REVIEW

Potential for Dietary ω-3 Fatty Acids to Prevent Nonalcoholic Fatty Liver Disease and Reduce the Risk of Primary Liver Cancer[1–3]

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) has increased in parallel with central obesity, and its prevalence is anticipated to increase as the obesity epidemic remains unabated. NAFLD is now the most common cause of chronic liver disease in developed countries and is defined as excessive lipid accumulation in the liver, that is, hepatosteatosis. NAFLD ranges in severity from benign fatty liver to nonalcoholic steatohepatitis (NASH), and NASH is characterized by hepatic injury, inflammation, oxidative stress, and fibrosis. NASH can progress to cirrhosis, and cirrhosis is a risk factor for primary hepatocellular carcinoma (HCC). The prevention of NASH will lower the risk of cirrhosis and NASH-associated HCC. Our studies have focused on NASH prevention. We developed a model of NASH by using mice with the LDL cholesterol receptor gene ablated fed the Western diet (WD). The WD induces a NASH phenotype in these mice that is similar to that seen in humans and includes robust induction of hepatic steatosis, inflammation, oxidative stress, and fibrosis. With the use of transcriptomic, lipidomic, and metabolomic approaches, we examined the capacity of 2 dietary ω-3 (n–3) polyunsaturated fatty acids, eicosapentaenoic acid (20:5ω-3; EPA) and docosahexaenoic acid (22:6ω-3; DHA), to prevent WD-induced NASH. Dietary DHA was superior to EPA at attenuating WD-induced changes in plasma lipids and hepatic injury and at reversing WD effects on hepatic metabolism, oxidative stress, and fibrosis. The outcome of these studies suggests that DHA may be useful in preventing NASH and reducing the risk of HCC. *Adv Nutr* 2015;6:694–702.

Keywords: fatty liver disease, liver cancer, inflammation, oxidative stress, fibrosis, metabolomics, ω-3 PUFAs

Introduction

Primary hepatocellular carcinoma (HCC)[4] is the fifth most common human cancer in men and the seventh most common cancer in women in the Western societies, and HCC represents the third most frequent cause of cancer deaths worldwide (1–3). High rates of HCC are seen in Eastern and Southeastern Africa and Asia and lower levels in Western countries. Risk factors for HCC include age and sex (male), hepatitis virus infection (hepatitis B and C viruses), exposure to toxins (aflatoxin), chronic alcohol abuse, cirrhosis, tobacco use, and genetic disorders (hereditary hemochromatosis, α1-antitrypsin deficiency, and primary biliary cirrhosis) (1, 2).

The unabated increase in the incidence of obesity, type 2 diabetes (T2D), and nonalcoholic fatty liver disease (NAFLD) is driving the concern for an increased HCC incidence in Western societies (4) (**Figure 1**). This is because NAFLD can progress to nonalcoholic steatohepatitis (NASH) and cirrhosis; cirrhosis is a risk factor for HCC. Chronic fatty liver disease sets the stage for poorly regulated regeneration of hepatic parenchymal cells resulting from hepatic inflammation, parenchymal cell death, and fibrosis, thus, increasing HCC risk. Current treatment options for HCC are limited to surgery and drugs such as the multi-kinase inhibitor, sorafenib. Because diet is a main driver of NAFLD and NASH progression, our focus was on developing nutritional strategies to prevent NASH. This report focuses on the use of dietary 20–22-carbon ω-3 PUFAs to prevent NASH.

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[4] Abbreviations used: *CD14*, CD14 molecule gene; *Col1A1*, collagen 1A1 gene; DNL, de novo lipogenesis; HCC, hepatocellular carcinoma; *MCP1*, monocyte chemoattractant protein 1 gene; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; *NOX2*, NADPH oxidase 2 gene; Nrf2, nuclear factor (erythroid-derived 2)-like 2; TLR, toll-like receptor; T2D, type 2 diabetes; WD, Western diet; 17,18-DiHETE, 17,18-dihydroxy-eicosatetraenoic acid; 18-HEPE, 18-hydroxy-eicosapentaenoic acid.

