Omega-3 Fatty Acids, Hepatic Lipid Metabolism, and Nonalcoholic Fatty Liver Disease

E. Scorletti[1] and C.D. Byrne[1,2]

[1]Nutrition and Metabolism, Human Development and Health Academic Unit, University of Southampton and National Institute for Health Research Southampton Biomedical Research Center, Southampton University Hospitals National Health Service Trust, Southampton General Hospital, Southampton SO16 6YD, United Kingdom

[2]Endocrinology and Metabolism Unit, Southampton General Hospital, University of Southampton, Southampton SO16 6YD, United Kingdom; email: c.d.byrne@soton.ac.uk

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Keywords

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Abstract

Long-chain omega-3 fatty acids belong to a family of polyunsaturated fatty acids that are known to have important beneficial effects on metabolism and inflammation. Such effects may confer a benefit in specific chronic noncommunicable diseases that are becoming very prevalent in Westernized societies [e.g., nonalcoholic fatty liver disease (NAFLD)]. Typically, with a Westernized diet, long-chain omega-6 fatty acid consumption is markedly greater than omega-3 fatty acid consumption. The potential consequences of an alteration in the ratio of omega-6 to omega-3 fatty acid consumption are increased production of proinflammatory arachidonic acid–derived eicosanoids and impaired regulation of hepatic and adipose function, predisposing to NAFLD. NAFLD represents a spectrum of liver fat–related conditions that originates with ectopic fat accumulation in liver (hepatic steatosis) and progresses, with the development of hepatic inflammation and fibrosis, to nonalcoholic steatohepatitis (NASH). If the adipose tissue is inflamed with widespread macrophage infiltration, the production of adipokines may act to exacerbate liver inflammation and NASH. Omega-3 fatty acid treatment may have beneficial effects in regulating hepatic lipid metabolism, adipose tissue function, and inflammation. Recent studies testing the effects of omega-3 fatty acids in NAFLD are showing promise and suggesting that these fatty acids may be useful in the treatment of NAFLD. To date, further research is needed in NAFLD to (*a*) establish the dose of long-chain omega-3 fatty acids as a treatment, (*b*) determine the duration of therapy, and (*c*) test whether there is benefit on the different component features of NAFLD (hepatic fat, inflammation, and fibrosis).

Contents

INTRODUCTION

Omega-3 fatty acids were first discovered in 1929 by George Burr and his wife at the University of Minnesota Medical School. Omega-3 and omega-6 fatty acids were found to be of primary importance for normal growth and the prevention of dermatitis, and thus they were termed "essential fatty acids" (29). In 1960, the introduction of parenteral nutrition preparations devoid of lipids enabled researchers to study the effects of essential fatty acids deficiency. It was shown that deficiency of omega-3 and omega-6 fatty acids caused numbness, tingling, weakness, psychological disturbance, and blurred vision (30). Concurrently, it was also found that these symptoms were avoided by the introduction of α-linolenic acid (omega-3 fatty acid) and linoleic acid (omega-6 fatty acid) in the preparations. More recently, several studies have analyzed the relevance of essential fatty acids deficiency (12) in the development of metabolic syndrome (53), insulin resistance, cardiovascular disease, hypertension (77), dyslipidemia, fatty liver accumulation [e.g., nonalcoholic fatty liver disease (NAFLD)], and hepatic steatosis [e.g., nonalcoholic steatohepatitis (NASH)] (49, 61).

NAFLD comprises a spectrum of fat-related conditions. The first stage is characterized by simple steatosis described as the presence of droplets of triglycerides (TGs) in the cytoplasm in more than 5% of the hepatocytes. Steatosis can progress to NASH characterized by hepatocyte injury due to inflammation and collagen deposition. Approximately 20% of patients with NASH progress to cirrhosis, a condition in which hepatocytes are replaced by collagen tissue (3). Finally, cirrhosis can progress to hepatocellular carcinoma (42).

NAFLD is a worldwide health problem affecting one-third of the global population. The fate of patients with cirrhosis secondary to NASH is often to develop liver-related cardiovascular disease (4), liver failure, and/or liver cancer. Liver transplantation for NASH has increased by 8.5% between 2001 and 2009, and NASH is now the third most common indication for liver transplantation (17). In this review, we aim to describe the effects of omega-3 fatty acids on hepatic lipid metabolism and on inflammatory pathways affecting development of NAFLD. Furthermore, we discuss the role of omega-3 fatty acids as a potential treatment for NAFLD.

NAFLD: nonalcoholic fatty liver disease

NASH: nonalcoholic steatohepatitis

TGs: triglycerides

OMEGA-3 FATTY ACIDS

Chemical Structure and Nomenclature

Omega-3 fatty acids together with omega-6 fatty acids belong to the family of polyunsaturated fatty acids; these are long-chain fatty acids characterized by the presence of more than two double bonds in the molecule. Polyunsaturated fatty acids contain a carboxyl group at one end and a methyl group at the other end of the carbon chain; the first double bond is counted from the methyl end (or omega or *n* end) of the carbon chain.

These fatty acids are classified on the basis of distinct systems of nomenclature. Common names (e.g., α-linolenic acid, the simplest

omega-3) are vernacular names that often do not follow any classification. Systematic names (e.g., *all-cis*-9,12,15-octadecatrienoic acid) describe the molecule counting the double bond beginning from the carboxylic group; the double bond is labeled as *cis* or *trans* depending on the orientation of the double bond and the shape of the molecule. Shorthand nomenclature is based on the number of carbon atoms in the molecule and the number and position of double bonds. There are three different shorthand nomenclatures: (*a*) delta-x (Δ^x), in which the double bond is counted from the carboxylic acid and the prefix *cis* or *trans* indicates the stereochemical conformation of the molecule (e.g., *cis*-Δ^9, *cis*-Δ^{12} octadecadienoic acid); (*b*) *n* minus x or omega-x (ω-x), in which "x" is the number of the position of the double bond counting from the terminal "*n* or ω" methyl group (CH_3) (e.g., α-linolenic acid is an n-3 or ω-3 or omega-3 fatty acid); and (*c*) lipid number, whereby the molecule is described according to the number of carbon atoms forming the fatty acid chain and the number of double bonds present in the fatty acid chain (e.g., α-linolenic acid is described as 18:3) (1).

Sources of Omega-3 Fatty Acids and Dietary Intake

Plants can synthesize α-linolenic acid from linoleic acid in a reaction catalyzed by the enzyme Δ-15 desaturase; mammals do not possess this enzyme and thus they have to obtain this fatty acid from the diet. α-Linolenic acid can be found in green leafy vegetables, seeds such as linseed and its oil, nuts, and legumes, and a small percentage of α-linolenic acid is also found in corn oil, sunflower oil, or safflower oil. Seafood is a good source of omega-3 fatty acids. Nevertheless, it is necessary to distinguish between different types of fish, as the amount and type of omega-3 fatty acids vary. Lean fishes such as cod store lipid in their liver; fatty fishes such as salmon, tuna, mackerel, and sardines store lipid throughout their bodies (13).

At present, in Westernized countries the intake of omega-3 fatty acids falls short of recommended dietary intake. By contrast, the consumption of omega-3 is typically high in Eskimos, who live in the circumpolar regions of the globe, such as Canada, Siberia, Greenland, and Alaska; consumption also is high in the Japanese population (70). Fish consumption in these countries is typically high in part for cultural reasons, and it markedly exceeds dietary consumption in Australia, the United States, France, and the United Kingdom. The omega-3 fatty acid intake among Eskimos and Japanese people is approximately 3 to 4 g/day and 5 to 6 g/day, respectively (51). In contrast, the mean intake of omega-3 fatty acids among the Australian population is estimated to be 0.189 g/day (52), and in Europe and North America the intake of omega-3 fatty acids is approximately 0.15 to 0.25 g/day (5).

In adults, the estimated minimal daily intake of omega-3 fatty acids to prevent deficiency is approximately 0.35 to 0.40 g/day (0.5% of total fat), with a minimum intake of α-linolenic acid of 0.3 to 0.4 g/day (0.2% to 0.3% of total fat). The recommended intake of α-linolenic acid is 1.6 g/day for men and 1.1 g/day for women (≥0.5% total fat), whereas an adequate intake of eicosapentaenoic acid and docosahexaenoic acid ranges between 0.25 and 2 g/day (corresponding to two fish meals per week, with at least one oily fish meal consumed) (25, 79). The American Heart Association recommends two portions of fatty fish per week to prevent hypertriglyceridemia and cardiovascular disease. Moreover, in the presence of hypertriglyceridemia, the American Heart Association suggests treatment with 2 to 4 g/day of eicosapentaenoic acid and docosahexaenoic acid to reduce TG levels by 20% to 50% (40, 85).

