes and enzymes, such as sterol regulatory element-binding protein 1c (SREBP-1c), carbohydrate responsive element-binding protein (ChREBP), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and HMG-CoA reductase. Also, PPAR activity and expression can be linked to increasing lipoprotein lipase (LPL)

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Key words: *hepatic steatosis, liver, metabolism, n-3 fatty acids, pre-clinical models*

Received: November 02, 2016; **Accepted:** December 14, 2016; **Published:** December 19, 2016

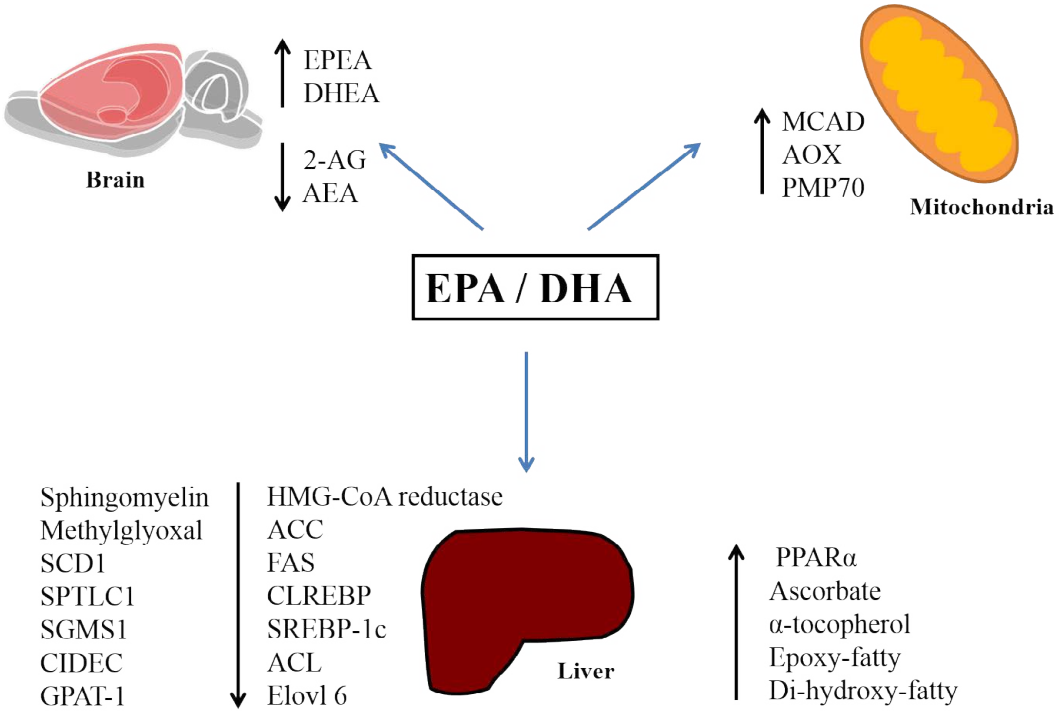

Figure 1. Summary of EPA and DHA molecular effects related to the reduction in hepatic lipid accumulation. Abbreviations: EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, EPEA: eicosapentaenoyl ethanolamide, DHEA: docosahexaenoyl ethanolamide, 2-AG: 2-Arachidonoylglycerol, AEA: N-arachidonoylethanolamine, MCAD: medium-chain acyl-CoA dehydrogenase, AOX: acyl-CoA oxidase, PMP70: peroxisomal membrane protein 70, SCD1: stearoyl-CoA desaturase-1, SPTLC1: serine-palmitoyl transferase long chain base subunit-1, SGMS1: phosphatidylcholine:ceramide choline phosphotransferase 1, CIDEC: death inducing DFFA like effector c, GPAT-1: glycerol-3-phosphate acyltransferase, ACC: acetyl-CoA carboxylase, FAS: fatty acid synthase, SREBP-1c: sterol regulatory element-binding protein 1c, ACL, Elovl 6: ELOVL family member 6, PPARα: peroxisome proliferator activated receptor-α, ChREBP: carbohydrate responsive element-binding protein.

activity, carnitine palmitoyltransferase (CPT) activity, and β-oxidation flux, promoting higher oxidation of fatty acids [32,45-50]. Mice supplemented with fish oil (a source of EPA and DHA) and fed with high-fat diet have shown that increasing the expression of enzymes related to lipogenesis, and enzymes involved in the oxidative capacity are important for controlling the accumulation of liver lipids [51]. This study showed an increase in the activity of medium-chain acyl-CoA dehydrogenase (MCAD), acyl-CoA oxidase (AOX), and increased content of the peroxisomal membrane protein 70 (PMP70) in mice fed with fish oil [51]. Such data indicate that supplementation of fish oil reduces accumulation of the fatty acids in liver promoting increased oxidative capacity.

