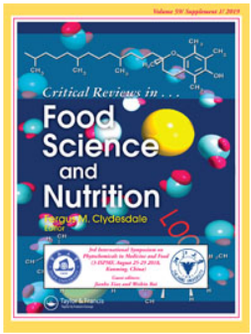

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REVIEW

n-3 Polyunsaturated fatty acids for the management of alcoholic liver disease: A critical review

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ABSTRACT

Excess alcohol exposure leads to alcoholic liver disease (ALD), a predominant cause of liver-related morbidity and mortality worldwide. In the past decade, increasing attention has been paid to understand the association between n-3 polyunsaturated fatty acids (n-3 PUFAs) and ALD. In this review, we summarize the metabolism of n-3 PUFAs, animal model of ALD, and the findings from recent studies determining the role of n-3 PUFAs in ALD as a possible treatment. The animal models of acute ethanol exposure, chronic ethanol exposure and chronic-plus-single binge ethanol feeding have been widely used to explore the impact of n-3 PUFAs. Although the results of studies regarding the role of n-3 PUFAs in ALD have been inconsistent or controversial, increasing evidence has demonstrated that n-3 PUFAs may be useful in alleviating alcoholic steatosis and alcohol-induced liver injury through multiple mechanisms, including decreased *de novo* lipogenesis and lipid mobilization from adipose tissue, enhanced mitochondrial fatty acid β-oxidation, reduced hepatic inflammation and oxidative stress, and promoted intestinal homeostasis, positively suggesting that n-3 PUFAs might be promising for the management of ALD. The oxidation of n-3 PUFAs *ex vivo* in an experimental diet was rarely considered in most n-3 PUFA-related studies, likely contributing to the inconsistent results. Thus, the role of n-3 PUFAs in ALD deserves greater research efforts and remains to be evaluated in randomized, placebo-controlled clinic trial.

KEYWORDS

Alcoholic liver disease; alcoholic steatosis; intestinal homeostasis; inflammation; n-3 polyunsaturated fatty acids; oxidative stress

Introduction

Alcohol has been found to contribute to at least 60 different health conditions (Mukherjee et al., 2007). The harmful use of alcohol leads to approximately 3.3 million deaths each year and 5.1% of the global burden of diseases (World Health Organization 2014). Prolonged, excessive alcohol consumption results in alcoholic liver disease (ALD), which is a leading risk factor for both morbidity and mortality among people with alcohol abuse worldwide. The spectrum of ALD classically ranges from simple hepatic steatosis to more advanced forms, including alcoholic steatohepatitis, progressive fibrosis, cirrhosis, and even hepatocellular carcinoma (Bataller and Brenner 2005; Day and James 1998). Alcoholic fatty liver (simple hepatic steatosis), characterized by lipid accumulation in the liver, is an initial stage of ALD and a reversible pathological condition. Although up to 90% of heavy drinkers develop hepatic steatosis, only approximately 10–20% of patients may progress to severe forms, such as alcoholic hepatitis or cirrhosis (Mathurin and Bataller 2015).

In recent years, n-3 polyunsaturated fatty acids (n-3 PUFAs), including eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), have been well documented to exert health promotion and beneficial effects on many diseases, such as cardiovascular diseases, metabolic disorders and inflammation-associated diseases (Bellenger et al. 2011; Wan et al. 2010) (Siriwardhana et al. 2012). Clinical studies have indicated that a low circulating level of n-3 PUFAs was found in the patients with ALD and nonalcoholic fatty liver disease (NAFLD) (Holman and Johnson 1981; Johnson et al. 1985; Lakshman 2004), and it is likely that the replenishment of n-3 PUFAs may alleviate ALD. The benefits of n-3 PUFAs in NAFLD and nonalcoholic steatohepatitis (NASH) have been well summarized (de Castro and Calder 2018; Jump et al. 2018). A large body of studies has addressed the roles of n-3 PUFAs or n-3 PUFA-rich fish oil in ALD. However, the outcomes of these studies have been inconsistent. An increasing number of studies has demonstrated that fish oil or n-3 PUFAs exerts protective effects on hepatic steatosis and liver injury induced by alcohol exposure (Sekiya et al. 2003; Song et al. 2008; Wada et al. 2008; Wang et al. 2017a; Wang et al. 2016b; Wang et al. 2016c), that are attributed to protecting mitochondrial dysfunction (Song et al. 2008), stimulating fatty acid β-oxidation by the activation of peroxisome proliferator-activated

