Omega-3 fatty acids and metabolic partitioning of fatty acids within the liver in the context of non-alcoholic fatty liver disease

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Abstract

Purpose of review: Non-alcoholic fatty liver disease (NAFLD) is now the most prevalent form of liver disease globally, affecting about 25% of the world's adult population. It is more common in those living with obesity, where it may affect as many as 80% of individuals. The aim of this article is to describe recent human studies evaluating the influence of omega-3 fatty acids on *de novo* lipogenesis (DNL) and hepatic fatty acid partitioning between incorporation into triacylglycerols (TAGs) and β-oxidation, to discuss the relevance of these effects in the context of NAFLD, and to provide an overview of the mechanisms that might be involved.

Recent findings: The omega-3 fatty acids EPA and DHA decrease hepatic DNL and partition fatty acids away from TAG synthesis and towards β-oxidation. EPA and DHA affect multiple hepatic transcription factors resulting in down-regulation of the DNL pathway and upregulation of β-oxidation. The net result is decreased accumulation of hepatic TAG and lowering of circulating TAG concentrations. Human trials demonstrate that EPA and DHA can decrease liver fat in patients with NAFLD.

Summary: Increased intake of EPA and DHA may reduce the likelihood of hepatic TAG accumulation and could be used to reduce liver fat in patients with NAFLD.

Key words: Triglyceride, De novo lipogenesis, Beta-oxidation, Liver, Omega-3, Fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered to be the hepatic manifestation of the metabolic syndrome. NAFLD covers the spectrum of liver disease from simple steatosis to hepatocellular carcinoma [1*, 2*]. NAFLD is now the most prevalent form of liver disease globally and is estimated to affect about 25% of the world's adult population, although it is much more common in those living with obesity, where it may affect as many as 80% of individuals. Over the last few years experts have sought for a terminology that more accurately reflects the pathogenesis of the disease and there has been discussion that the term NAFLD does not reflect current knowledge, and metabolic (dysfunction) associated fatty liver disease (MAFLD) has been suggested as a more appropriate overarching term [3*, 4*, 5]. Nevertheless, in this article, the term NAFLD will be retained since that is the term used in all human studies that are discussed.

Hepatic steatosis ("fatty liver"), the first stage in the NAFLD spectrum, involves accumulation of triacylglycerols (TAGs) within hepatocytes. It is defined histologically as the presence of TAGs in > 5% of hepatocytes; when magnetic resonance imaging or spectroscopy is used, hepatic steatosis is defined as hepatic fat > 5.6%. Obesity and type-2 diabetes are major risk factors for the development of NAFLD [6*] and, in turn, NAFLD increases the risk of cardiovascular disease [7*, 8*], as well as other diseases including chronic kidney disease [9*].

The hepatic accumulation of fat represents an imbalance between the input and output of fatty acids by the liver. Fatty acid input can be as pre-formed fatty acids entering the liver (e.g. from circulating non-esterified fatty acids (NEFAs) or from TAGs in lipoprotein remnants) or from fatty acids formed by the *de novo* fatty acid synthesis pathway, typically using glucose or fructose as the substrate (Figure 1). Possible fates of hepatic fatty acids are esterification into TAGs that can be stored intracellularly or incorporated into very low density lipoproteins (VLDLs), or β-oxidation to yield carbon dioxide or ketone bodies (Figure 1) [10, 11*]. VLDLs are secreted by hepatocytes and act to deliver fatty acids to extrahepatic tissues such as adipose tissue and skeletal muscle. Hence, the partitioning of fatty acids between incorporation into TAGs and β-oxidation is likely to be an important determinant of hepatic TAG accumulation, and also of plasma/serum TAG concentration. Stable isotope tracer techniques have been used to assess the contribution of different fatty acid sources to hepatic TAGs. For example, Donnelly et al. [12] reported that the contributions of circulating NEFAs, *de novo* lipogenesis (DNL) and dietary fatty acids to hepatic TAGs were 59, 26 and 15%

respectively, although these contributions are likely to be affected by obesity, insulin resistance and diet as reviewed elsewhere [10, 11*].

The marine omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well known to lower fasting plasma/serum TAG concentrations when used together [13*] or individually [14, 15*]. A recent meta-analysis of 110 randomised controlled trials of EPA and DHA, most often used in combination, identified a mean difference of -0.368 mmol/L (95% confidence interval -0.427, -0.309) between omega-3 fatty acids and placebo [16]. This might represent a 10 to 40% reduction in plasma/serum TAG concentration. As a result of this action, these fatty acids have been recommended to be used for treatment of hypertriglyceridemia [17]. The TAG-lowering effect of EPA and DHA suggests that they might influence the partitioning of hepatic fatty acids towards β-oxidation and away from incorporation into TAGs [18], hence reducing hepatic VLDL-TAG secretion and so lowering circulating TAG concentrations. The aim of this article is to describe recent human studies evaluating the influence of omega-3 fatty acids on DNL and hepatic fatty acid partitioning, to discuss the relevance of this effect in the context of NAFLD, and to provide an overview of the mechanisms that might be involved.