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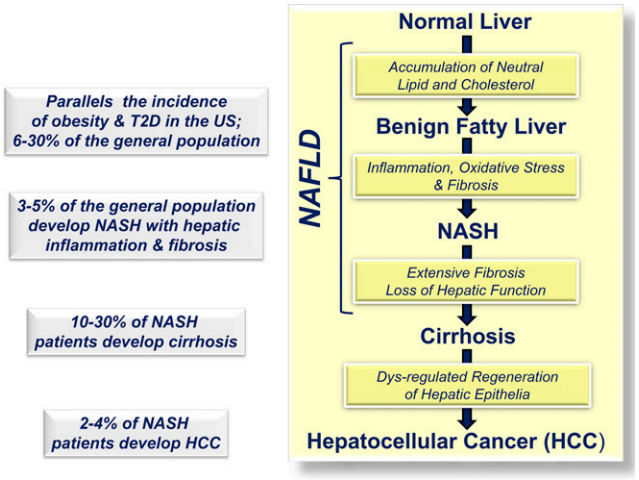

FIGURE 1 Transition from normal liver to primary HCC. HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; T2D, type 2 diabetes.

NAFLD and NASH

Current data from the CDC estimate that nearly 78.6 million obese adults and 12.7 million obese children (aged 2–19 y) are in the United States (5, 6). Obesity is a risk factor for developing NAFLD and NASH. As such, the prevalence of NAFLD and NASH has increased in parallel with the incidence of central obesity in Western societies (7, 8). NAFLD is the most common fatty liver disease in developed countries (9) and is defined as excessive lipid accumulation in the liver, that is, hepatosteatosis (10, 11). NAFLD is the hepatic manifestation of metabolic syndrome (MetS) (12), and MetS risk factors include obesity, elevated plasma TGs and LDL cholesterol, reduced HDL cholesterol, high blood pressure, and fasting hyperglycemia (13). The prevalence of NAFLD in the general population is estimated to range from 6% to 30%, depending on the method of analysis and population studied (14) (Figure 1).

NAFLD ranges from benign hepatosteatosis to NASH (15), which is defined as hepatosteatosis with inflammation and hepatic injury (16). Approximately 30–40% of patients with steatosis develop NASH (17), representing ~3–5% in the general population (14). NAFLD and NASH have high prevalence (≥60%) in the population with T2D (18). Patients undergoing bariatric surgery have NAFLD (93%) or NASH (26%) (19). Patients with NASH have higher mortality rates than patients with NAFLD, and both are higher than in the general population (20–22). Over a 10-y period, cirrhosis and liver-related death occurs in 20% and 12% of patients with NASH, respectively (23). Given the increasing prevalence of NASH and its adverse clinical outcome, NASH is rapidly becoming an important public health burden. NASH can progress to cirrhosis and HCC (8, 17). By the year 2020, cirrhosis resulting from NASH is projected to be the leading cause of liver transplantation in the United States (24).

Multi-Hit Hypotheses for NASH Development

The development of NASH was proposed to follow a multi-hit model (25–27). The first hit involves excessive neutral lipid accumulation in the liver which sensitizes the liver to the second hit (26) (Figure 2). The second hit is characterized by hepatic inflammation, oxidative stress, and hepatic insulin resistance. These events promote hepatic damage that is associated with increased blood concentrations of hepatic enzymes/proteins (alanine aminotransferase, aspartate aminotransferase, C-reactive protein, serum amyloid A1, and plasminogen activator inhibitor-1) (7, 8, 28). This proinflammatory state leads to hepatocellular death and necrosis (necroinflammation), and cell death promotes fibrosis, that is, the third hit. Fibrosis is mediated by activation of hepatic stellate cells and myofibrillar cells; these cells produce extracellular matrix proteins, such as collagen [collagen 1A1 gene, (*Col1A1*)] and smooth muscle α2 actin (29). Dietary (excess fat, cholesterol, glucose, and fructose), metabolic (plasma and hepatic FA profiles, hepatic ceramide, oxidized LDL), endocrine/paracrine (insulin, leptin, adiponectin, and TGF-β), gut (endotoxin, microbial metabolites), and genetic (e.g., patatin-like phospholipase domain containing 3 polymorphisms) factors contribute to NASH progression (30–38).