Furthermore, a recent study showed that purified EPA and DHA have different effects on atherogenic high fat (AHF) NAFLD development of diet-induced disease in mice. EPA and DHA reduced the SREBP-1 protein and expression of lipogenic genes. However, EPA was more effective than DHA in reducing mRNA expression of FAS, (ELOVL family member 6) Elovl6 and glycerol-3-phosphate acyltransferase (GPAT-1). The authors also showed that cell expression death inducing DFFA like effector c (CIDEC), a protein located in lipid droplets playing a key role in fatty liver formation, was significantly suppressed in the AHF + EPA, but not in the AHF + DHA group [52]. Therefore, it is important to consider that these PUFAs (EPA and DHA) have specific targets but can contribute together to attenuate the accumulation of fatty acids in the liver.

A study with LDLR -/- mice with Nonalcoholic Steatohepatitis induced by Western diet (WD) showed that diets containing EPA and DHA have a hepatoprotective effect [53]. The authors showed that dietary EPA and DHA attenuates hepatic inflammation by suppressing saturated (SFA), monounsaturated fatty acids (MUFA), and sphingomyelin production. Apparently, this was achieved by suppressing substrate availability (citrate) and the expression of enzymes (FAS, ATP citrate lyase-ACL, stearoyl-CoA desaturase-1-SCD1, serine-palmitoyl transferase long chain base subunit-1-SPTLC1, phosphatidylcholine: ceramide choline phosphotransferase 1-SGMS1) involved in these pathways. Furthermore, DHA and EPA seemed to control cellular levels of antioxidants such as ascorbate and α-tocopherol, and increased the formation of oxidized lipids that can be hepatoprotective (epoxy and di-hydroxy-fatty derivatives of EPA and DHA). In particular, this study showed that DHA improved methylglyoxal detoxification induced by WD, this finding was important to understand how DHA regulates glucose and lipid metabolism [53]. Thus, a diet with DHA>EPA reduced the progression of hepatic steatosis by controlling the activation of transcription factors involved in lipid metabolism, oxidative stress, and inflammation. This shows that, besides acting together in some experimental models, DHA and EPA can modulate some molecular pathways and the respective outcomes differently.

Additional mechanisms have been investigated to further elucidate the effects of EPA and DHA on hepatic lipid metabolism. Mice fed a WD containing cod (a fish source of EPA and DHA) showed a significant increase in the concentrations of EPA and DHA, and an attenuation in hepatic fat accumulation, accompanied by a change in the endocannabinoid system tone. The presence of higher concentrations of EPA and DHA, when compared to arachidonic acid,

leads to the reduction of synthesis of 2-arachidonoylglycerol (2-AG), N-arachidonoylethanolamine (AEA), and increases the availability of substrate for the formation of endocannabinoid derivatives from EPA and DHA (*e.g.* eicosapentaenoyl ethanolamide (EPEA) and docosahexaenoyl ethanolamide (DHEA), respectively). This mechanism can partly explain the attenuation of the increase of hepatic lipids, and the development of obesity in WD and cod fed mice [54].

Thus, the most recent literature shows that EPA and DHA (n-3 PUFAs present in fish oil and oily fish) can attenuate the accumulation of lipids in the liver in pre-clinical models. Such effects are associated not only with n-3 fatty acids influencing the activity of proteins involved in lipogenesis and β-oxidation but also with metabolic oxidative enzymes and changes in endocannabinoid system tone (Figure 1). Further research should explore these mechanisms, especially distinct EPA and DHA effects on specific molecular targets. This could lead us to the next generation of effective therapeutic approaches.

Conflict of interest

The authors confirm that there no conflict of interest.

Authorship contributions

The authors together contributed to the preparation of the article design, writing and critical review of the intellectual content of the article, as well as approval of the final version.

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