Effect of omega-3 fatty acids on DNL and hepatic fatty acid partitioning in humans

Studies report that supplemental EPA+DHA increase whole body fat oxidation in healthy community-dwelling older females [19] and in patients with type-2 diabetes [20], as assessed using indirect calorimetry, although a study in healthy young males did not see this effect [21]. DNL and partitioning of fatty acids were studied in 16 patients with NAFLD who were involved in a RCT of high dose omega-3 fatty acids (~3.5 g EPA+DHA daily for 15 to 18 months versus olive oil as placebo) [22]; these patients were a subgroup of those studied in the full trial [23]. A two-step hyperinsulinaemic euglycaemic clamp with a deuterated glucose (6,6 $^{2}H_{2}$ glucose) infusion was used to assess hepatic and peripheral insulin sensitivity. Approximately 2 weeks after the assessment of insulin sensitivity, fasting hepatic DNL was assessed. Patients consumed deuterated water ($^{2}H_{2}O$; 3 g/kg body water) the evening before the study day to achieve a body water enrichment of 0.3%. The next morning they consumed a standard test meal containing 200 mg of [U^{13}C]palmitic acid to trace the fate of dietary fatty acids. Serial blood and breath samples were taken throughout the 5 hr postprandial period. Production of ketone bodies derived from the oxidation of dietary [U^{13}C]palmitate was assessed by measuring the ^{13}C enrichment of plasma β-hydroxybutyrate. Hepatic DNL was

assessed by measuring the incorporation of deuterium from $^{2}H_2O$ in plasma water into VLDL-TAG palmitate. These measures were all made prior to and after the omega-3 fatty acid or placebo intervention. Patients were separated into those with < 2% (n = 7) or ≥ 2% (n = 9) enrichment in erythrocyte DHA over the course of the intervention period. In the < 2% group, mean erythrocyte DHA at the start and end of the intervention was 4.62% and 4.81% of total fatty acids, respectively. In the ≥ 2% group, mean erythrocyte DHA at the start and end of the intervention was 3.68% and 7.08% of total fatty acids, respectively. Fasting plasma TAG and VLDL-TAG concentrations were significantly decreased by 0.6 mmol/ L and 0.7 mmol/L, respectively, in the DHA ≥ 2% group, while concentrations remained unchanged in the DHA < 2% group. The changes in the ≥ 2% DHA group were 28% and 52% decreases from study entry, respectively, consistent with the well-described TAG-lowering effect of EPA ad DHA. The absolute concentration of VLDL-TAG derived from DNL decreased by 50% in the DHA ≥ 2% group between baseline and end of study but there was no change in the DHA < 2% group. The appearance of [U^{13}C]palmitate in VLDL-TAG significantly decreased (by 50%) and the incorporation of ^{13}C into plasma β-hydroxybutyrate significantly increased (by 6-fold) in the DHA ≥ 2% group but neither changed in the DHA < 2% group. These observations indicate that increased DHA status (as indicated by erythrocyte DHA content) is associated with decreased DNL and increased partitioning of hepatic fatty acids away from esterification into TAGs and towards β-oxidation in patients with NAFLD. Hepatic insulin sensitivity also significantly increased in the DHA ≥ 2% group.

In another study, 38 healthy adult males consumed high dose omega-3 fatty acids (~3.5 g EPA+DHA daily) or olive oil as placebo for 8 weeks [24*]. Similar approaches to those used by Hodson et al. [22] were used to monitor DNL and β-oxidation. In addition, indirect calorimetry was performed in the fasted state and 2 hours after consuming the test meal in order to determine whole-body CO_2 production, whole-body respiratory exchange ratio and substrate utilisation rates. Furthermore, breath samples were collected over 6 hours to measure expired $^{13}CO_2$ enrichment (from the [U^{13}C]palmitate). Fasting plasma TAG and VLDL-TAG concentrations significantly decreased (mean 18% and 20%, respectively) in the omega-3 fatty acid group but did not change in the placebo group. The ratio of VLDL-TAG to VLDL-apolipoprotein (apo) B was significantly decreased (by 48%) in the omega-3 fatty acid group. This would suggest smaller VLDL particles, perhaps due to lower TAG content, rather than fewer VLDL particles. Omega-3 fatty acids decreased fasting (and post-prandial) hepatic DNL by 30% and increased hepatic and whole body oxidation, as assessed by $^{13}CO_2$ production from

labelled palmitate, by 10% and 14%, respectively. The authors calculated that omega-3 fatty acids increased post-prandial fat oxidation and decreased post-prandial carbohydrate oxidation.

