

Regulation of Cholesterol Metabolism in Liver: Link to NAFLD and Impact of n-3 PUFAs

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Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease that affects one-third of adults in westernized countries. NAFLD represents a wide spectrum of hepatic alterations, ranging from simple triglyceride accumulation in the liver to steatohepatitis. Several pharmaceutical approaches to NAFLD management have been examined, but no particular treatment has been considered both safe and highly effective. Growing evidence reveal that supplemental fish oil, seal oil and purified n-3 fatty acids can reduce hepatic lipid content in NAFLD through extensive regulation by inhibiting lipogenesis, promoting fatty acid oxidation and suppressing inflammatory responses. Recently, the *fat-1* transgenic mice capable of converting n-6 to n-3 polyunsaturated fatty acids (PUFAs) have been used to examine the effects of endogenous n-3 PUFAs on NAFLD. The increased n-3 PUFAs in *fat-1* transgenic mice reduced diet-induced hyperlipidemia and fatty liver through induction of CYP7A1 expression and activation of cholesterol catabolism to bile acid. This article introduces the n-3 PUFAs, and addresses the evidence and mechanisms by which endogenously synthesized n-3 PUFAs or increased dietary n-3 PUFAs may ameliorate NAFLD.

Key Words: Cholesterol metabolism, Hepatic steatosis, Lipid metabolism, Non-alcoholic fatty liver disease, n-3 polyunsaturated fatty acids

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by pathological fat accumulation in hepatocytes in the absence of excessive alcohol consumption, which comprises a wide spectrum of liver disease ranging from steatosis to steatohepatitis, cirrhosis and even hepatocellular carcinoma. It is clear that NAFLD is strongly associated with insulin resistance, obesity, dyslipidemia and diabetes but its pathogenesis is not fully understood [1]. According to the “two hit” hypothesis, hepatic steatosis and insulin resistance

occur first because of an increased uptake of lipids and/or decreased fatty acids oxidation, inadequate *de novo* synthesis of triglycerides, and/or decreased synthesis or secretion of very low density lipoproteins (VLDLs) in liver [2]. This induces a chronic inflammatory condition characterized by the release of pro-inflammatory cytokines and oxidative stress. Both of which are responsible of the “second hit”, which induces the progression from steatosis to more advanced stages of liver damage. This theory, however, should be revised since the increased ratio of saturated-to-unsaturated fatty acids within the liver directly induce hepatic inflammation and insulin resistance, which may worsen NAFLD progression [3].

Currently, the first-line treatment of NAFLD is based on diet and lifestyle modification. Studies of the dietary habits of NAFLD patients showed low intake of n-3 poly-

Received: February 17, 2013, Accepted: March 10, 2013

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unsaturated fatty acids (PUFAs) and/or a lower relative concentration of n-3 PUFAs in tissues and blood [4-6]. Indeed, insufficient intake of n-3 PUFAs is associated with increased risk of NAFLD independent of traditional risk factors and data strongly support the detrimental role of high n-6/n-3 ratio on metabolic parameters [4]. Deficiency of n-3 PUFAs leads to hepatic steatosis and insulin resistance, whereas dietary supplementation of n-3 PUFAs appears to safely reduce nutritional hepatic steatosis in adults [7]. The present review discusses the potential mechanisms through which n-3 PUFAs may show its benefits in NAFLD, and the current data supporting its use.

METABOLISM OF n-3 PUFAs

Fatty acids, essential components of all cell membranes, play a number of key roles in metabolism such as being a major metabolic fuel (storage and transport energy), and the ligands for transcription factors. Although animals can synthesize saturated fatty acids and some monounsaturated fatty acids from carbon groups in carbohydrates and proteins, they lack the enzymes, desaturases, which are neces-