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receptor - alpha (PPARα) (Sekiya et al. 2003), inhibiting lipogenesis by the regulation of sterol regulatory element-binding protein (SREBP)-1 (Sekiya et al. 2003; Wada et al. 2008), ameliorating gut-derived endotoxin-mediated inflammation (Wang et al. 2016c) and ameliorating lipid homeostasis at the adipose tissue-liver axis (Wang et al. 2016b) in the liver. Nevertheless, several studies have shown that fish oil causes severe liver injury and promotes the pathogenesis of ALD (Donohue et al. 2007; Nanji et al., 1995; Nanji et al., 1994b; Sachan et al., 2002). Therefore, in the present review, we summarized the available findings of n-3 PUFAs used to treat ALD and their underlying molecular mechanisms, which will offer more current and comprehensive evidence in the use of n-3 PUFAs in ALD.

n-3 PUFAs and Metabolism

n-3 PUFAs are the principal structural components of the cell membrane and play pivotal roles in several biological processes and metabolic pathways (Simopoulos, 1991, 2008). Although saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) can be biosynthesized from acetyl-CoA that are mainly derived from carbohydrates via the glycolytic pathway, n-3 PUFAs cannot be endogenously synthesized or converted from n-6 PUFAs in mammals due to the lack of corresponding desaturases to introduce a cis double bond at the n-3 position of acyl chains with regiospecificity. Thus, n-3 PUFAs are defined as essential fatty acids that are mostly obtained from the diet. α-Linolenic acid (ALA, 18:3 n-3) is a plant-derived n-3 PUFA that is abundant in flaxseed oil, walnut and perilla oils (Barcelo-Coblijn and Murphy, 2009). Dietary ALA acts as a precursor of the n-3 PUFA family and can be synthesized to long-chain n-3 PUFAs, including EPA and DHA, by progressive elongation and desaturation (**Figure 1**) (Brenna, 2002). However, this conversion efficiency appears to be limited (less than 5%), particularly with a background diet high in linoleic acid (LA, 18:2 n-6), because LA competes with ALA for identical elongases and desaturases (Brenna et al., 2009; Fu and Sinclair, 2000). EPA and DHA, the marine-derived n-3 PUFAs, can also be directly obtained from seafoods, such as salmon and sardines (Calder, 2015). n-3 PUFAs are the important precursors for a series of bioactive molecules, termed eicosanoids. EPA can be metabolized to 3-series prostaglandins (e.g., PGD_3 and PGE_3) and 3-series thromboxanes (e.g., TXA_3 and TXB_3) by cyclooxygenases (COX). EPA is also the precursor of 5-series leukotrienes (e.g., LTA_5 and LTB_5) and E-series resolvins (e.g., RvE_1 and RvE_2) by lipoxygenases (LOX) and cytochrome P450 enzymes, respectively (Astarita et al., 2015). DHA can be metabolized to D-series resolvins (e.g., RvD_1 to RvD_6), protectins (e.g., PD1) and maresins (e.g., MaR_1 and MaR_2) (Serhan et al., 2015). These n-3 PUFA-derived eicosanoids are predominantly anti-inflammatory, inhibit platelet aggregation and dilate blood vessels, which are functionally adverse with n-6 PUFA-derived eicosanoids (Saini and Keum, 2018). Until now, the impact of eicosanoids or pro-resolution lipid mediators derived from n-3 PUFAs on ALD has never been addressed.