Endogenous Synthesis and Metabolism

In the liver, dietary α-linolenic acid is first metabolized to stearidonic acid [SDA; 18:4(ω-3)] by Δ6-desaturase. This first reaction is a rate-limiting step and competes with the conversion of linoleic acid to arachidonic acid [20:4(ω-6)] in omega-6 fatty acid metabolism. SDA can

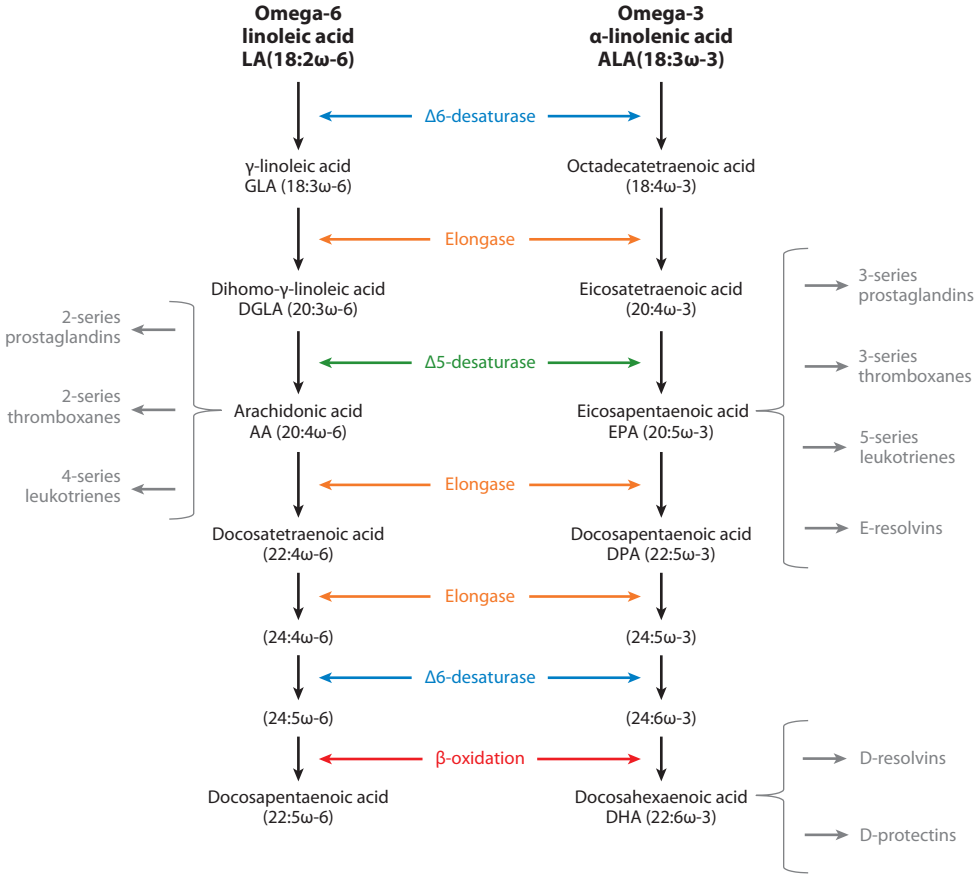

Figure 1

Synthesis and metabolites of omega-6 and omega-3 fatty acids. In the liver, dietary α-linolenic acid is metabolized to stearidonic acid by Δ6-desaturase; this first reaction is a rate-limiting step and competes with the conversion of linoleic acid to arachidonic acid in omega-6 fatty acid metabolism. In omega-3 fatty acid metabolism, Δ6-desaturase participates twice, once in the first step in which α-linolenic acid is desaturated to stearidonic acid and subsequently in the conversion of docosapentaenoic acid to docosahexaenoic acid; in contrast, Δ6-desaturase activity is utilized for omega-6 fatty acid metabolism only once. Δ5-Desaturase and Δ6-desaturase compete to metabolize these two polyunsaturated fatty acids. Omega-6 fatty acids produce 2- and 4-series prostaglandins, thromboxanes, and leukotrienes, whereas omega-3 fatty acids produce 3- and 5-series prostaglandins and leukotrienes as well as resolvins and protectins. Figure modified from Reference 74.

be elongated to form eicosatetraenoic acid that can be desaturated by Δ5-desaturase to form eicosapentaenoic acid [EPA; 20:5(ω-3)] (**Figure 1**). At this stage EPA can be further elongated to form docosapentaenoic acid [DPA; 22:5(ω-3)], and DPA can be converted to docosahexaenoic acid [DHA; 22:6(ω-3)] by Δ6-desaturase with the involvement of limited peroxisomal β-oxidation. In omega-3 fatty acid metabolism, Δ6-desaturase participates twice, once in the first step in which α-linolenic acid is desaturated to SDA and subsequently in the conversion of DPA to DHA. In contrast, Δ6-desaturase activity is utilized for omega-6 fatty acid metabolism only once (9, 13).

The optimal ratio between omega-6 and omega-3 fatty acids should be 1–4:1. In a Western diet (22), however, omega-6 fatty

EPA: eicosapentaenoic acid

DPA: docosapentaenoic acid

DHA: docosahexaenoic acid

acid consumption is significantly higher than omega-3 fatty acid consumption; as a result, the aforementioned ratio can increase to 10:1 or even 20:1. In the presence of an optimal ratio of omega-6 and omega-3 fatty acids, Δ5-desaturase and Δ6-desaturase compete to metabolize these two polyunsaturated fatty acids, although both enzymes have a greater affinity for omega-3 fatty acids. It has been suggested that an alteration in the ratio of omega-6 fatty acid to omega-3 fatty acid and the consequent increase of arachidonic acid-derived eicosanoids may be important in the pathogenesis of NAFLD (54).

Several factors can influence the equilibrium of Δ5-desaturase and Δ6-desaturases, including diet, insulin concentration, oxidative stress, and liver disease. Notably, decreased activity of both these enzymes has been shown in the liver of obese NAFLD patients (2). High omega-6 fatty acid intake in the diet alters the activity of Δ5-desaturase and Δ6-desaturase, increasing the production of arachidonic acid and curtailing that of EPA. In physiological and pathological conditions, cyclooxygenases and lipoxygenases can convert arachidonic acid and EPA into eicosanoids. Eicosanoids are lipid molecules with signaling functions that have an important role in regulating inflammation. Eicosanoids include prostaglandins, prostacyclins, thromboxanes, and leukotrienes (68). A diet rich in omega-6 fatty acids causes an accumulation of arachidonic acid in the cell membrane, influencing cell transport and favoring the production of arachidonic acid–derived eicosanoids. High arachidonic acid–derived eicosanoids lead to the synthesis of 2-series prostaglandins and thromboxanes (with two double bonds in the carbon chain) and 4-series leukotrienes (with four double bonds in the carbon chain). These eicosanoids play a pivotal role in regulating the production of proinflammatory cytokines. Overproduction of eicosanoids is associated with an increased release of proinflammatory cytokines, neutrophil activation, increased production of reactive oxygen species, and increased vascular permeability. This effect increases risk of platelet aggregation, hemorrhage, and vasoconstriction. EPA-derived eicosanoids are 3-series prostaglandins and thromboxanes (with three double bonds in the carbon chain) and 5-series leukotrienes (with five double bonds in the carbon chain). These EPA-derived eicosanoids have an anti-inflammatory effect compared with arachidonic acid–derived eicosanoids (41).

PPARα: peroxisome proliferator–activated receptor alpha

In addition, omega-3 fatty acids have a role as a substrate for a novel group of lipid mediators: resolvins and protectins. The E-series resolvins are lipid mediators enzymatically derived from EPA and comprise resolvin E1 (RvE1) and resolvin E2 (RvE2). The D-series resolvins are enzymatically derived from DHA. Resolvins have an anti-inflammatory effect, as they regulate the trafficking and activation of cells causing inflammation, such as granulocytes, macrophages, and lymphocytes (**Figure 2**) (11, 74). Protectins comprise another group of lipid mediators generated from 17S-hydroxy-DHA, which is an intermediate product of DHA. Protectins have a role akin to that of resolvins in the regulation of inflammation; moreover, they exert a specific action to prevent asthma, inflammation of the airway, and infiltration of the lung by T-cells (43).