sary to insert a cis double bond at the n-6 or the n-3 position of a fatty acid. Consequently, linoleic acid (LA, C18:2; n-6) and alpha-linolenic acid (ALA, C18:3; n-3) are essential nutrients that must be taken from food [8]. Human can synthesize long-chain n-6 PUFAs, such as arachidonic acid (AA; C20:4;n-6) from LA and long-chain n-3 PUFAs, such as eicosapentaenoic acid (EPA; C20:5; n-3) and docosahexaenoic acid (DHA; C22:6; n-3) from ALA (Fig. 1). However, EPA and DHA have to be obtained from diet because mammals inefficiently convert ALA to EPA and DHA [9]. n-3 PUFAs are enriched in fish oil, flaxseed and some nuts whereas n-6 PUFAs are found predominantly in grain and vegetable oil. It has been estimated that the ratio of n-6 to n-3 PUFAs in the diet of early humans was 1:1 [10], but the ratio in the typical Western diet is now almost 10:1 due to increased use of vegetable oils rich in LA as well as reduced fish consumption [11]. A lower intake in dietary sources of n-3 PUFAs seems to be associated with NAFLD [4,12]. In fact, patients with hepatic steatosis present a lower n-3/n-6 PUFA ratio in liver tissue biopsies, namely in phospholipids subfractions, and in red blood cells [5,6].

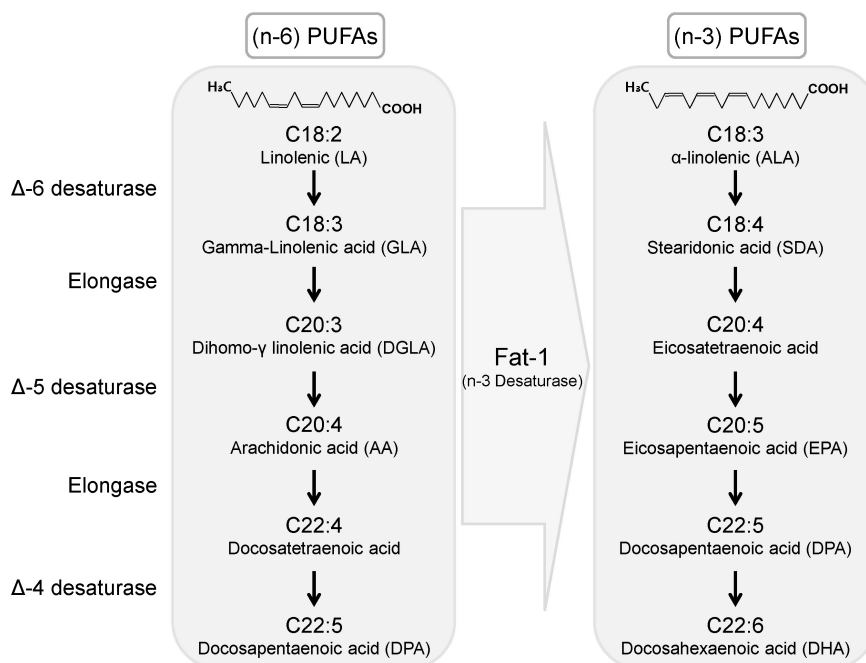


Fig. 1. Metabolism of n-3 and n-6 polyunsaturated fatty acids (PUFAs). Linolenic acid (LA) and alpha-linolenic acid (ALA) are the parent PUFAs for n-6 and n-3, respectively. The *fat-1* gene encodes an n-3 desaturase that converts n-6 to n-3 fatty acid.

THE EFFECTS OF DIETARY n-3 PUFAs AND UNDERLYING MECHANISMS

Health benefits of dietary n-3 PUFAs include decrease of triglyceridemia and hepatic steatosis in human and rodent models of obesity. Indeed, n-3 PUFAs supplementation lessened the hepatic steatosis in *ob/ob* mice [13-16] and in rats fed a high-fat diet (HFD) [17,18]. In addition to improvement in hepatic steatosis, these rodent models had lowered plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels [14,19,20]. The lipid-lowering effect of n-3 PUFAs in rats fed high fat/cholesterol has been associated with the downregulation of *de novo* lipogenesis and upregulation of lipid oxidation.