To increase n-3 PUFA levels in animals, supplemental intervention of fish oil, flaxseed oil, or pure EPA and DHA was used in the most studies. The transgenic *fat-1* mouse was generated to increase the tissue level of endogenous n-3 PUFAs (Kang et al., 2004) and has been widely used to illustrate the benefits of long-chain n-3 PUFAs and their molecular mechanisms (Kang, 2007), including ALD (Huang et al., 2015; Nieto, 2007; Wang et al., 2017a), as a well-controlled animal model. Transgenic *fat-1* mice, expressing the *fat-1* gene derived from *Caenorhabditis elegans*, encode the n-3 desaturase that endogenously synthesizes n-3 from n-6 PUFAs by introducing a cis-double bond at the n-3 position, leading to the elevated level of n-3 PUFAs in their tissues and organs, without supplemental intervention of n-3 PUFAs (Kang et al., 2004). Specifically, n-6 type PUFAs, including linoleic acid (LA, 18:2 n-6), γ-linolenic acid (GLA, 18:3 n-6), dihomo-γ-linolenic acid (DGLA, 20:3 n-6), arachidonic acid (AA, 20:4 n-6), docosatetraenoic acid (DTA, 22:4 n-6) and n-6 docosapentaenoic acid (n-6 DPA, 22:5 n-6), can be converted to n-3 type PUFAs, ALA (18:3 n-3), stearidonic acid (SDA, 18:4 n-3), eicosatetraenoic acid (ETA, 20:4 n-3), EPA(20:5 n-3), n-3 DPA (22:5 n-3) and DHA (22:6 n-3), respectively, in *fat-1* mice (Figure 1). When fed the same diet containing abundant n-6, but deficient in n-3 PUFAs, the *fat-1* mice and their wild-type (WT) littermates can produce high and low n-3 PUFA profiles, respectively. Thus, the potential confounding factors from the different diets, including flavor, fat oxidation, and unwanted components (e.g., antioxidants, cholesterol and contaminants), may be well eliminated in the comparative study (Kang, 2007).

Animal Models of ALD

The challenge of experimental study in ALD is the absence of a reliable animal model that closely mimics all relevant features of ALD, ranging from hepatic steatosis to alcoholic hepatitis, and fibrosis, in humans (Bertola et al., 2013; Ghosh Dastidar et al., 2018). Either the animal species or drinking pattern has been shown to greatly affect alcoholic liver injury. The pathogenesis of ALD in nonhuman primates is more similar to that in humans than in other animals, but the usefulness of nonhuman primates is limited by the prodigious cost and availability (Pawlosky and Salem, 2004). It is well known that rodents are instinctively resistant to alcohol hepatotoxicity due to species-related differences in the metabolism of alcohol (Holmes et al., 1986). Thus, most animal models used to investigate alcoholic liver injury in rodents only induce mild steatosis, slight liver inflammation, but no fibrosis, mimicking early stages of human ALD (Bertola et al., 2013). Additionally, the pattern of alcohol exposure in animal models is another important factor for ALD. Binge drinking is the simplest model of ethanol-induced liver injury, and if animals are gavaged by a single dose or multiple doses of alcohol over a few hours or several days to induce acute liver damage, binge drinking is well suited to closely mimic acute alcohol consumption with