Omega-3 DPA is a metabolite formed by the elongation of EPA, mediated by the enzyme fatty acid elongase-2 and 5. DPA can also be retro converted to EPA by peroxisomal acyl-coA oxidase and one cycle of β-oxidation. DPA has been shown to be particularly effective in inhibiting platelet aggregation when compared to EPA and DHA. DPA can interfere with cyclooxygenase-1 activity, resulting in the suppression of thromboxane synthesis from arachidonic acid; DPA can also cause an acceleration of lipoxygenase pathway, thus inhibiting platelet aggregation (71). Moreover, in hepatocytes, DPA has additional effects: interfering with peroxisome proliferator–activated receptor alpha (PPARα) in the regulation of β-oxidation and suppression of lipogenic genes in cultured liver cells. Nevertheless, the effects of DPA in the liver are overshadowed by those of EPA and DHA, which have greater affinity

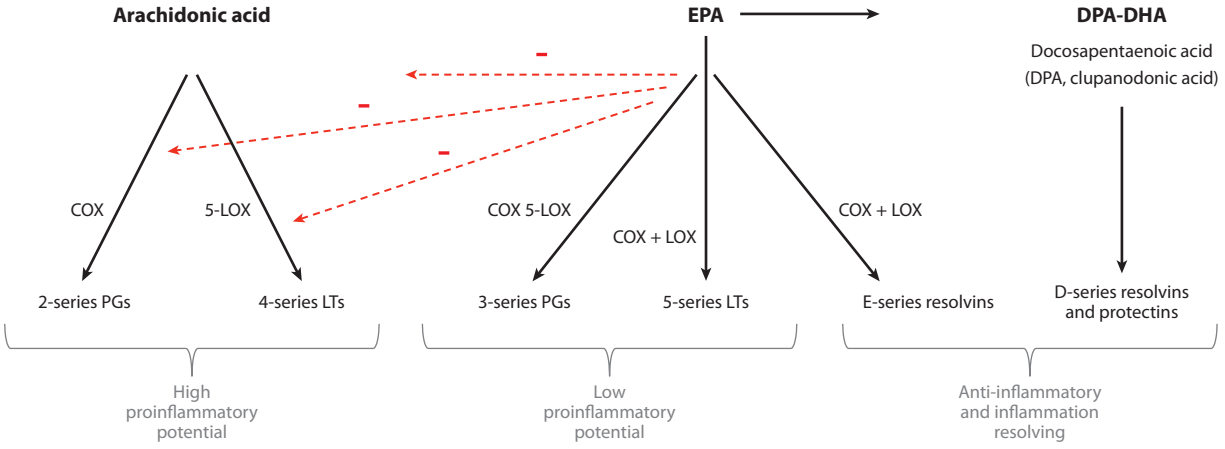

Figure 2

Potential proinflammatory and anti-inflammatory metabolites of n-3 and n-6 long-chain fatty acids. Cyclooxygenases (COXs) and lipoxygenases (LOXs) can convert arachidonic acid (AA) and eicosapentaenoic acid (EPA) into eicosanoids. Eicosanoids are lipid molecules with a signaling function and have an important role in regulating inflammation; they include prostaglandins (PGs), prostacyclins, thromboxanes, and leukotrienes (LTs). High-AA-derived eicosanoids lead to the synthesis of 2-series PGs and thromboxanes and 4-series LTs. These eicosanoids have a pivotal role in regulating the production of proinflammatory cytokines. Overproduction of eicosanoids is associated with an increased release of proinflammatory cytokines, neutrophil activation, increased production of reactive oxygen species, and increased vascular permeability. EPA-derived eicosanoids are 3-series PGs and thromboxanes and 5-series LTs. These EPA-derived eicosanoids have an anti-inflammatory effect compared with AA-derived eicosanoids. In addition, omega-3 fatty acids have a role as a substrate for a novel group of lipid mediators: resolvins and protectins. These mediators have an anti-inflammatory effect, as they regulate the trafficking and activation of cells causing inflammation, such as granulocytes, macrophages, and lymphocytes. Abbreviation: DHA, docosahexaenoic acid. Figure modified from Reference 11.

for PPARα and a more significant impact on the regulation of lipogenesis (38).

Hepatic and Adipose Tissue Function, Inflammation, and Nonalcoholic Fatty Liver Disease

The liver has a central anatomic location in the gastrointestinal tract and is the major metabolic organ for anabolic and catabolic processes. The liver is linked to the intestine (*a*) through a unique vasculature that converges in the portal vein and (*b*) through the enterohepatic biliary circulation. The liver has a wide range of functions, inter alia detoxification, hormone production, and plasma protein synthesis. A major function of the liver is to affect lipid metabolism (e.g., cholesterol synthesis, de novo lipogenesis, and synthesis of apolipoprotein B100). Among these other functions, the liver stores glycogen, lipid soluble vitamins, iron, and copper. The liver is not designed to store lipid, and lipid accumulation in hepatocytes is toxic. The quantity and the composition of food are relevant factors with regard to fat accumulation in the hepatocytes. Over the past three decades, the food habits of the general population have been changing toward a diet characterized by an increased consumption of fat and carbohydrate.

VLDL: very-low-density lipoprotein

NAFLD is a spectrum of fat-related liver conditions, and hepatic lipid accumulation plays a pivotal role in the pathogenesis and progression of NAFLD. In the jejunum, dietary TGs are hydrolyzed by pancreatic lipase, resulting in the release of fatty acids and monoacylglycerol that in turn are absorbed in the small intestine and utilized to synthesize TG. Subsequently, TG can be synthesized and stored in adipose tissue or metabolized in the liver (64). Dietary TG is packaged into chylomicrons, and hepatic TG is packaged into very-low-density lipoproteins (VLDLs) in order to transport TG to peripheral tissues. Chylomicrons are assembled in the enterocytes during the absorptive

phase (exogenous pathway), whereas VLDL particles are secreted from the liver, particularly during the fasting state (endogenous pathway).

In the exogenous pathway, chylomicrons are formed from the coalescence of dietary TG together with apolipoproteins apoB48, apoAIV, and apoAV. Subsequently, chylomicrons are released into the lymphatic system, and later they acquire apoAI, apoAII, apoC (CI, CII, CIII), and apoE. ApoCIII is an inhibitor of lipoprotein lipase (LPL), and hypertriglyceridemia can be further exacerbated by low activity of LPL or by high level of apoCIII. In the peripheral tissues, TGs from the chylomicrons are hydrolyzed by LPL into free fatty acids, and Petersen et al. (69) have shown that a genetic variation of apoCIII is associated with NAFLD, which suggests that variation in LPL activity may be important in the pathogenesis of NAFLD.

In the endogenous pathway, TGs derived from de novo lipogenesis and circulating nonesterified fatty acids (NEFAs) contribute to form nascent VLDL particles that are secreted from the liver. Nascent VLDL contains only apoB100; subsequently, it acquires apoA (AI, AII, AIV), apoC (CI, CII and CIII), and apoE. TGs from VLDL particles are hydrolyzed by LPL to form VLDL remnants, and then they are internalized in the liver, binding to the apoE receptor. In the liver, VLDL remnants are hydrolyzed by hepatic triglyceride lipase to form low-density lipoprotein (LDL) particles. This overflow from the transport pathway is clinically crucial, as an excessive transport of VLDL remnants to the liver increases LDL production, perhaps contributing to NAFLD. Ceramide and sphingolipids can be transported by VLDL, and in the liver, intestine, and heart, de novo ceramide could be secreted as part of apolipoprotein B–containing lipoproteins; this alteration of lipoprotein structure may affect hepatic degradation of lipoproteins, with consequent intracellular accumulation of lipid (27, 50), thereby potentially predisposing to NAFLD.

The effect of omega-3 fatty acids on TGs primarily involves the suppression of hepatic VLDL apoB production and apoB pool size. Several tracer studies have demonstrated the effects of omega-3 fatty acids on VLDL metabolism. Chan et al. (16) showed a reduction of TG plasma concentration in obese people after six weeks of treatment with high doses (4 g) of fish oil capsules comprising 45% EPA and 39% DHA. This reduction was mainly due to the effects of omega-3 fatty acids on VLDL apoB pool size; the effect of omega-3 fatty acids on VLDL particles was to favor the conversion of VLDL to LDL (16). This effect involves a decrease in triacylglycerol synthesis by 35% and an increase in fatty acid mitochondrial oxidation. In particular, omega-3 fatty acids induce the aggregation of apoB after its secretion from the endoplasmic reticulum (ER). In the Golgi, this aggregate material is oxidized and remains in the cell, where it is susceptible to the autophagic process (63). In a study using cultured hepatocytes, the effects of incubation with palmitic acid, oleic acid, and DHA on ER stress and apoB100 secretion were compared (15). The investigators found that a long period of incubation with oleic acid provoked ER stress. In contrast, a short incubation period with palmitic acid was sufficient to cause the same effect on ER stress. In addition, palmitic acid favored ceramide production. DHA did not induce ER stress at any time. All three fatty acids inhibited the secretion of apoB100, but only DHA induced autophagic degradation. Furthermore, it has been shown that DHA may interfere with *apoCIII* gene transcription, thereby potentially decreasing the negative effect of apoCIII on LPL activity (59).