Lipogenesis is thought to be regulated via the transcription factors carbohydrate response element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c), which are activated by glucose and insulin, respectively [21,22]. On the contrary, peroxisome proliferator activated receptor α (PPAR α) is a transcription factor known to decrease plasma lipids and induce enzymes and other proteins involved in lipid oxidation within the mitochondria, microsomes and peroxisomes [23]. The increased hepatic SREBP-1c/PPAR α ratio and steatosis are strongly correlated with insulin resistance and n-3 PUFA depletion in obese patients [24]. Administration of n-3 PUFAs to obese patients and NAFLD-prone rodents decreased the nuclear abundance of ChREBP/SREBP-1c while induced PPAR α in the liver [14,17]. Furthermore, the expression of ChREBP/SREBP-1c-dependent, glycolytic/lipogenic enzymes were reduced, while that of PPAR α -dependent, oxidative enzymes were increased by n-3 feeding [23,25].

Fish oil feeding is also associated with elevated concentration of adiponectin in the circulation and visceral adipose tissue [13,17,26]. Adiponectin, one of the insulin-sensitizing adipokines, has been shown to reduce hepatic lipids by activating fatty acid oxidation and inhibiting hepatic lipogenesis in liver [27,28]. Various pathological conditions are associated with lower plasma adiponectin concentration. Specifically, NAFLD patients have lower levels of adiponectin whereas fish oil supplementation to the obese increases adiponectin in most [29-31], but not all studies [32].

Besides the function on metabolic homeostasis, there is evidence from several studies that n-3 PUFAs have also shown the anti-inflammatory and anti-oxidant effects to protect the cells from ROS-induced damage [20,33]. Overall, these results suggest that n-3 PUFAs reduce hepatic lipids, markers of inflammation, and improve insulin sensitivity, all major events in NAFLD development and/or progression.

THE *FAT-1* TRANSGENIC MICE

Although increasing the ratio of dietary n-3 to n-6 PUFAs might be beneficial in the prevention and treatment of dyslipidemia, obesity, insulin resistance, NAFLD and diabetes, it is difficult to ascertain the contribution of certain n-3 PUFAs per se, without the potential confounding effects of other dietary components. Since no pure n-3 and n-6 fatty acids are available, the major sources of the required n-3 and n-6 PUFAs are from fish oils and plant seed/vegetable oils, respectively. These two kinds of oils contain different bioactive compounds, such as saturated fatty acids, monounsaturated fatty acids, cholesterol, antioxidants, contaminants and other unidentified substances, that affect the study outcomes. Indeed, while it is generally accepted that fish oil improves the serum lipid profile, it is controversial whether dietary flaxseed oil has a similar effect [34]. Studying the effects of dietary n-3 PUFAs in the context of high fat feeding has proven to be rather complicated because incorporation of n-3 PUFAs into rodent diets often prevents weight gain [35].

Therefore it is ideal to develop a transgenic mouse capable of converting n-6 to n-3 PUFAs so that the results obtained in such an animal model will be more reliable and easy to interpret in terms of the effects of n-3 and n-6 PUFAs. As mentioned, n-3 PUFAs are not synthesized in mammals including humans. Kang et al. generated *fat-1* transgenic (*fat-1*) mice expressing the *Caenorhabditis elegans fat-1* gene encoding an n-3 fatty acid desaturase that converts n-6 PUFAs to n-3 PUFAs (Fig. 1) [36]. This allows production of two different FA profiles (low vs high n-6/n-3 ratios) in the *fat-1* mice and wild-type littermates by using just a single diet, thus eliminating the potential diet variations. Hence, the *fat-1* mice are valuable *in vivo* system for elucidating the role of n-3 PUFAs and the n-6/n-3 ratio

in NAFLD development and progression.