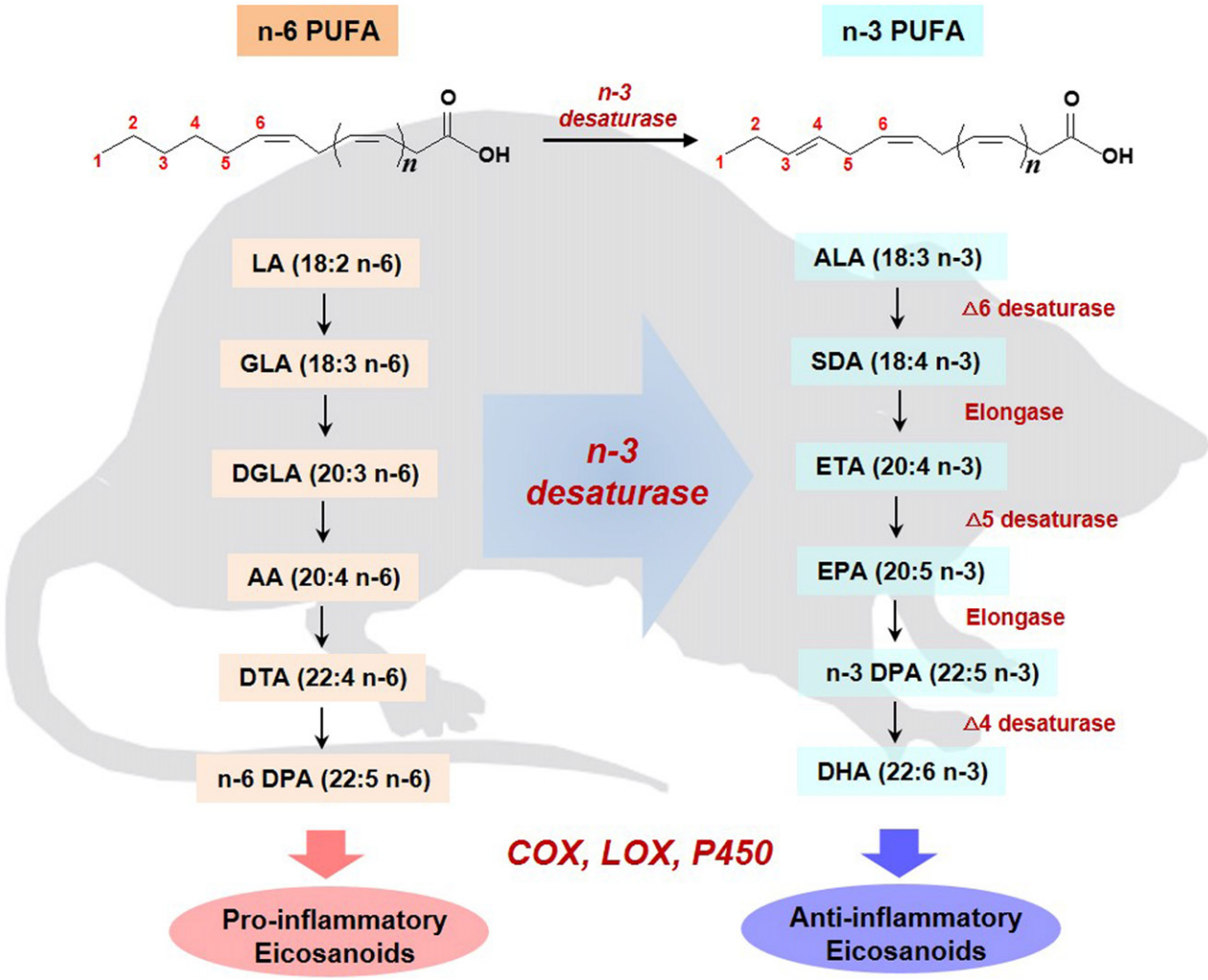

Figure 1. Metabolic pathway scheme of long-chain polyunsaturated fatty acids (PUFAs) from dietary linolenic acid (LA) and alpha-linolenic acid (ALA). n-6 and n-3 PUFAs share the same desaturases and elongases. The *fat-1* gene encodes an n-3 desaturase that synthesizes n-3 PUFAs from n-3 PUFAs.

an equivalent blood alcohol level, behavioral responses and physiological changes to those of binge drinking in humans (Ding et al., 2015; Huang et al., 2013). The rodent model of feeding the Lieber-DeCarli liquid diet containing alcohol for 4-6 weeks has been extensively used to produce chronic alcoholic liver injury. However, such a model only induces mild hepatic steatosis, a slight increase in the circulating level of aminotransferases, and little hepatic inflammation (Bertola et al., 2013). Improved feeding with the Lieber-DeCarli diet that gradually increased the ethanol concentration for 12 weeks was developed and shown to induce obvious steatosis (Zhou et al., 2005). In several studies (Zhong et al., 2013), mild hepatic steatosis is also induced by long-term feeding of a Lieber-DeCarli control diet, in which 36% of the energy compositions is provided by fat. A mouse model of chronic-plus-single-binge ethanol feeding was developed to mimic acute-on-chronic alcohol-induced liver injury in humans (Bertola et al., 2013). In this model, animals were fed the Lieber-DeCarli liquid diet for 10 days, and then were gavaged with a single-dose ethanol at day 11, to achieve a higher blood concentration of ethanol over acute or chronic ethanol feeding alone, leading to synergistically induced hepatic steatosis, inflammation and liver damage in mice and rats (Aroor et al., 2011; Bertola et al., 2013).

Can n-3 PUFAs protect against ALD?

In recent years, n-3 PUFAs have been attracted increasing attention in the treatment of ALD. In this review, 22 studies involving fish oil, flaxseed oil, endogenous n-3 PUFAs, EPA, DHA and ALA were summarized and revealed the potential role of n-3 PUFAs in ALD (**Table 1**). Acute or chronic alcohol-fed mice (e.g., C57BL/6 and transgenic *fat-1* mice) and rats (e.g., Wistar, Wistar-Furth, and Long-Evans rats) are commonly used experimental animals; rhesus monkeys (Pawlosky and Salem, 2004) and rat hepatocytes (Aliche-Djoudi et al., 2011; Aliche-Djoudi et al., 2013) are also employed to study the impacts of n-3 PUFAs in ALD. The enzyme activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are widely used as biochemical markers to clinically monitor liver function or liver injury (Ding et al., 2012). Most of these studies have shown that n-3 PUFAs can protect against liver injury induced by alcohol, as characterized by reversing the circulating AST and ALT activities elevated by alcohol exposure.