Relevance of the effect of omega-3 fatty acids on DNL and hepatic fatty acid partitioning to NAFLD

An omega-3 fatty acid induced decrease in DNL and increase in fatty acid oxidation would be expected to reduce hepatic accumulation of TAG, as well as to lower blood TAG concentrations. The regulatory effect of omega-3 fatty acids on hepatic fatty acid synthesis and oxidation has two important implications for both prevention and treatment of NAFLD, or at least the earliest "fatty liver" stage. The first implication is that a low status of EPA and DHA, mainly determined by low dietary intake of these fatty acids, but also perhaps by low endogenous synthesis from the precursor α-linolenic acid, might predispose individuals to a balance between DNL and β-oxidation that favours the former and hepatic TAG accumulation. The second implication is that patients who have developed fatty liver could be treated with EPA and DHA with the aim of decreasing DNL and increasing β-oxidation, acting to decrease the amount liver fat.

There are reports that liver lipids and phospholipids and red blood cells contain less EPA, DHA, and some other polyunsaturated fatty acids in patients with NAFLD than in control subjects (see [25, 26]). Whilst these observations fit with the ideas that low omega-3 fatty acid exposure might predispose to hepatic fat accumulation and development of NAFLD, the direction of causality is uncertain. While very low dietary intake or low endogenous synthesis would result in lower EPA and DHA in the liver and in red blood cells, it is also possible that changes linked to NAFLD result in lower omega-3 fatty acid levels; these could include altered complex lipid metabolism in NAFLD with decreased incorporation or increased release of omega-3 fatty acids or utilization and losses of these fatty acids driven by inflammation and oxidative stress.

Numerous studies in different animal models have reported that EPA and DHA decrease liver fat [27-30], an effect often linked to decreased expression and activity of lipogenic enzymes such as fatty acid synthase and increased expression and activity of oxidative enzymes such as carnitine palmitoyl transferase 1. Dietary EPA and DHA both have these effects. Quite a number of human studies have been performed giving patients with NAFLD supplemental EPA and DHA; 17 controlled trials in adults or children published between 2004 and 2016 have been extensively reviewed elsewhere [26]. In 2012 Parker et al.

[31] published the first meta-analysis on RCTs of omega-3 fatty acids (EPA and DHA) and fatty liver. They included 9 trials, 6 in patients with NAFLD and 3 in patients with related conditions. Trials used EPA+DHA doses of between 0.83 and 13.7 g/day and were of duration between 8 weeks and 12 months. Seven trials were included in the meta-analysis exploring the effect of omega-3 fatty acids on liver fat, measured using different approaches. Six of these trials individually reported that omega-3 fatty acids reduced liver fat compared with control, so it is no surprise that aggregating the findings of these trials identified a significant effect of omega-3 fatty acids. Since then, more trials have been performed and at least another eight meta-analyses of omega-3 fatty acids in NAFLD have been published including [32, 33, 34*, 35*]. Most of these report that the combination of EPA and DHA (or DHA alone in some studies mainly in children) decreases liver fat in patients with NAFLD. Musa-Veloso et al. [32] reported that omega-3 fatty acids decreased liver fat assessed by magnetic resonance imaging or spectroscopy when findings from 4 trials in adults and one trial in children were aggregated. Furthermore, grade of steatosis, as determined by ultrasonography, was improved by omega-3 fatty acids when findings from 4 trials in adults and 3 trials in children were aggregated. More recently, Lee et al [34*] identified that liver fat was decreased by omega-3 fatty acids when assessed either by ultrasound (4 studies) or by magnetic resonance imaging or spectroscopy (5 studies). The most recent meta-analysis [35*] only included 3 trials assessing liver fat, but showed a significant improvement with omega-3 fatty acids. Although most trials in adults have used a combination of EPA and DHA, Scorletti et al. [23] identified that the increase in red blood cell DHA, but not EPA, was an independent predictor of fat loss in patients receiving high dose omega-3 fatty acids (~3.5 g EPA plus DHA daily) for 15 to 18 months. This suggests that DHA may be more active than EPA in reducing liver fat in patients with NAFLD. In accordance with this, studies in children using DHA in the absence of EPA report a reduction in liver fat [36].