ENDOGENOUSLY SYNTHESIZED n-3 PUFAs EFFECTS ON NAFLD

Studies on *fat-1* mice yielded interesting results including reduction of inflammation, bone loss, colitis, colon cancer, melanoma growth, invasiveness of lung cancer cells, and seizure susceptibility [37-42]. White et al. [43] reported that transgenic restoration of n-3 PUFAs in insulin target tissues improved resolution capacity and alleviated obesity-linked inflammation and insulin resistance in HFD-fed mice. The authors proposed that the endogenous n-3 PUFAs exerted their protective effects through their lipid oxygenation products, which reduced macrophage infiltration and inflammation in the expanding adipose tissue of obese mice. However, the hepatic lipid accumulation was not changed in both HFD-fed wild-type and *fat-1* mice, showing different results from dietary n-3 PUFAs supplementation reversed hepatic steatosis in *ob/ob* mice [15].

We have recently reported that endogenously synthesized n-3 PUFAs could ameliorate HFD-induced fatty liver and hyperlipidemia in *fat-1* mice, leading to significant attenuation of NAFLD and hepatic injury, as reflected by significant decreases in either triglyceride (TG) or liver enzymes, including AST and ALT [44]. Protection against hyperlipidemia in *fat-1* mice was associated with significant upregulation of genes involved in cholesterol uptake (*Ldlr*), bile acid metabolism (*Cyp7a1*) or excretion (*Abcg5* and *Abcg8*), and downregulation of *Apoa4*, a component of chylomicrons and HDL. These results suggested that endogenously synthesized n-3 PUFAs ameliorated fatty liver and hypercholesterolemia through modulating cholesterol and bile acid metabolism. Recently, cholesterol accumulation is proposed to participate to the pathogenesis of NAFLD and non-alcoholic steatohepatitis [45]. Moreover, patients with NAFLD also exhibited high hepatic-free cholesterol levels [45]. The sole pathways for hepatic cholesterol elimination are bile acid synthesis and cholesterol exported into the bile [46]. CYP7A1 is the rate-limiting enzyme in the bile acid synthetic pathway that converts cholesterol into bile acids in the liver. Recent studies have shown that transgenic mice overexpressing CYP7A1 were protected against HFD-in-

duced hypercholesterolemia, obesity, and insulin resistance [47]. Moreover, the hepatic cholesterol transporters *Abcg5* and *Abcg8* were significantly induced in *Cyp7a1*-tg mice [47], as shown in *fat-1* mice [44]. Therefore, the findings regarding to the regulation of *Ldlr* and *Cyp7a* by endogenous n-3 PUFAs paralleled its effects on circulating cholesterol, TG, and LDL cholesterol levels during HFD treatment and provided mechanistic evidence for decreased cholesterol accumulation and increased hepatic uptake of circulating LDL cholesterol.

CONCLUSION

The prevalence of NAFLD is increasing worldwide. Although simple steatosis is relatively benign and principally reversible, steatohepatitis is the progressive form of NAFLD and can develop cirrhosis, hepatic failure, and hepatocellular carcinoma. The only approved management of NAFLD is lifestyle modification including increased physical activity, dietary behaviors, and weight reduction. Therefore, an effective pharmaco-therapy is required in NAFLD management.

n-3 PUFAs have been suggested as a treatment for NAFLD. Dietary n-3 PUFAs reduce hepatic steatosis, inflammation and oxidative stress and improve insulin sensitivity. Studies on *fat-1* mice revealed that endogenously synthesized n-3 PUFAs ameliorated fatty liver and hypercholesterolemia resulting from enhanced hepatic CYP7A1 expression. These findings underscore the importance of endogenous n-3 PUFAs to maintain whole-body bile acid homeostasis, which plays a key role in lipid, glucose, and energy homeostasis. To confirm the clinical relevance of the preventive effect of exogenous n-3 PUFAs on NAFLD, the physiological concentration of n-3 PUFAs required to produce similar effects on the fatty livers of *fat-1* mice should be determined. For this purpose it will be helpful to investigate the concentrations of n-3 PUFAs in the liver after administration of various doses exogenous n-3 FAs in WT mice compared to *fat-1* mice.

ACKNOWLEDGEMENTS

This work was supported by the Basic Science Research Program through the National Research Foundation of

Korea (NRF) funded by the Ministry of Education, Science and Technology [NRF-2012R1A1A3018738 to J-Y Cha].

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