Hepatosteatosis develops because of an imbalance of hepatic lipid metabolism that leads to the accumulation of hepatic neutral lipids as TGs and diacylglycerols and cholesterol esters. FA sources of hepatic TGs and cholesterol esters include nonesterified FAs mobilized from adipose tissue, de novo lipogenesis (DNL), and the diet via the portal circulation. Hepatic FA oxidation and VLDL assembly and secretion represent 2 pathways for removal of fat from the liver. Hepatosteatosis develops when lipid storage exceeds lipid export and oxidation (39). In humans with NAFLD, ~60% of the FAs appearing in the liver are derived from circulating nonesterified FAs mobilized from adipose tissue; 26% are from DNL and 15% from diet (40). Both hepatic

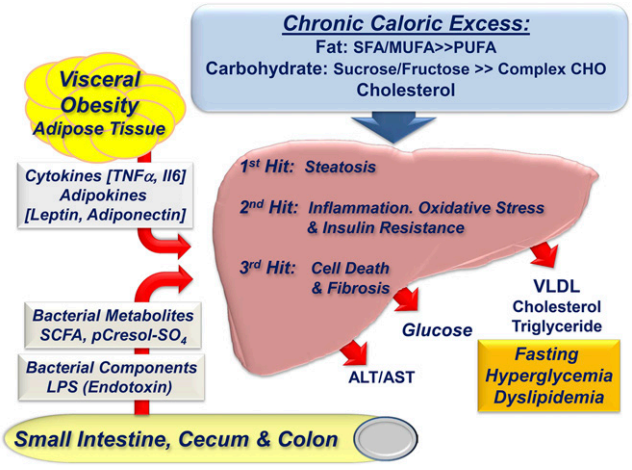

FIGURE 2 Factors contributing to the onset and progression of NASH. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHO, carbohydrate; NASH, nonalcoholic steatohepatitis.

and peripheral insulin resistance also contribute to the disruption of these pathways and to the development of hepatosteatosis (39).

Patients with NASH consume a lower ratio of PUFAs to SFAs than the general population (41, 42). Consumption of a low ratio of ω-3 PUFAs to ω-6 PUFAs is also associated with NAFLD development, whereas increased dietary long-chain ω-3 PUFAs decrease hepatic steatosis (43–45). Mice fed a ω-3 PUFA-deficient diet developed hepatosteatosis and insulin resistance (46). Livers of these mice showed a major decline in α-linolenic acid (18:3ω-3), EPA (20:5ω-3), and DHA (22:6ω-3), but no change in hepatic ω-6 PUFAs, such as linoleic acid (18:2ω-6) or arachidonic acid (20:4ω-6). Depletion of hepatic ω-3 PUFAs lowered FA oxidation, a PPARα-regulated mechanism, and increased DNL and TG accumulation, which are sterol regulatory element binding protein 1, carbohydrate regulatory element binding protein, and max-like factor X-regulated pathways. PPARα, sterol regulatory element binding protein 1, and the carbohydrate regulatory element binding protein/max-like factor X heterodimer are well-established targets of 20–22-carbon ω-3 PUFA control (47). Although *trans*-FA (TFA) consumption is associated with insulin resistance and cardiovascular disease, the impact of TFA consumption on NAFLD in humans is less clear (48). Studies that used mice suggest that TFA consumption is associated with hepatic steatosis and injury (49, 50). Thus, reduced hepatic ω-3 PUFAs and increased concentrations of TFAs may account for changes in hepatic lipid metabolism that promote NAFLD.

Excess dietary cholesterol contributes to NASH (51) by promoting hepatic inflammation (32, 52–54). In the global ablation of the LDL receptor gene ($Ldlr^{-/-}$) mouse model, high-fat/high-cholesterol diets promote NASH (55). Kupffer cells, that is, resident hepatic macrophage, become engorged with oxidized-LDL which induces inflammatory cytokine secretion. These locally secreted cytokines act on neighboring hepatic cells to promote a proinflammatory state, leading to cell injury. Kupffer cells also secrete chemokines [monocyte chemoattractant protein-1 gene, (*MCP1*)] that recruit monocytes to the liver, further amplifying hepatic inflammation. Controlling hepatic inflammation is an attractive target for NASH management and therapy.