Additionally, other circulating biomarkers, such as triglyceride (TG), total cholesterol (TC), alkaline phosphatase (ALP) and total bilirubin (TB), were also utilized to assess the hepatoprotective effects of ALA-rich flaxseed oil (Wang et al., 2016a) and endogenous n-3 PUFAs (Wang et al., 2017a). Excess lipid deposition in the liver is the early response to alcohol exposure, and this abnormal retention of lipids greatly affects hepatocellular physiological functions, increasing the susceptibility of hepatocytes to hepatotoxins (Tamai et al., 2002). In most of these studies, n-3 PUFAs can protect against hepatic steatosis induced by either acute or chronic alcohol exposure, as evaluated by measurement of triglycerides in the liver and histopathological observation, including hematoxylin-eosin (H&E) and Oil red O staining. The inflammatory response in the liver is another important feature of ALD. Because n-3 PUFAs are well known to have anti-inflammatory properties, most studies showed that n-3 PUFAs inhibit the hepatic production of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and monocyte chemotactic protein-1 (MCP-1), and suppress macrophage infiltration in the liver (Huang et al., 2015; Wang et al., 2016a; Zhang et al., 2017).

However, the impact of n-3 PUFAs on ALD remains controversial. Yi Wang *et al.* reported that DHA failed to attenuate hepatic steatosis induced by chronic alcohol exposure (Wang et al., 2016d). Additionally, numerous studies have demonstrated that the combination of dietary fish oil and alcohol exacerbates hepatic steatosis, necrosis, hepatic inflammation and liver injury (Nanji et al., 2004; Nanji et al., 2001; Nanji et al., 1995). Oral alcohol feeding with fish oil as a fat source has been used as an animal model for pathological alcoholic liver injury (Tipoe et al., 2008). An explicative hypothesis proposed is that a high level of n-3 PUFAs from fish oil provides a substrate of lipid peroxidation, further accentuating oxidative stress and inflammation and leading to exacerbated liver damage (Donohue et al., 2007; Nanji et al., 1995; Nanji et al., 1994b). Alcohol consumption increases the production of reactive oxygen species (ROS) in the liver that can react with PUFAs located in the cell membrane. The supplementation of n-3 PUFAs with a high degree of unsaturation may serve as the preferential substrate and reduce the potential of ROS to react with PUFAs in the cell membrane, resulting in the protection of cell membrane damage. Furthermore, fish oil was added to the animal diets as the source of n-3 PUFAs in these studies with negative results. n-3 PUFA oxidation *ex vivo* during the preparation and storage of the experimental diet, and the feeding procedure was rarely considered in most n-3 PUFA-related studies. Long-chain n-3 PUFAs, such as EPA and DHA, are highly unstable and vulnerable to be oxidized more easily than other types of fatty acids (Kelly, 1991). Fish oils may contain a high level of oxidized substances that may be derived from poor preservation and a long storage period (Kang, 2007). The oxidation of n-3 PUFAs *ex vivo* prior to feeding leads to the loss of beneficial nutrients and production of genotoxic and cytotoxic substances. Awada M *et al.* revealed that dietary oxidized n-3 PUFAs can trigger oxidative stress and inflammation in mice (Awada et al., 2012). Our findings also indicate that oxidized fish oil aggravates hepatic steatosis, hepatic inflammation and liver injury induced by alcohol, but these changes induced by alcohol can be ameliorated by fish oil containing a high content of EPA and DHA (unpublished data). It remains unclear whether a high level of oxidative stress observed in previous studies with negative results is caused by supplemented n-3 PUFAs or lipid peroxides already existing in the fish oil used (Huang et al., 2013). It is intriguing that the positive results were always obtained in transgenic *fat-1* mice, in which n-6 PUFAs are endogenously converted to the n-3 PUFAs, thus avoiding oxidation of n-3 PUFAs *ex vivo*. Additionally, neither the concentration of n-3 PUFAs nor that of the other components, including SFAs, MUFAs, n-6 PUFAs, cholesterol and antioxidants, in fish oil used was depicted in fish oil-related studies. Therefore, the oxidation and doses of n-3 PUFAs in fish oil might contribute to the conflicting outcomes of n-3 PUFAs in ALD.