Fatty acids may have different chain lengths (short, medium, long, and very long) and may be saturated or unsaturated. Fatty acids have different metabolic fates, depending on their chain length and degree of saturation. Short-chain (2–5 carbon atoms) and medium-chain (6–12 carbon atoms) fatty acids are directly absorbed from the intestine into the blood through intestinal branching capillaries. Medium-chain fatty acids can form medium-chain triglycerides that are not incorporated into chylomicrons. Medium-chain fatty acids can be

LPL: lipoprotein lipase

NEFAs: nonesterified fatty acids

rapidly oxidized to form acetyl-CoA in the liver and thus are not stored in adipose tissue (66).

Long-chain (13–21 carbons) and very-long-chain (22 or more carbons) fatty acids are constituents of glycerolipids (mono-, di-, and triglycerides) and sphingolipids with a backbone comprising ceramide. Increased intake of these fatty acids promotes the accrual of long-chain fatty acyl CoAs, diacylglycerol, sphingolipids, and ceramide in tissue lipid deposits. Increased plasma levels of sphingolipids have been implicated in the pathogenesis of obesity, insulin resistance, and NAFLD. Ceramide can be produced by three pathways: (*a*) de novo ceramide synthesis from palmitate and serine, (*b*) sphingomyelin hydrolysis by a neutral sphingomyelinase and acidic sphingomyelinase, and (*c*) ceramide salvage by the catabolism of other complex sphingolipids. A diet rich in glycerolipids and n-6 polyunsaturated fatty acids supplies substrate for de novo ceramide synthesis (26). Sphingomyelinase activity has been found to be increased in the adipose tissue of obese mice fed a high-fat diet, and these mice had increased plasma ceramide levels (6). Ceramide may be implicated in the pathogenesis of NASH (62). Recently, Moles et al. (55) demonstrated that the liver expression of acidic sphingomyelinase is increased in subjects with NASH. These authors found that acidic sphingomyelinase contributes to the pathogenesis of liver fibrosis through activation of hepatic stellate cells (55). Haus et al. (28) have shown that ceramide also induces liver inflammation through activation of nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNF-α), and proinflammatory cytokines that in turn induce ceramide synthesis through activation of plasma membrane enzyme sphingomyelinase. The accumulation of ceramides in the liver increases the production of reactive oxygen species that together with proinflammatory cytokines may contribute to the progression of liver steatosis. Furthermore, ceramide may inhibit insulin action by decreasing phosphorylation and activation of Akt, a protein kinase involved in the regulation of glucose and lipid metabolism, contributing to insulin resistance (28). All of these mechanisms are implicated in the pathogenesis of NAFLD.

Ceramide can be produced in caveolae in response to inflammation. Caveolae are dynamic plasma membrane–located assemblies of cholesterol, sphingolipids (sphingomyelin, ceramide), glycerophospholipids, TNF-α, and interleukin (IL)-1β receptors. In the presence of inflammation, the action of TNF-α and IL-1β can stimulate apoptosis and sphingomyelinase activity, with consequent production of ceramide (23, 45). Ma and colleagues (47) noted that feeding mice omega-3 fatty acids, but not omega-6 fatty acids, altered the caveolae microenvironment and modified membrane lipid composition by increasing phospholipid omega-3 fatty acyl content. This modification positively influences cellular signaling and apoptosis and reduces cholesterol content in caveolae by 46% compared with omega-6 fatty acids (47). Furthermore, the incorporation of DHA into the phospholipid in caveolae inhibits the activity of sphingomyelinase, with a consequent reduction of ceramide production that in turn decreases activity of proinflammatory cytokines TNF-α and IL-1β (60).

High concentrations of glucose and insulin activate glycolytic/lipogenic enzymes such as glucokinase and liver-pyruvate kinase (L-PK). Glucose activates L-PK, which in turn catalyzes the formation of acetyl-CoA from pyruvate, providing citrate for fatty acid synthesis. The metabolism of fatty acids is regulated by several nuclear receptors and transcription factors; the former include the PPAR family, retinoid X receptor α (RXRα), liver X receptor α (LXRα), and hepatic nuclear factor α and γ (HNF4 α and γ); among the latter are sterol regulatory element–binding protein-1 (SREBP-1), carbohydrate response element–binding protein (ChREBP), and max-like factor X (MLX). Notably, these transcription factors have a pivotal role in controlling hepatic carbohydrate and lipid synthesis and oxidation relevant to the pathogenesis of NAFLD (56). PPARs are transcriptional factors that regulate the expression of genes involved in lipids, carbohydrates, and protein metabolism. There

NF-κB: nuclear factor-κB

TNF-α: tumor necrosis factor-α

IL: interleukin

RXRα: retinoid X receptor α

LXRα: liver X receptor α

HNF: hepatic nuclear factor

SREBP-1: sterol regulatory element–binding protein-1

ChREBP: carbohydrate response element–binding protein

MLX: max-like factor X

are three types of PPAR: PPARα, PPARβ/δ, and PPARγ. PPARα is mainly expressed in the liver and is involved in fatty acid uptake, transport, and oxidation. Omega-3 fatty acids are potent ligands for PPARs. The activation of PPARα by omega-3 fatty acids promotes mitochondrial and peroxisomal fatty acid oxidation, decreasing their intracellular accumulation. PPARγ is expressed in liver, muscle, and adipose tissue, and its activation decreases fatty acid synthesis and TG formation. PPARβ/δ is expressed in the liver, adipose tissue, and intestine, and the activation of PPARβ/δ enhances fatty acid oxidation (75, 90).

In the presence of high insulin levels and oxysterols (oxidized derivatives of cholesterol) there is an increased activation of LXRα that stimulates the fatty acid biosynthetic pathway through upregulation of SREBP-1c (18, 32, 65). SREBP-1c is one of the three helix-loop-helix leucine zipper transcription factors (SREBP-1a, SREBP-1c, and SREBP-2) involved in the regulation of enzymes that catalyze lipogenesis, such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and TG synthesis (e.g., glycerol-phosphate acyltransferase). SREBP-1c is expressed in the liver, adipose tissue, and skeletal muscle and is very important in the regulation of lipid metabolism.

High-carbohydrate diets increase hepatic de novo lipogenesis through a multistage process in which (*a*) dietary glucose is transported into the liver by glucose transporter-2, (*b*) dietary glucose is phosphorylated by the enzyme glucokinase and converted by liver-specific pyruvate kinase into acetyl-CoA, (*c*) acetyl-CoA is converted into malonyl-CoA in the presence of insulin that activates acetyl CoA carboxylase, and (*d*) malonyl-CoA is the committed substrate on the fatty acid synthesis pathway. The activity of the glycolytic and lipogenic enzymes is controlled at the transcriptional level by SREBP-1c and ChREBP. Hepatic de novo lipogenesis accounts for 5% of the total lipid production in healthy subjects but can increase to 26% in subjects with NASH (24).

Dietary omega-3 fatty acids have an important metabolic and physiological role through their metabolism and biosynthesis of EPA and DHA. In hepatocytes, omega-3 fatty acids downregulate gene expression of several genes involved in lipogenesis by inhibiting SREBP-1c (82) and upregulate lipid oxidation by activation of PPARα (57), which facilitates fatty acid transfer into the mitochondria. In hepatocytes, both omega-3 and omega-6 fatty acids downregulate the activity of SREBP-1c, depressing its lipogenic function (58, 76). In particular, DHA appears to have a specific effect on SREBP-1c gene expression. Jump et al. (34) have shown that DHA has a unique role in the suppression of the mature nuclear form of SREBP through the inhibition of 26S proteasome by accelerating 26S proteasome–dependent degradation of nuclear SREBP-1 (7, 34).

Increased concentrations of blood glucose stimulate hepatic lipogenesis, activating ChREBP, which is a transcriptional regulator expressed in the liver and modulated by glucose (84). Under basal conditions, ChREBP is phosphorylated and localized in the cytosol of hepatocytes. High glucose concentrations stimulate the glycolytic pathway, resulting in increased production of glyceraldehyde 3-phosphate and fructose-6-P, which results in the formation of xylulose 5-phosphate (Xu5P). Xu5P activates protein phosphatase that dephosphorylates ChREBP, and the protein phosphatase thereby stimulates translocation of ChREBP from the cytosol to the nucleus (35). Dephosphorylated ChREBP moves into the hepatocyte nuclei in the form of a heterodimer ChREBP/MLX. In the nucleus, the heterodimer binds to its response element (ChoRE), thereby activating glycolytic and lipogenic gene expressions that include L-PK, ACC, and FAS (33). HNF4α binds the L-PK element adjacent to the ChoRE for full glucose activation of L-PK gene transcription. SREBP-1c and ChREBP are upregulated in conditions of hyperinsulinemia and hyperglycemia, respectively. Thus, in situations of increased insulin and increased glucose concentrations, such as in type 2 diabetes, liver lipid

ACC: acetyl-CoA carboxylase

FAS: fatty acid synthase

AMPK: adenosine monophosphate-activated protein kinase

accumulation is promoted, potentially affecting NAFLD development. Interestingly, it has been shown that DHA reduces ChREBP activity by reducing ChREBP and MLX nuclear abundance and by interacting with HNF4α and L-PK promoter activity (87), therefore suggesting a novel mechanism by which DHA may act to decrease lipogenesis in NAFLD.