Excessive consumption of simple sugar was implicated in hepatosteatosis and NASH progression. Over the past 30 y there was a dramatic increase in obesity and NAFLD in the United States. Although total fat consumption has remained steady, carbohydrate and total caloric intake have increased (56–60). As such, elevated carbohydrate and specifically fructose consumption were linked to NAFLD and NASH progression (61–63). The liver expresses the fructose-specific transporter (glucose transporter 5 gene). Moreover, the liver metabolizes up to 70% of dietary fructose (62, 63), and fructose metabolism is independent of insulin regulation. Compared with glucose, fructose more readily enters the pathways for DNL and TG synthesis. Fructose promotes all aspects of MetS, including hepatosteatosis, insulin resistance, dyslipidemia, hyperglycemia, obesity, and hypertension. In contrast to fructose, hepatic glucose metabolism is well regulated by insulin in healthy individuals, and glucose is converted to glycogen for storage. Excess glucose consumption does not promote hepatosteatosis as aggressively as excess fructose consumption. Fructose also affects several biochemical events that exacerbate NASH development, including formation of advanced glycation end-products and reactive oxygen species (64–67).

Development of Mouse Models of NASH

Several mouse models of NAFLD and NASH were developed. Four such models include the genetic models (*ob/ob* and *db/db* mice), a dietary model (methionine-choline–deficient diets), and chemically induced model (intraperitoneal carbon tetrachloride) (68, 69). These models recapitulate some aspects of human NAFLD/NASH but not other aspects of the disease. $Ldlr^{-/-}$ mice develop hypercholesteremia because of elevated plasma VLDL and LDL when fed a high-cholesterol diet (70). Although $Ldlr^{-/-}$ mice were used to study atherosclerosis, we and others observed that when $Ldlr^{-/-}$ mice are fed a high-fat/high-cholesterol diet, such as the Western diet (WD), mice develop a NASH phenotype similar to that seen in humans (32, 36, 54, 71–74). Because humans and $Ldlr^{-/-}$ mice develop NAFLD and NASH in a context of obesity and insulin resistance, these mice appear to be a useful preclinical model to investigate the development, progression, and remission of NASH.

The WD (Research Diets; D12079B) used in our studies is moderately high in saturated and *trans*-fat (41% total calories), sucrose (30% total calories), and cholesterol (0.15 g %, wt:wt) and is similar to the fast-food diet (75) and human diets linked to obesity in the United States (76, 77). Both the WD and fast-food mouse models induced a NASH phenotype that recapitulates many of the clinical features of human NASH with MetS, including dyslipidemia, hyperglycemia, hepatosteatosis, hepatic damage (plasma alanine aminotransferase and aspartate aminotransferase), hepatocyte ballooning, induction of hepatic markers of inflammation (*MCP1*), oxidative stress [NADPH oxidase 2 gene (*NOX2*) and other *NOX* components], and fibrosis (TGF-β1 gene, *proCol1A1*, tissue inhibitor of metalloprotease-1 gene) (54, 73, 75, 78–80) (**Figure 3**). Moreover, NASH is associated with a major enrichment of both plasma and liver with SFAs and MUFAs and depletion of hepatic ω-3 PUFAs (54, 73, 78). The development of this phenotype was attributed to a diet high in saturated and *trans*-fat, sucrose, and cholesterol (32, 52–55, 62, 71–74, 81, 82).

Potential for Dietary 20–22-Carbon ω-3 PUFAs to Prevent NASH

20–22-Carbon ω-3 PUFAs are pleiotropic regulators of cell function; they have well-established effects on membrane structure, cell signaling, gene expression, lipid and carbohydrate metabolism, and inflammation (47). As such, these FAs appear to be an ideal bioactive nutrient to combat NASH. A meta-analysis of 9 clinical studies indicated that dietary supplementation with 20–22-carbon ω-3 PUFAs