n-3 PUFAs protect against alcoholic hepatic steatosis

Hepatic steatosis, the early response to alcohol exposure, is the initial stage of ALD and a reversible pathological condition. The liver with large lipid accumulation is more susceptible to further injury that leads to the advanced stages of ALD (Tamai et al., 2002). Thus, reducing lipid accumulation in the liver is a crucial strategy to halt or postpone the progression of ALD (Wang et al., 2017a). Based on these studies with positive results, n-3 PUFAs can efficiently protect against alcoholic hepatic steatosis by inhibiting *de novo* lipogenesis, enhancing fatty acid β-oxidation, and ameliorating lipid homeostasis at the adipose tissue-liver axis.

De novo lipogenesis

It is well recognized that sterol regulatory element-binding protein (SREBP)-1c, a transcription factor, plays a deleterious role in *de novo* lipogenesis by regulating the expression of over 30 target lipogenic genes in the liver. SREBP-1c-deficient mice are resistant to hepatic steatosis induced by intragastric infusion of alcohol for 4 weeks (Ji et al., 2006). Either chronic or acute alcohol exposure directly upregulates the transcription of SREBP-1c gene and stimulates its maturation via acetaldehyde, a primary metabolite of alcohol (Horton et al., 2002; Lian et al., 2010). Additionally, alcohol exposure indirectly increases SREBP-1c expression by the activation of endoplasmic reticulum (ER) stress (Gao and Bataller, 2011). Endogenous n-3 PUFA augmentation and fish oil supplementation can decrease both the transcription and protein expression of SREBP-1c in the liver that upregulated by acute alcohol exposure (Huang et al., 2015; Wada et al., 2008). These inhibitory effects of n-3 PUFAs on SREBP-1c expression might be mediated by suppressing the binding of the liver X receptor (LXR)/retinoid X receptor (RXR) heterodimer to the LXR response elements (LXREs) in the SREBP-1c promoter, a process crucial for SREBP-1c expression (Yoshikawa et al., 2002).

Table 1. A summary of the role of n-3 PUFAs in ethanol-induced liver injury ("↓" and "↑" indicate significant decrease and increase, respectively ($p < 0.05$), "-" indicates no significant difference)