Patients with NAFLD often develop insulin resistance and type 2 diabetes mellitus (81). Moreover, the presence of NAFLD and type 2 diabetes increases the risk of NASH. With normal insulin sensitivity, insulin binds to the insulin receptor substrate protein, resulting in the phosphorylation of tyrosine residues. Tyrosine phosphorylation of insulin receptor substrate-1 and -2 initiates a cascade of events that lead to translocation of glucose transporter-4 into the cell membrane for glucose transport into the cytoplasm (8). With liver fat accumulation, an excess of diacylglycerol in hepatocytes causes insulin resistance by inhibiting the tyrosine kinase activity (72).

Omega-3 fatty acids can favorably affect lipid metabolism and insulin sensitivity by inducing phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) in the liver and in adipose tissue. DHA activates AMPK and stimulates lipid oxidation in hepatocytes, adipocytes, and skeletal muscle, modulating glucose and lipid metabolism. Similarly, EPA-activated AMPK stimulates muscle glucose uptake, improving insulin sensitivity (46).

Adipose tissue has an important role in regulating energy homeostasis and produces several proinflammatory adipokines, such as TNF-α, IL-8, IL-6, IL-1β, and monocyte chemoattractant protein-1, as well as hormones such as leptin, adiponectin, and resistin. Excessive calorie intake may induce defective adipose tissue expansion, leading to adipocyte injury, inflammation, and death; this typically occurs in centrally obese people. In adipocytes overloaded with lipids, insulin fails to suppress hormone-sensitive lipase-mediated lipolysis, increasing release of NEFAs into the circulation. When this occurs, NEFAs are redirected to the liver, where they are oxidized or re-esterified into TG and secreted via VLDL. The aforementioned rise of NEFAs in the circulation combined with insulin resistance caused by excessive calorie intake produces an increase in CD36 fatty acid transporter expression (86).

CD36 is a lipid chaperone for oxidized LDL and NEFAs. In monocytes and macrophages, its expression is regulated by PPARα/γ. CD36 interacts with oxidized LDL and jun N-terminal kinase, triggering adipocyte inflammation. Additionally, the Toll-like receptor (TLR)/CD36 complex stimulates NF-κB, causing adipocyte secretion of adipokines, furthering inflammation (10). Proinflammatory adipokines may cause liver inflammation, and DHA (and to a lesser extent EPA) suppresses PPARα/γ-mediated upregulation of CD36 expression (48).

Adiponectin is a hormone produced by adipose tissue that is decreased in obesity and NAFLD. Adiponectin has a beneficial effect on the liver by decreasing lipid accumulation and by protecting the liver from inflammation and fibrosis. Inflammation of adipose tissue causes decreased production of adiponectin, resulting in a failure to suppress intracellular production of reactive oxygen species, with consequent upregulation of NF-κB. Omega-3 fatty acids can increase levels of adiponectin secondary to PPARγ stimulation (73), thereby potentially decreasing the risk of progression from hepatic steatosis to NASH.

The production of proinflammatory adipokines causes the recruitment of macrophages in adipose tissue, stimulating the production of TNF-α, IL-6, and reactive oxygen species and increasing adipocyte lipolysis. The combined effect increases the flow of fatty acids and cytokines to the liver, causing endoplasmic reticulum stress and activating Kupffer cells. Subsequently, endoplasmic reticulum stress and Kupffer cell activation trigger liver inflammation, potentially promoting development of NASH (20).

Enterohepatic Circulation and Nonalcoholic Fatty Liver Disease

Imbalances in gut microbiota can increase fat absorption and energy harvest, causing liver fat accumulation (31), and in addition to the mechanisms discussed above, recent studies have shown the potential role of the gut microbiota in the pathogenesis and progression of NAFLD.

The liver, biliary tract, intestine, portal venous circulation, colon, systemic circulation, and kidney are all involved in the enterohepatic circulation of bile acids. Bile acid, water, electrolytes, phosphatidylcholine, cholesterol, and bilirubin are all components of bile, an iso-osmotic micellar solution produced by the liver. Bile acid synthesis is important for lipid digestion and absorption, cholesterol catabolism, fat-soluble vitamin absorption, and glucose and energy homeostasis. Bile acids are produced by cholesterol in two pathways: (*a*) a "classic" or natural pathway in which cholesterol is converted to 7-α hydroxycholesterol by a rate-limiting enzyme, cholesterol 7α-hydroxylase (CYP7A1); and (*b*) an "alternate" acidic pathway, in which cholesterol is converted to 27-hydroxy-cholesterol with 27-hydroxylase (21). These two pathways form the primary bile acids, i.e., chenodeoxycholic acid and cholic acid. In the intestine, gut microbiota deconjugate and dehydroxylate primary bile acids to form secondary bile acids, i.e., urodeoxycholic acids, deoxycholic acid, and lithocholic acid. Bile acids are natural ligands for farnesoid X receptor (FXR), a nuclear receptor expressed in the liver, intestine, kidney, and adipose tissue. Chenodeoxycholic acid is the most effective endogenous ligand for FXR (78). CYP7A1 is a rate-limiting enzyme that has a pivotal role in the regulation of bile acid synthesis. CYP7A1 transcription is inhibited by bile acids, steroid hormones, inflammatory cytokines, insulin, and growth factors.

In physiological conditions, insulin stimulates CYP7A1 expression. In contrast, in the presence of insulin resistance that is characteristic of patients with NAFLD, high concentrations of insulin activate SREBP-1c, which inhibits CYP7A1 expression. CYP7A1 expression is also inhibited by increased bile acid synthesis and by an increased bile acid pool size, returning cholesterol to the liver via the enterohepatic circulation (19). Inhibition of CYP7A1 decreases chenodeoxycholic acid production and FXR activation, causing hypercholesterolemia. Moreover, reduced activity of FXR decreases biliary cholesterol content and decreases the expression of hepatic but not intestinal expression of cholesterol transporters (ABCG5/G8) (44). Therefore, inhibition of CYP7A1 leads to accumulation of cholesterol and alteration of enterohepatic circulation, with consequent hepatic lipotoxicity that may have a deleterious impact on the liver in NAFLD. There is evidence that omega-3 fatty acids can affect lipid metabolism not only in the liver but also in the intestine. Omega-3 fatty acids increase mRNA expression of the intestinal cholesterol gene transporter (*Abcg5/8*) and bile acid transporters that promote cholesterol excretion in feces (37).

LXR and FXR are two nuclear receptors involved in cholesterol and bile acid metabolism. The activation of LXR by oxysterols (oxygenated derivatives of cholesterol) promotes the conversion of cholesterol into bile acids that in turn increases hepatic triglyceride synthesis and storage by promoting the expression of SREBP-1c. FXR is activated by bile acids to prevent bile acid accumulation. Moreover, FXR activation decreases hepatic and plasma triglycerides through suppression of the expression of SREBP-1c. Bile acid–activated FXR also induces the expression of apoCII that stimulates LPL activity, and it suppresses the expression of apoCIII (an LPL inhibitor). FXR, via induction of SHP, represses the expression of SREBP-1c and ChREBP, which are responsible for the hepatic de novo lipogenesis (36). Consequently, several potential pathways and mechanisms can be altered to cause a disturbance in liver lipid homeostasis and may be important in the pathogenesis of NAFLD.

FXR: farnesoid X receptor

There is evidence that omega-3 fatty acids act as FXR ligands and regulate FXR to affect lipid metabolism (88). In transgenic mice capable of converting omega-6 fatty acids into omega-3 fatty acids, Kim et al. (39) have demonstrated that endogenously synthesized omega-3 fatty acids have beneficial effects on high-fat-diet-induced NAFLD. In this study, endogenously synthesized omega-3 fatty acids are shown to upregulate genes involved in cholesterol uptake (*Ldl-r*), bile acid synthesis (*Cyp7a1*), and excretion (*Abcg5* and *Abcg8*) (39). Thus, these collective data suggest that omega-3 fatty acids increase primary bile acid synthesis and bile acid excretion from the liver and that increased bile acid synthesis activates FXR, induces the expression of apoCII, and suppresses the expression of apoCIII, thereby suggesting a mechanism of potential benefit by which omega-3 fatty acids may ameliorate NAFLD.