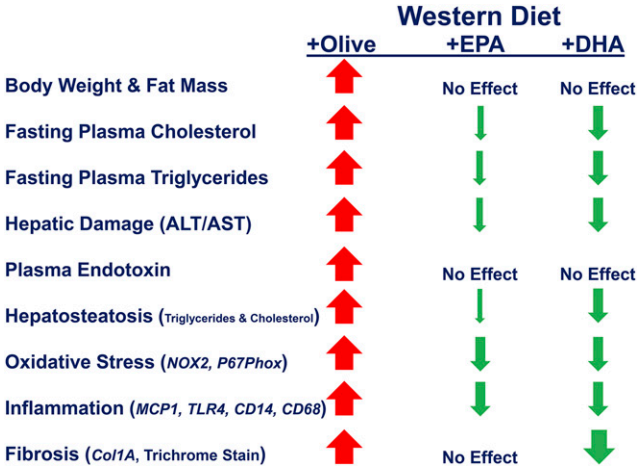

FIGURE 3 Effects of the Western diet and 20–22-carbon ω-3 PUFAs on the prevention of NASH *Ldlr*$^{-/-}$ mice. The size of the arrow indicates effect size. No effect indicates no changes from WD + olive oil-fed mice. Olive oil was added to the WD to keep all diets isocaloric. ALT, alanine aminotransferase; AST, aspartate aminotransferase; *CD14*, CD14 molecule gene; *CD68*, CD68 molecule gene; *Col1A*, collagen 1A1 gene; *Ldlr*$^{-/-}$, global ablation of the LDL receptor gene; MCP1, monocyte chemoattractant protein-1; NASH, nonalcoholic steatohepatitis; *NOX2*, NADPH oxidase 2 gene; P67Phox, 67 kDa neutrophil cytosolic factor 2; *TLR4*, toll-like receptor 4 gene; WD, Western diet.

decreased liver fat (83), and clinical trials suggest 20–22-carbon ω-3 PUFAs may lower liver fat in children and adults with NAFLD (84–89). Of 235 clinical trials that assessed NASH and NASH therapies (90), 23 trials used 20–22-carbon ω-3 PUFAs as a treatment strategy. In most trials, diets were supplemented with fish oil or a combination of EPA + DHA; few studies used EPA or DHA alone.

Preclinical assessment of the efficacy of ω-3 PUFA supplementation to prevent NASH in* Ldlr$^{-/-}$ *mice. Diets supplemented with fish oil, EPA, or DHA prevent high-fat diet-induced NASH to varying degrees (47, 54, 73, 78). The amount of EPA and DHA in these high-fat diets was at ~2% of total calories. This dose of 20–22-carbon ω-3 PUFAs is comparable with the dose consumed by patients taking Lovaza (GlaxoSmithKline) for the treatment of dyslipidemia (91). Humans who consumed EPA + DHA ethyl esters (4 g/d for 12 wk) showed increased plasma EPA + DHA from 5.5 mol% before treatment to 16.2 mol% after treatment (92). Supplementing human diets with a DHA-enriched fish oil (6 g/d for 8 wk) increased plasma DHA from 4 mol% before treatment to 8 mol% after treatment (93, 94). Plasma concentrations of DHA and total 20–22-carbon ω-3 PUFAs [EPA, docosapentaenoic acid (22:5ω-3), and DHA] in *Ldlr*$^{-/-}$ mice fed a WD for 16 wk was 4.3 and 6.7 mol %, respectively. Feeding *Ldlr*$^{-/-}$ mice a WD that contained DHA (at 2% total calories) for 16 wk increased plasma DHA and total 20–22-carbon ω-3 PUFAs to 9 and 15.2 mol%, respectively. Our protocol for 20–22-carbon ω-3 PUFA supplementation of diets yields a change in blood 20–22-carbon ω-3 PUFAs that is comparable with that seen in humans who consumed 4–6 g 20–22-carbon ω-3 PUFAs/d.

Dietary ω-3 PUFAs do not prevent WD-induced systemic inflammation. Systemic inflammation is a main driver of NASH. Inflammatory signals that affect NASH progression include gut-derived microbial products (e.g., endotoxin/LPS, oxidized LDL (34, 55, 80, 95), adipokines (leptin and adiponectin) and cytokines (TNFα) (96), and products from hepatocellular death (27, 97) (Figure 2). Supplementation of the WD with either EPA or DHA does not attenuate WD-induced endotoxinemia (78). The appearance of endotoxin in the plasma of WD-fed *Ldlr*$^{-/-}$ mice (98) may represent a problem with gut physiology such as microbial overgrowth, increased gut permeability (leaky gut), or cotransport of microbial lipids with chylomicron (34, 98–100). A link between the gut microbiome and NAFLD was established (34, 101, 102).