Mechanisms by which omega-3 fatty acids might influence DNL and hepatic fatty acid partitioning

The older [19,20] and newer [22, 24*] studies described above demonstrate that the combination of the omega-3 fatty acids EPA and DHA increases fat oxidation in humans, with the new studies [22, 24*] additionally reporting that omega-3 fatty acids decrease DNL and promote fatty acid partitioning away from TAG synthesis and towards hepatic β-oxidation in humans. This might account for the reductions in liver fat observed in patients with NAFLD

given omega-3 fatty acids, as reviewed in the previous section, and for the well described plasma/serum TAG-lowering effect of these fatty acids [13-17]. Effects on DNL and fatty acid partioning in the liver suggests that it is likely that omega-3 fatty acids modulate the enzymatic machinery involved in hepatic fatty acid and TAG metabolism. Indeed, over a long period, many studies using many different cell and animal models have identified that omega-3 fatty acids influence the amount and/or the activity of several hepatic nuclear receptors (transcription factors) that play a role in regulating hepatic DNL and β-oxidation and alter the amount or activity of key enzymes involved in the relevant pathways. The nuclear receptors affected by omega-3 fatty acids include liver X receptor α (LXRα), hepatocyte nuclear factor-4α (HNF-4α), farnesoid X receptor (FXR), peroxisome proliferator activated receptor-α (PPAR-α) and the sterol regulatory element binding proteins (SREBPs). Effects of omega-3 fatty acids on these nuclear receptors have been reviewed and discussed in detail elsewhere [26] and will be summarised here (see also Table 1).

LXR is activated by oxysterols and controls reverse cholesterol transport, promotes *de novo* fatty acid synthesis by increasing expression of SREBP-1c and carbohydrate response element binding protein (ChREBP), and promotes glycolysis via the phosphofructokinase-2/fructose-bisphosphatase-2 system [37*]. DHA can inhibit LXRα activation [38], which would act to decrease fatty acid synthesis.

FXR is highly expressed in the liver and has a central role in bile acid metabolism downregulating synthesis, secretion and reabsorption [39*]. FXR is also able to decrease cholesterol, fatty acid and TAG synthesis through down regulation of SREBP-1c, SREBP-2 and LXR [40, 41*]. FXR also controls lipoprotein metabolism by stimulating expression of apolipoproteins, especially apoC-II [42], an activator of lipoprotein lipase, the enzyme responsible for TAG clearance from the bloodstream. DHA is an FXR ligand resulting in its activation [43], an effect which would decrease hepatic fatty acid and TAG synthesis, and also promote clearance of circulating TAGs.

HNF-4α is a nuclear receptor which regulates several genes related to lipoprotein and carbohydrate metabolism and bile acid synthesis [44]. Omega-3 fatty acids decrease the synthesis of HNF-4α: ρat hepatocytes cultured with fish oil rich chylomicron remnant-like particles showed a decrease in HNF-4α mRNA and protein and a reduced expression of genes encoding apoB and microsomal transfer protein, which are regulated by HNF-4α [45].

SREBP-1a, -1c and -2 are key lipogenic transcription factors [46]. SREBP-1c and SREBP-2 are highly expressed in the liver. SREBP-1c increases expression of genes connected

with fatty acid and TAG synthesis [47*], while SREBP-2 increases expression of genes encoding enzymes involved in synthesis of cholesterol [48]. Expression of SREBP-1c is regulated by LXRα. Omega-3 fatty acids decrease SREBP-1c gene expression [49, 50], which may involve inhibition of the binding of LXRα to its response element in the promoter region of the SREBP-1c gene. Omega-3s also impair maturation of active SREBP [51]; older studies suggest that this relates to inhibition of migration of SREBP from the endoplasmic reticulum to the Golgi complex. This effect seems to be related to enrichment of intracellular membranes with EPA and DHA which causes cholesterol migration from highly concentrated areas, such as the plasma membrane, to less concentrated membranes, such as endoplasmic reticulum membrane. Reduced expression of genes involved in *de novo* fatty acid synthesis, such as fatty acid synthase, by EPA and DHA have often been related to decreased expression and activity of SREBP-1c [50-52] and this is linked to ameliorated hepatic steatosis by suppressing SREBP-1 expression [51, 52].

ChREBP increases the expression of L-pyruvate kinase, a glycolytic enzyme, and the expression of lipogenic genes, such as malic enzyme, ATP-citrate lyase, acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase and fatty acid elongases [53*]. ChERBP expression is stimulated by glucose, consequently activating glycolysis and *de novo* fatty acid synthesis. EPA and DHA were able to downregulate ChREBP gene expression through accelerating ChREBP mRNA decay [54]. This could be another mechanism by which omega-3 fatty acids decrease DNL.