Treatment for Nonalcoholic Fatty Liver Disease

Although weight loss and an improvement of lifestyle through a healthy diet and increased physical activity have beneficial effects on liver fat accumulation, to date there are no licensed treatments for NAFLD.

Several studies have researched the effects of omega-3 fatty acids on subjects with NAFLD/NASH. Capanni et al. (14) tested the effects of 1 g EPA per day in 42 patients with NAFLD for a period of 12 months. Following treatment, there was an improvement in liver fat quantity measured by ultrasound as well as reductions in TGs and liver enzyme in the treatment group compared to the control group (14). Spadaro et al. (80) compared the effects of 2 g per day omega-3 fatty acid supplementation combined with American Heart Association (AHA) diet recommendations versus the effects of AHA diet recommendations alone in patients with NAFLD. After a six-month treatment period, there was an improvement of liver fat content measured with ultrasound, decreased serum levels of TG, alanine aminotransferase, and TNF-α, and an improvement in insulin resistance in the omega-3 fatty acids group compared with the group with only dietary advice (80). Zhu et al. (89) studied the effects of 6 g per day EPA plus DPA and DHA in subjects with NAFLD and hypertriglyceridemia; 144 patients were randomly assigned to treatment or placebo. The study lasted six months, and the authors observed an improvement in liver fat content measured with ultrasound and an improvement in serum levels of TG and liver enzyme in the treatment group compared with the placebo group (89). Tanaka et al. (83) observed a reduction of hepatic steatosis after 12 months with 2.7 g per day EPA supplementation in patients with biopsy-proven NASH. The effects of omega-3 fatty acid treatment in NAFLD were recently summarized in a meta-analysis and systematic review (67). The authors concluded that although there was significant heterogeneity between studies, the pooled data suggested that omega-3 long-chain fatty acid supplementation may decrease liver fat. However, the authors noted that the optimal treatment dose is currently not known and stated that well-designed randomized controlled trials that quantify the magnitude of effect of omega-3 PUFA supplementation on liver fat are needed (67).

CONCLUSIONS

Complex mechanisms operate to regulate hepatic lipid homeostasis in normal physiology, and it is plausible that some or all of these mechanisms are perturbed at least in part in NAFLD. The accumulation of liver lipid occurs as a result of disturbances in several metabolic and inflammatory pathways that are affected by (*a*) diet, (*b*) regulation of de novo lipogenesis, (*c*) adipose tissue function, (*d*) regulation of bile synthesis and excretion, and (*e*) regulation of the enterohepatic circulation.

Currently, only weight loss and increases in physical activity (where appropriate) decrease hepatic fat, and to date no therapy is licensed for NAFLD. Drugs used to treat

hypertriglyceridemia (e.g., omega-3 fatty acids) may contribute to decreased fatty liver deposition and liver inflammation. Omega-3 fatty acids regulate hepatic lipid metabolism through several discrete mechanisms that may be beneficial in NAFLD:

1. activation of PPARs, which in turn increases hepatic fatty acid β-oxidation, apoB100 secretion, and autophagic degradation, causing a reduction in VLDL synthesis;
2. inhibition of SREBP-1c and ChREBP activity, reducing hepatic de novo lipogenesis; and
3. reduction of inflammation by inhibiting NF-κB, inhibition of production of inflammatory cytokines including TNF-α and IL-1β receptors, and reduction of arachidonic acid–derived eicosanoids.

Furthermore, omega-3 fatty acids may have beneficial effects (*a*) on lipid storage by regulating intra-adipocyte lipolysis, (*b*) by downregulating the production of proinflammatory cytokine, and (*c*) by upregulating bile synthesis (see **Figure 3**).

In summary, further research is needed to elucidate the mechanisms of disturbance in lipid metabolism in NAFLD and to understand the

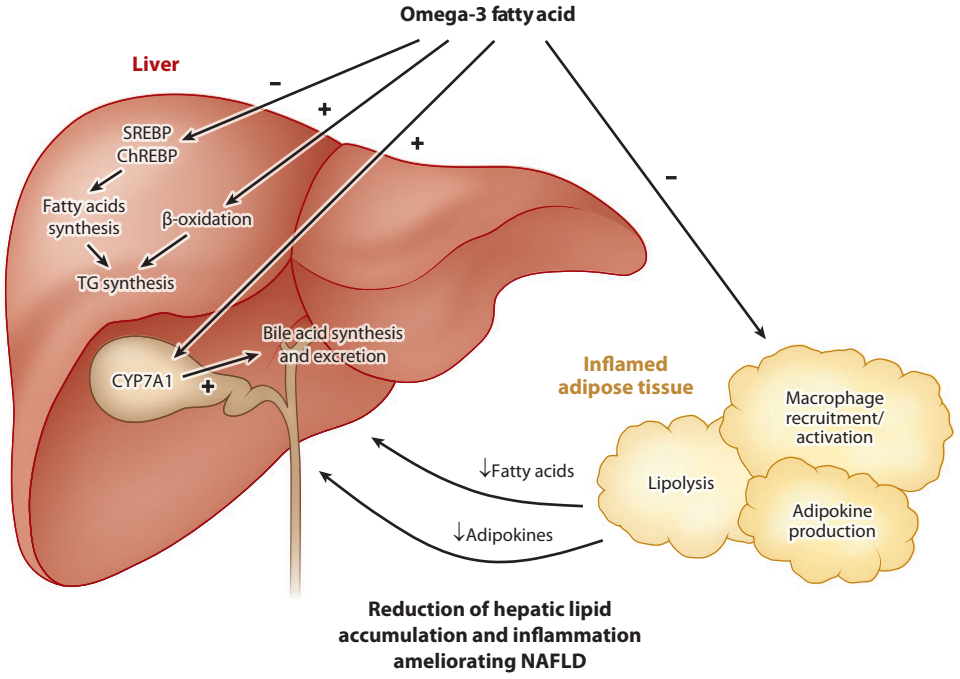

Figure 3

Potential beneficial effects of omega-3 fatty acids in liver and adipose tissue to ameliorate nonalcoholic fatty liver disease. In liver, long-chain omega-3 fatty acids regulate hepatic lipid metabolism by increasing hepatic fatty acid oxidation and inhibition of SREBP-1c and ChREBP activity (nuclear transcription factors that stimulate hepatic de novo lipogenesis). In adipose tissue, omega-3 fatty acids have a potential anti-inflammatory effect by inhibiting macrophage recruitment and activation; decreasing fatty acid release; decreasing adipokine and cytokine secretion, and favorably affecting the enterohepatic circulation. Furthermore, omega-3 fatty acids upregulate CYP7A1 expression, increasing bile acid synthesis and excretion. The potential beneficial consequence of these effects is to ameliorate NAFLD. Abbreviations: ChREBP, carbohydrate regulatory element–binding protein; CYP7A1, cholesterol 7α-hydroxylase; NAFLD, nonalcoholic fatty liver disease; SREBP, sterol regulatory element–binding protein; TG, triglyceride.

relative impact of the functions of other tissues, such as inflamed adipose tissue and the intestine, in NAFLD. New treatments for NAFLD are urgently needed that focus not only on decreasing liver lipid accumulation but also on liver inflammation and fibrosis. Presently, studies testing an effect of omega-3 fatty acids in NAFLD are showing promise, but more work is needed to establish the optimal dose and duration of therapy.

DISCLOSURE STATEMENT

The authors conducted the WELCOME study [Wessex Evaluation of fatty Liver and Cardiovascular markers in NAFLD (nonalcoholic fatty liver disease) with OMacor thErapy] in people with NAFLD. The WELCOME study is a phase IV trial that tested the effects of high-dose purified n-3 long-chain fatty acids (Omacor-Solvay/Abbott/Pronova 4 grams o.d.) on a range of liver and cardio-metabolic outcomes. The trial was completed in 2012 (**http://www.clinicaltrials.gov**; registration number NCT00760513).