ω-3 PUFAs attenuate hepatic inflammation. Despite the absence of an effect of 20–22-carbon ω-3 PUFAs on systemic inflammation markers, such as endotoxin, gene expression analyses showed that DHA was more effective than EPA at attenuating WD-induced expression of hepatic toll-like receptor gene (*TLR*) subtypes (*TLR2, TLR4, TLR9*), CD14 molecule gene (*CD14*; binds endotoxin), downstream targets of TLRs, such as NF-κB (p50 subunit) nuclear abundance and downstream targets of NF-κB such as chemokines (*MCP1*), cytokines (IL-1β gene), inflammasome components (nucleotide-binding domain, leucine-rich containing family, pyrin domain containing protein 3 gene), and oxidative stress (*NOX2*, and its subunits) markers (73, 78). These studies suggest that EPA and DHA attenuate the hepatic (cellular) response to plasma inflammatory factors by downregulating key cellular mediators of inflammation, such as *TLRs, CD14* (binds LPS, effect on *CD14* mRNA and protein), NF-κB-p50 nuclear abundance.

ω-3 PUFAs have selective effects on hepatic oxidative stress. Hepatic oxidative stress increases with NASH and is reflected by a substantial increase in gene expression and metabolite markers of oxidative stress that appear in liver and urine (54, 73). A response to increased oxidative stress is the induction of nuclear factor (erythroid-derived 2)-like 2 (*Nrf2*), a key transcription factor involved in the antioxidant response (78). Nrf2 regulates the expression of multiple transcripts linked to the antioxidant stress response, such as hemeoxygenase 1 and glutathione *S*-transferase α 1 genes and several *NOX* subunits. Adding EPA or DHA to the WD did not prevent the WD-mediated increase in hepatic nuclear content of *Nrf2* or expression of hemeoxygenase 1 or glutathione *S*-transferase α 1 gene. The EPA- and DHA-containing diets, however, significantly lowered WD-mediated induction of multiple NOX subunits (*Nox2*, *P22phox*, *P40phox*, and *P67phox*) (73). *NOX* subtypes are a main source of superoxide and hydrogen peroxide. As such, the NOX pathway is a main target of WD and 20–22-carbon ω-3 PUFAs.

ω-3 PUFAs attenuate hepatic fibrosis. Hepatic fibrosis (scarring) develops as a result of cell death and activation of hepatic stellate cells and myofibrillar cells to produce extracellular matrix proteins. Key regulators of fibrosis include TGF-β, connective tissue growth factor, platelet-derived growth factor, NOX, inflammatory mediators (endotoxin, TLR agonist), and leptin (38, 80, 103). A fibrotic liver can progress to a cirrhotic liver (Figure 1), and 90% of HCCs arise from cirrhotic livers (104).

Addition of DHA to the WD attenuated the WD-mediated fibrosis as quantified by suppression of expression of *Col1A1*, tissue inhibitor of metalloprotease 1 gene, TGF-β1 gene, plasminogen activated inhibitor-1 gene, and staining of liver for fibrosis with the use of trichrome, a collagen stain (54, 73). Interestingly, EPA did not prevent WD-induced fibrosis. On the basis of these studies, DHA is the preferred ω-3 PUFA to prevent NASH-associated fibrosis.

WD and 20–22-Carbon ω-3 PUFAs affect all major hepatic metabolic pathways. The impact of the WD and 20–22-carbon ω-3 PUFAs on liver metabolism was studied by using a global nontargeted metabolomic approach. The analysis identified 320 known biochemicals (78). Compared with mice fed an unpurified diet, both the WD + olive oil- and WD + DHA-containing diets significantly affected the abundance of metabolites in all major hepatic metabolic pathways, including amino acids and peptides, carbohydrate and energy, lipid, nucleotide, and vitamins and cofactors. Our studies have identified gene expression and metabolite signatures for NASH (73, 78). The gene expression signature for NASH includes increased expression of chemokines (*MCP1*), Kupffer cell surface marker (CD68 molecule gene), TLRs and their components (*TLR4, CD14*), enzymes involved in oxidative stress *(NOX2*), stearoyl CoA desaturase 1 gene, and collagen (*Col1A1*). The metabolomic signature for NASH includes increased hepatic content of palmitoylsphingomyelin, MUFAs (16:1ω-7, 18:1ω-7, and 18:1ω-9), α-tocopherol (vitamin E), 5-methyl tetrahydrofolate and decreased hepatic content of EPA, DHA, and oxidized lipids derived from EPA, specifically 18-hydroxyeicosapentaenoic acid (18-HEPE) and 17,18-dihydroxyeicosatetraenoic acid (17,18-DiHETE). A volcano plot of the metabolomic and gene expression data illustrates the impact of diet on the hepatic amount of these molecules (**Figure 4**). The metabolites and mRNAs that comprise the metabolomic and gene expression signature were changed dramatically by the WD + olive oil diet, compared with mice fed the unpurified diet. These changes were reversed in mice fed the WD + DHA diet.