PPAR-α up-regulates expression of genes encoding enzymes involved in hepatic β-oxidation, such as carnitine palmitoyl transferase 1 [55*, 56*]. EPA and DHA both increase the expression and activity of hepatic PPAR-α [57], meaning that they act to promote β-oxidation. EPA- and DHA-derived oxylipins are also potent PPAR agonists [58].

Thus overall, EPA and DHA act to control gene expression, ultimately co-ordinately suppressing hepatic lipogenesis (fatty acid and TAG synthesis and TAG-rich lipoprotein assembly) through SREBP- 1c inhibition and up-regulating hepatic fatty acid oxidation through PPAR-α activation. The combination of these effects would have the net result of decreasing DNL and partitioning fatty acids away from esterification and towards oxidation, resulting in a) less hepatic TAG accumulation and b) less hepatic TAG export. Therefore, through these effects EPA and DHA act to reduce both hepatic and circulating TAGs.

Conclusions

The omega-3 fatty acids EPA and DHA are well known to lower plasma/serum TAG concentrations. In various animal models they lower hepatic TAG content and this has been shown to involve altered expression and activity of several transcription factors with associated effects on expression and activity of enzymes involved in fatty acid and TAG synthesis, which are decreased, and in β-oxidation, which is increased. Recent studies show that these omega-3 fatty acids decrease hepatic DNL and partition fatty acids away from TAG synthesis and towards β-oxidation in humans, including in patients with NAFLD. The net result is decreased accumulation of hepatic TAG and lowering of circulating TAG concentrations. Human trials demonstrate that EPA and DHA can decrease liver fat in patients with NAFLD, as confirmed through several meta-analyses. Increased intake of EPA and DHA may reduce the likelihood of hepatic TAG accumulation, so lowering the risk of developing NAFLD, and could be used to reduce liver fat in patients with NAFLD.

Conflicts of interest

PCC acts as an advisor/consultant to DSM, BASF AS, Cargill, Smartfish, Fresenius-Kabi, Bayer Consumer Care and GSK Consumer Healthcare.

Key points

- The omega-3 fatty acids EPA and DHA decrease hepatic *de novo* lipogenesis (fatty acid and triacylglycerol (TAG) synthesis)
- EPA and DHA favour partioning of hepatic fatty acids away from TAG synthesis towards β-oxidation
- The effects of EPA and DHA on hepatic *de novo* lipogenesis and fatty acid partitioning involve altered expression and activation of nuclear receptors that act as transcription factors regulating expression of genes encoding key proteins involved in those metabolic processes
- Higher intake of EPA and DHA may reduce the risk of developing non-alcoholic fatty liver disease
- EPA and DHA have been demonstrated in some studies to decrease liver fat in patients with non-alcoholic fatty liver disease, an effect supported by findings of several meta-analyses

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Figure 1. Overview of hepatic fatty acid and triglyceride metabolism. The pathway of *de novo* fatty acid and triacylglycerol (TAG) synthesis starting with glucose or fructose and occurring via pyruvate, acetyl CoA, citrate, acetyl CoA (again) and malonyl CoA is shown with dashed arrows. The pathway of fatty acid β-oxidation occurring via acetyl CoA and producing CO_2 or ketone bodies is shown with a dotted arrows. Fatty acyl CoA can be derived from de novo synthesis, non-esterified fatty acids (NEFAs) released from adipose tissue, or lysosomal degradation of chylomicron remnants. The TAG formed can be incorporated into very low density lipoproteins (VLDL) and secreted into the bloodstream or retained within the hepatocyte. Three key enzymes are shown: acetyl CoA carboxylase (ACC), carnitine palmitoyl transferase 1 (CPT1) and fatty acid synthase (FAS). Malonyl CoA is an inhibitor of ACC. OAA, oxaloacetate.

Table 1. Hepatic transcription factors of relevance to hepatic TAG accumulation and the effect of EPA and DHA.

Transcription factor	Role in hepatic fatty acid and TAG metabolism	Effect of EPA and DHA	Likely impact on hepatic TAG
LXR	↑ DNL (via ↑ SREBP-1c and ↑ ChREBP)	Decreased activation	↓
FXR	↓ DNL (via ↓ SREBP-1c and ↓ LXR)	Increased activation	↓
HNF-4α	↑ VLDL assembly and secretion	Decreased synthesis	?
SREBP-1c	↑ DNL	Decreased synthesis and decreased maturation	↓
ChREBP	↑ DNL	Decreased synthesis	↓
PPAR-α	↑ β-oxidation	Increased synthesis and increased activation	↓