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LITERATURE CITED

1. 1977. The nomenclature of lipids. *Eur. J. Biochem.* 79:11–21
2. Araya J, Rodrigo R, Pettinelli P, Araya AV, Poniachik J, Videla LA. 2010. Decreased liver fatty acid Δ-6 and Δ-5 desaturase activity in obese patients. *Obesity* (*Silver Spring*) 18:1460–63
3. Argo CK, Northup PG, Al-Osaimi AM, Caldwell SH. 2009. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. *J. Hepatol.* 51:371–79
4. Bhatia LS, Curzen NP, Calder PC, Byrne CD. 2012. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? *Eur. Heart J.* 33:1190–200
5. Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. 2011. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *Am. J. Clin. Nutr.* 93:950–62
6. Boini KM, Zhang C, Xia M, Poklis JL, Li PL. 2010. Role of sphingolipid mediator ceramide in obesity and renal injury in mice fed a high-fat diet. *J. Pharmacol. Exp. Ther.* 334:839–46
7. Botolin D, Wang Y, Christian B, Jump DB. 2006. Docosahexaenoic acid (22:6,n-3) regulates rat hepatocyte SREBP-1 nuclear abundance by Erk- and 26S proteasome-dependent pathways. *J. Lipid Res.* 47:181–92
8. Bugianesi E, McCullough AJ, Marchesini G. 2005. Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology* 42:987–1000
9. Burdge GC. 2006. Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukot. Essent. Fatty Acids* 75:161–68
10. Cai L, Wang Z, Ji A, Meyer JM, van der Westhuyzen DR. 2012. Scavenger receptor CD36 expression contributes to adipose tissue inflammation and cell death in diet-induced obesity. *PLoS ONE* 7: e36785
11. Calder PC. 2010. Omega-3 fatty acids and inflammatory processes. *Nutrients* 2:355–74
12. Calder PC, Dangour AD, Diekman C, Eilander A, Koletzko B, et al. 2010. Essential fats for future health. Proceedings of the 9th Unilever Nutrition Symposium, 26–27 May 2010. *Eur. J. Clin. Nutr.* 64(Suppl. 4):S1–13
13. Calder PC, Yaqoob P. 2009. Understanding omega-3 polyunsaturated fatty acids. *Postgrad. Med.* 121:148–57

14. Capanni M, Calella F, Biagini MR, Genise S, Raimondi L, et al. 2006. Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study. *Aliment. Pharmacol. Ther.* 23:1143–51
15. Caviglia JM, Gayet C, Ota T, Hernandez-Ono A, Conlon DM, et al. 2011. Different fatty acids inhibit apoB100 secretion by different pathways: unique roles for ER stress, ceramide, and autophagy. *J. Lipid Res.* 52:1636–51
16. Chan DC, Watts GF, Mori TA, Barrett PH, Redgrave TG, Beilin LJ. 2003. Randomized controlled trial of the effect of n-3 fatty acid supplementation on the metabolism of apolipoprotein B-100 and chylomicron remnants in men with visceral obesity. *Am. J. Clin. Nutr.* 77:300–7
17. Charlton MR, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. 2011. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 141:1249–53
18. Chen G, Liang G, Ou J, Goldstein JL, Brown MS. 2004. Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. *Proc. Natl. Acad. Sci. USA* 101:11245–50
19. Chiang JY. 2009. Bile acids: regulation of synthesis. *J. Lipid Res.* 50:1955–66
20. Clària J, González-Périz A, López-Vicario C, Rius B, Titos E. 2011. New insights into the role of macrophages in adipose tissue inflammation and fatty liver disease: modulation by endogenous omega-3 fatty acid-derived lipid mediators. *Front. Immunol.* 2:49
21. Clayton PT. 2011. Disorders of bile acid synthesis. *J. Inherit. Metab. Dis.* 34:593–604
22. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, et al. 2005. Origins and evolution of the Western diet: health implications for the 21st century. *Am. J. Clin. Nutr.* 81:341–54
23. D'Alessio A, Al-Lamki RS, Bradley JR, Pober JS. 2005. Caveolae participate in tumor necrosis factor receptor 1 signaling and internalization in a human endothelial cell line. *Am. J. Pathol.* 166:1273–82
24. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. 2005. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J. Clin. Invest.* 115:1343–51
25. Elmadfa I, Kornsteiner M. 2009. Fats and fatty acid requirements for adults. *Ann. Nutr. Metab.* 55:56–75
26. Frangioudakis G, Garrard J, Raddatz K, Nadler JL, Mitchell TW, Schmitz-Peiffer C. 2010. Saturated- and n-6 polyunsaturated-fat diets each induce ceramide accumulation in mouse skeletal muscle: reversal and improvement of glucose tolerance by lipid metabolism inhibitors. *Endocrinology* 151:4187–96
27. Gatt S, Bierman EL. 1980. Sphingomyelin suppresses the binding and utilization of low density lipoproteins by skin fibroblasts. *J. Biol. Chem.* 255:3371–76
28. Haus JM, Kashyap SR, Kasumov T, Zhang R, Kelly KR, et al. 2009. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* 58: 337–43
29. Holman RT. 1988. George O. Burr and the discovery of essential fatty acids. *J. Nutr.* 118:535–40
30. Holman RT. 1998. The slow discovery of the importance of ω3 essential fatty acids in human health. *J. Nutr.* 128:427–33S
31. Iacono A, Raso GM, Canani RB, Calignano A, Meli R. 2011. Probiotics as an emerging therapeutic strategy to treat NAFLD: focus on molecular and biochemical mechanisms. *J. Nutr. Biochem.* 22: 699–711
32. Ikegami T, Hyogo H, Honda A, Miyazaki T, Tokushige K, et al. 2012. Increased serum liver X receptor ligand oxysterols in patients with non-alcoholic fatty liver disease. *J. Gastroenterol.* 47:1257–66
33. Ishii S, Iizuka K, Miller BC, Uyeda K. 2004. Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription. *Proc. Natl. Acad. Sci. USA* 101:15597–602
34. Jump DB, Botolin D, Wang Y, Xu J, Demeure O, Christian B. 2008. Docosahexaenoic acid (DHA) and hepatic gene transcription. *Chem. Phys. Lipids* 153:3–13
35. Kabashima T, Kawaguchi T, Wadzinski BE, Uyeda K. 2003. Xylulose 5-phosphate mediates glucose-induced lipogenesis by xylulose 5-phosphate-activated protein phosphatase in rat liver. *Proc. Natl. Acad. Sci. USA* 100:5107–12
36. Kalaany NY, Mangelsdorf DJ. 2006. LXRS and FXR: the yin and yang of cholesterol and fat metabolism. *Annu. Rev. Physiol.* 68:159–91

37. Kamisako T, Tanaka Y, Ikeda T, Yamamoto K, Ogawa H. 2012. Dietary fish oil regulates gene expression of cholesterol and bile acid transporters in mice. *Hepatol. Res.* 42:321–26
38. Kaur G, Cameron-Smith D, Garg M, Sinclair AJ. 2011. Docosapentaenoic acid (22:5n-3): a review of its biological effects. *Prog. Lipid Res.* 50:28–34
39. Kim EH, Bae JS, Hahm KB, Cha JY. 2012. Endogenously synthesized n-3 polyunsaturated fatty acids in fat-1 mice ameliorate high-fat diet-induced non-alcoholic fatty liver disease. *Biochem. Pharmacol.* 84:1359–65
40. Kris-Etherton PM, Harris WS, Appel LJ. 2003. Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. *Arterioscler. Thromb. Vasc. Biol.* 23:151–52
41. Le HD, Meisel JA, de Meijer VE, Gura KM, Puder M. 2009. The essentiality of arachidonic acid and docosahexaenoic acid. *Prostaglandins Leukot. Essent. Fatty Acids* 81:165–70
42. Levene AP, Goldin RD. 2012. The epidemiology, pathogenesis and histopathology of fatty liver disease. *Histopathology* 61:141–52
43. Levy BD. 2010. Resolvins and protectins: natural pharmacophores for resolution biology. *Prostaglandins Leukot. Essent. Fatty Acids* 82:327–32
44. Li T, Matozel M, Boehme S, Kong B, Nilsson LM, et al. 2011. Overexpression of cholesterol 7α-hydroxylase promotes hepatic bile acid synthesis and secretion and maintains cholesterol homeostasis. *Hepatology* 53:996–1006
45. Liu P, Anderson RG. 1995. Compartmentalized production of ceramide at the cell surface. *J. Biol. Chem.* 270:27179–85
46. Lorente-Cebrian S, Bustos M, Marti A, Martinez JA, Moreno-Aliaga MJ. 2009. Eicosapentaenoic acid stimulates AMP-activated protein kinase and increases visfatin secretion in cultured murine adipocytes. *Clin. Sci.* (*Lond.*) 117:243–49
47. Ma DW, Seo J, Davidson LA, Callaway ES, Fan YY, et al. 2004. n-3 PUFA alter caveolae lipid composition and resident protein localization in mouse colon. *FASEB J.* 18:1040–42
48. Madonna R, Salerni S, Schiavone D, Glatz JF, Geng YJ, De Caterina R. 2011. Omega-3 fatty acids attenuate constitutive and insulin-induced CD36 expression through a suppression of PPARα/γ activity in microvascular endothelial cells. *Thromb. Haemost.* 106:500–10
49. Marsman HA, Heger M, Kloek JJ, Nienhuis SL, van Werven JR, et al. 2011. Reversal of hepatic steatosis by omega-3 fatty acids measured non-invasively by ^{1}H-magnetic resonance spectroscopy in a rat model. *J. Gastroenterol. Hepatol.* 26:356–63
50. Merrill AH Jr, Lingrell S, Wang E, Nikolova-Karakashian M, Vales TR, Vance DE. 1995. Sphingolipid biosynthesis de novo by rat hepatocytes in culture. Ceramide and sphingomyelin are associated with, but not required for, very low density lipoprotein secretion. *J. Biol. Chem.* 270:13834–41
51. Meyer BJ. 2011. Are we consuming enough long chain omega-3 polyunsaturated fatty acids for optimal health? *Prostaglandins Leukot. Essent. Fatty Acids* 85:275–80
52. Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair AJ, Howe PR. 2003. Dietary intakes and food sources of ω-6 and ω-3 polyunsaturated fatty acids. *Lipids* 38:391–98
53. Mirmiran P, Hosseinpour-Niazi S, Naderi Z, Bahadoran Z, Sadeghi M, Azizi F. 2012. Association between interaction and ratio of ω-3 and ω-6 polyunsaturated fatty acid and the metabolic syndrome in adults. *Nutrition* 28:856–63
54. Molendi-Coste O, Legry V, Leclercq IA. 2011. Why and how meet n-3 PUFA dietary recommendations? *Gastroenterol. Res. Pract.* 2011:364040
55. Moles A, Tarrats N, Morales A, Dominguez M, Bataller R, et al. 2010. Acidic sphingomyelinase controls hepatic stellate cell activation and in vivo liver fibrogenesis. *Am. J. Pathol.* 177:1214–24
56. Musso G, Gambino R, Cassader M. 2009. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). *Prog. Lipid Res.* 48:1–26
57. Neschen S, Morino K, Dong J, Wang-Fischer Y, Cline GW, et al. 2007. n-3 Fatty acids preserve insulin sensitivity in vivo in a peroxisome proliferator-activated receptor-α-dependent manner. *Diabetes* 56:1034–41
58. Novak EM, Keller BO, Innis SM. 2012. Metabolic development in the liver and the implications of the n-3 fatty acid supply. *Am. J. Physiol. Gastrointest. Liver Physiol.* 302:G250–59