The oxidized lipids identified in these studies are generated by enzymatic and nonenzymatic processes. 18-HEPE is a resolvin E1 precursor, and resolvins are anti-inflammatory oxidation products of EPA (106). 17,18-DiHETE is an oxidized lipid generated first by cytochrome P450 2C-catalyzed formation of 17,18-epoxy-eicosatetraenoic acid from EPA; this epoxy FA is converted to the di-hydroxy FA by a epoxide hydrolase to form 17,18-DiHETE. The metabolomic analysis did not detect the 17,18- epoxy-eicosatetraenoic

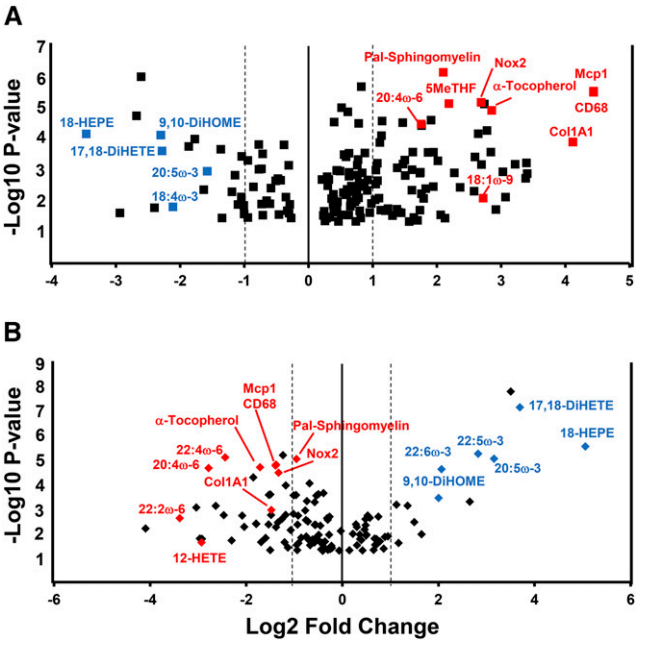

FIGURE 4 Volcano plots of WD effects on hepatic metabolites. A metabolomic and transcriptomic analysis was performed as described (78). More than 300 hepatic metabolites and 6 mRNA markers of NASH were examined with the use of MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/MetaboAnalyst/) (105). The outcome of this analysis provided a volcano plot. Results are plotted as log2 fold change vs. log10 *P* value. Several metabolites and RNA transcripts are labeled to illustrate the impact of diet on hepatic abundance of these molecules. (A) The comparison of hepatic molecules from unpurified diet-fed compared with WD + olive oil-fed *Ldlr*$^{-/-}$ mice. (B) The comparison of hepatic molecules from WD + olive oil-fed mice compared with WD + DHA-fed *Ldlr*$^{-/-}$ mice. CD68, CD68 molecule; Col1A1, collagen 1A1; *Ldlr*$^{-/-}$, global ablation of the LDL receptor gene; Mcp1, monocyte chemoattractant protein 1; NASH, nonalcoholic steatohepatitis; Nox2, NADPH oxidase 2; WD, Western diet; 5-MeTHF, 5-methyl tetrahydrofolate; 9,10-DiHOME, 9,10-dihydroxy-12Z-octadecenoic acid; 17,18-DiHETE, 17,18-dihydroxy-eicosatetraenoic acid; 18-HEPE, 18-hydroxy-eicosapentaenoic acid.

acid, suggesting that this lipid does not accumulate as a non-esterified lipid. Compared with mice fed the unpurified diet, WD + olive oil-fed mice have >60% reduction in hepatic content of 18-HEPE and 17,18-DiHETE. Compared with WD + olive oil-fed mice hepatic amounts of 18-HEPE and 17,18-DiHETE increased ≥40-fold in mice fed the WD that contained EPA or DHA. These dramatic changes in oxidized derivatives of EPA are inversely associated with the severity of NASH. A recent report suggest the Cyp450 epoxygenase pathway may play a key role in regulating hepatic inflammation in fatty liver disease (107). As such, the generation of these oxidized ω-3 PUFAs may be hepatoprotective.