59. Olivieri O, Martinelli N, Sandri M, Bassi A, Guarini P, et al. 2005. Apolipoprotein C-III, n-3 polyunsaturated fatty acids, and "insulin-resistant" T-455C *APOC3* gene polymorphism in heart disease patients: example of gene-diet interaction. *Clin. Chem.* 51:360–67
60. Opreanu M, Lydic TA, Reid GE, McSorley KM, Esselman WJ, Busik JV. 2010. Inhibition of cytokine signaling in human retinal endothelial cells through downregulation of sphingomyelinases by docosahexaenoic acid. *Invest. Ophthalmol. Vis. Sci.* 51:3253–63
61. Pachikian BD, Neyrinck AM, Cani PD, Portois L, Deldicque L, et al. 2008. Hepatic steatosis in n-3 fatty acid depleted mice: focus on metabolic alterations related to tissue fatty acid composition. *BMC Physiol.* 8:21
62. Pagadala M, Kasumov T, McCullough AJ, Zein NN, Kirwan JP. 2012. Role of ceramides in nonalcoholic fatty liver disease. *Trends Endocrinol. Metab.* 23:365–71
63. Pan M, Maitin V, Parathath S, Andreo U, Lin SX, et al. 2008. Presecretory oxidation, aggregation, and autophagic destruction of apoprotein-B: a pathway for late-stage quality control. *Proc. Natl. Acad. Sci. USA* 105:5862–67
64. Pan X, Hussain MM. 2012. Gut triglyceride production. *Biochim. Biophys. Acta* 1821:727–35
65. Pannu PS, Allahverdian S, Francis GA. 2013. Oxysterol generation and liver X receptor-dependent reverse cholesterol transport: Not all roads lead to Rome. *Mol. Cell. Endocrinol.* 368:99–107
66. Papamandjaris AA, MacDougall DE, Jones PJ. 1998. Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. *Life Sci.* 62:1203–15
67. Parker HM, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. 2012. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J. Hepatol.* 56: 944–51
68. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. 2012. Health implications of high dietary omega-6 polyunsaturated fatty acids. *J. Nutr. Metab.* 2012:539426
69. Petersen KF, Dufour S, Hariri A, Nelson-Williams C, Foo JN, et al. 2010. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N. Engl. J. Med.* 362:1082–89
70. Petrenya N, Dobrodeeva L, Brustad M, Bichkaeva F, Menshikova E, et al. 2011. Fish consumption and socio-economic factors among residents of Arkhangelsk city and the rural Nenets autonomous area. *Int. J. Circumpolar Health* 70:46–58
71. Phang M, Garg ML, Sinclair AJ. 2009. Inhibition of platelet aggregation by omega-3 polyunsaturated fatty acids is gender specific—redefining platelet response to fish oils. *Prostaglandins Leukot. Essent. Fatty Acids* 81:35–40
72. Postic C, Girard J. 2008. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J. Clin. Invest.* 118:829–38
73. Puglisi MJ, Hasty AH, Saraswathi V. 2011. The role of adipose tissue in mediating the beneficial effects of dietary fish oil. *J. Nutr. Biochem.* 22:101–8
74. Schmitz G, Ecker J. 2008. The opposing effects of n-3 and n-6 fatty acids. *Prog. Lipid Res.* 47:147–55
75. Shapiro H, Tehilla M, Attal-Singer J, Bruck R, Luzzatti R, Singer P. 2011. The therapeutic potential of long-chain omega-3 fatty acids in nonalcoholic fatty liver disease. *Clin. Nutr.* 30:6–19
76. Shearer GC, Savinova OV, Harris WS. 2012. Fish oil—how does it reduce plasma triglycerides? *Biochim. Biophys. Acta* 1821:843–51
77. Simopoulos AP. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp. Biol. Med.* (*Maywood*) 233:674–88
78. Smelt AH. 2010. Triglycerides and gallstone formation. *Clin. Chim. Acta* 411:1625–31
79. Smit LA, Mozaffarian D, Willett W. 2009. Review of fat and fatty acid requirements and criteria for developing dietary guidelines. *Ann. Nutr. Metab.* 55:44–55
80. Spadaro L, Magliocco O, Spampinato D, Piro S, Oliveri C, et al. 2008. Effects of n-3 polyunsaturated fatty acids in subjects with nonalcoholic fatty liver disease. *Dig. Liver Dis.* 40:194–99
81. Sung KC, Jeong WS, Wild SH, Byrne CD. 2012. Combined influence of insulin resistance, overweight/obesity, and fatty liver as risk factors for type 2 diabetes. *Diabetes Care* 35:717–22
82. Takeuchi Y, Yahagi N, Izumida Y, Nishi M, Kubota M, et al. 2010. Polyunsaturated fatty acids selectively suppress sterol regulatory element-binding protein-1 through proteolytic processing and autoloop regulatory circuit. *J. Biol. Chem.* 285:11681–91

83. Tanaka N, Sano K, Horiuchi A, Tanaka E, Kiyosawa K, Aoyama T. 2008. Highly purified eicosapentaenoic acid treatment improves nonalcoholic steatohepatitis. *J. Clin. Gastroenterol.* 42:413–18
84. Uyeda K, Yamashita H, Kawaguchi T. 2002. Carbohydrate responsive element-binding protein (ChREBP): a key regulator of glucose metabolism and fat storage. *Biochem. Pharmacol.* 63:2075–80
85. Whelan J, Rust C. 2006. Innovative dietary sources of n-3 fatty acids. *Annu. Rev. Nutr.* 26:75–103
86. Xu H, Barnes GT, Yang Q, Tan G, Yang D, et al. 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* 112:1821–30
87. Xu J, Christian B, Jump DB. 2006. Regulation of rat hepatic L-pyruvate kinase promoter composition and activity by glucose, n-3 polyunsaturated fatty acids, and peroxisome proliferator-activated receptor-α agonist. *J. Biol. Chem.* 281:18351–62
88. Zhao A, Yu J, Lew JL, Huang L, Wright SD, Cui J. 2004. Polyunsaturated fatty acids are FXR ligands and differentially regulate expression of FXR targets. *DNA Cell Biol.* 23:519–26
89. Zhu FS, Liu S, Chen XM, Huang ZG, Zhang DW. 2008. Effects of n-3 polyunsaturated fatty acids from seal oils on nonalcoholic fatty liver disease associated with hyperlipidemia. *World J. Gastroenterol.* 14:6395–400
90. Zúñiga J, Cancino M, Medina F, Varela P, Vargas R, et al. 2011. n-3 PUFA supplementation triggers PPAR-α activation and PPAR-α /NF-κB interaction: anti-inflammatory implications in liver ischemia-reperfusion injury. *PLoS ONE* 6:e28502

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Errata

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