Can ω-3 PUFAs Be Used to Treat Human NASH?

Therapeutic strategies for human NASH start with lifestyle management (diet and exercise) and treating the comorbidities associated with NASH, that is, obesity, T2D, and

dyslipidemia. The best strategy for managing NASH, however, has not been established (108). Some clinical approaches to manage NASH included *1*) reduce overall body weight through diet management, exercise, or bariatric surgery; *2*) pharmaceutical and dietary supplements, that is, metformin, fibrates, thiazolidinediones, statins, ω-3 PUFAs; *3*) suppress inflammation with the use of TLR modifiers or ω-3 PUFAs; and *4*) suppress oxidative stress with the use of vitamin E, silybin, and other antioxidants (84, 109–114). Therapeutic regulators of fibrosis, however, are less well defined (80, 115).

Several clinical trials have reported that ω-3 PUFAs lower hepatic fat in obese children and adults with NAFLD (84–89, 116, 117), whereas others report that fish oil (116) and EPA-ethyl esters (117) do not attenuate the histologic features of the disease, such as fibrosis. As such, human studies with the use of ω-3 PUFAs to treat NAFLD/NASH have yielded mixed results.

The *Ldlr*$^{-/-}$ mouse studies described in the sections above suggest that ω-3 PUFAs may be an attractive dietary supplement to combat NAFLD and NASH, with the added benefit of preventing NASH-associated HCC. These FAs have well-defined effects on hepatic lipid metabolism and inflammation (47, 118) and more recently hepatic fibrosis (54, 73, 90). Although several human studies have provided evidence in support of using supplemental ω-3 PUFAs to treat NAFLD (84–89, 116, 117), some studies suggest there may be limitations to the use of ω-3 PUFAs to treat NASH (116, 117). For example, in a recent double-blind, placebo-controlled trial (89), NAFLD patients received placebo or Lovaza (GlaxoSmithKline) at 4 g/d (~50:50 mix of EPA- and DHA-ethyl esters) for 15–18 mo. Compared with the placebo-treated group, the Lovaza-treated group showed a substantial reduction in liver fat without a substantial reduction in fibrosis scores.

Because DHA attenuates fibrosis in 2 separate rodent models of liver injury, that is, WD-induced fibrosis in mice and bile duct ligation-induced fibrosis in rats (54, 73, 119), we speculate that failure of 20–22-carbon ω-3 PUFAs to decrease hepatic fibrosis in humans may be explained by study design. Likely explanations include the type and amount of ω-3 PUFAs used in the trial. Our studies established that DHA is more effective than EPA at attenuating the onset and progression of NASH (73). Human studies, however, have examined the impact of ω-3 PUFAs on patients with preexisting disease (84–89, 116, 117). We are unaware of preclinical rodent studies that have assessed the impact of ω-3 PUFAs to promote remission or regression of NASH or hepatic fibrosis. As such, more preclinical studies are required to establish the capacity of ω-3 PUFAs to attenuate NASH at various stages in the disease process.

Conclusions and Key Unanswered Questions

To date, several human studies have indicated that ω-3 PUFAs may be useful in reducing liver fat in obese patients with NAFLD. Moreover, preclinical studies in mice have established that DHA can prevent NASH and NASH-associated fibrosis. It remains unclear whether dietary ω-3 PUFAs have the capacity to reverse the NASH, cirrhosis, or HCC phenotypes once these diseases are established. Equally important is defining the molecular mechanisms for DHA control of hepatic fibrosis. Finally, changes in hepatic EPA and DHA content significantly affect oxidized lipids derived from ω-3 and ω-6 PUFAs. These oxidized lipids likely play a role in inflammation and will affect the onset and progression of NASH. Whether these oxidized lipids affect the development of NASH, cirrhosis, or HCC remains to be determined.

Acknowledgments

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