No.	n-3 PUFAs	ALD Model	Animal	Hepatotoxicity	Mechanisms	Ref.
1	Endogenous n-3 PUFAs	Chronic-plus-single binge ethanol feeding	*fat-1* mice	↓AST; ↓ALT; ↓TB; ↓ALP; ↓TG; ↓TC	Adipose lipolysis: ↑GPR120, ↑CaMKKβ, ↑PDE3B, ↓p-AMPK, ↓cAMP, ↓ HSL, ↓ATGL; Adipose inflammation: ↓IL-1β, ↓TNF-α; Hepatic FFA uptake: ↓CD36, ↓FATP5, ↓FATP2; Hepatic inflammation: ↓IL-1β, ↓TNF-α, ↓MCP-1	(Wang et al., 2017a)
2	Endogenous n-3 PUFAs	3 acute doses of ethanol every 12 h	*fat-1* mice	↓ ALT; ↓ TG; ↑ HDL-C	Lipogenesis: ↓SREBP-1c, ↓FAS, ↓SCD-1; β-oxidation: ↑PPARα; Inflammation: ↓IL-6, ↓TNF-α, ↓MCP-1; Oxidative stress: ↓CYP2E1, ↑HO-1	(Huang et al., 2015)
3	Flaxseed oil	Chronic-plus-single binge ethanol feeding	C57BL/6 mice	↓ AST; ↓ ALT; ↓ TG	Lipid homeostasis at the adipose tissue-liver axis FAs uptake: ↓CD36, -FATP2, ↓FATP5; TG synthesis: -Gpat, ↓Dgat2, -Dgat1; VLDL export: ↑MTTP; Endoplasmic reticulum stress: ↓p-IRE1α; ↓p-EIF2α	(Wang et al., 2016b)
4	Flaxseed oil	Chronic-plus-single binge ethanol feeding	C57BL/6 mice	↓ ALP; ↓ ALT; ↓ TG; ↓ TB	Oxidative stress: ↓MDA, ↑GSH, ↑SOD; Inflammation: ↓IL-6, ↓TNF-α, ↓MCP-1, ↓CD14, ↓TLR4, ↓MYD88, ↓NF-κB; Intestinal barrier: ↓LPS, ↓16S rRNA, ↑ZO-1, ↑Occludin, ↑Claudin-4	(Wang et al., 2016a)
5	Flaxseed oil	Chronic ethanol feeding for 6 wks	C57BL/6 mice	↓ AST; ↓ ALT	Inflammation: ↓IL-6, ↓TNF-α, ↓IL-1β, -IL-10; Modulation of gut microbiota: ↓LPS; ↓Proteobacteria, ↑Porphyromonadaceae	(Zhang et al., 2017)
6	Fish oil	a binge ethanol administration	C57BL/6 mice	↓ TG	Lipogenesis: ↓SREBP-1c, ↓SCD1, ↓FAS, ↓ACC1, ↓LPK, ↓ChREBP; β-oxidation: ↑ PPARα, ↑MCAD, ↑ACO, ↑CPT1; TG synthesis: -DGAT1, -DGAT2, -GPAT, -PPARγ	(Wada et al., 2008)
7	Fish oil	Chronic ethanol feeding for 25 days	COL1A2-βGal transgenic mice	↑AST; ↑ALT	Oxidative stress: ↓GSH, ↓GCLM, ↓GCLC, ↑CYP2E1, ↑MDA ↑collagen I, transactivate COL1A2 promoter	(Nanji et al., 1994a)
8	Fish oil	Chronic ethanol feeding for 8 wks	Wistar rats	↓ AST; ↓ ALT	Inflammation: ↓IL-6, ↓TNF-α, ↓IL-1β, ↓IL-10; Intestinal permeability:↓Urinary Lactulose/ Mannitol Ratio; Modulation of Gut microbiota:↓LPS; ↓E. coli; ↑Bifidobacterium	(Chen et al., 2016)
9	Fish oil	Chronic ethanol feeding for 1 month	Wistar rats	N.A.	Lipid peroxidation: ↑CYP2E1	(Marmillot et al., 2000)
10	Fish oil	Chronic ethanol feeding for 6 wks	Wistar-Furth rats	↓TG; ↓TC	Reversing lipid and lipoprotein abnormalities: ↓Plasma apo A, ↑HDL apo E	(Nieto,2007)
11	Fish oil	Chronic ethanol feeding for 8 wks	Wistar-Furth rats	↓TC	Restoring the impaired reverse cholesterol transport function of HDL	(Lakshman et al., 1988)
12	Fish oil	Chronic ethanol feeding for 8 wks	Wistar rats	↑AST; ↑ALT; ↑TG	Peroxidation: ↑MDA; Inflammation: ↑TNF-α	(Wada et al., 2008)
13	Fish oil	Chronic ethanol feeding for 4 wks	Wistar rats	↑Score for fatty liver, necrosis and inflammation	-PPARα, -FACO, -CYP4A1, -L-FABP; ↓FACO/PPAR ratio	(Donohue et al., 2007)
14	Fish oil	Continuous intragastric infusion of liquid diet for 1 month	Wistar rats	↑Pathology scores	Oxidative stress: ↑lipid peroxides, ↑8-isoprostanes; Stellate cell activation: ↑α-SMA; ↓proteasome chymotrypsin-like activity; ↓Chymotrypsin-like activity	(Nanji et al., 2004)
15	Fish oil	Chronic ethanol feeding for 8 wks	Wistar rats	↑Score for fatty liver, necrosis and inflammation	↑amounts of collagen and pericellular fibrosis; ↑Endotoxin; Lipid Peroxidation: ↑CDA232; Inflammation: ↑TNF-α, ↑Cox-2	(Donohue et al., 2004)
16	Fish oil	Chronic ethanol feeding for 4 wks	Wistar rats	↑Score for fatty liver, necrosis and inflammation	Lipid peroxidation: ↑ CDA232, ↑TBARS; Antioxidant Enzymes: ↓CuZnSOD, ↓Catalase, ↓GPX, -MnSOD	(Nanji et al., 1997)

(*continued*)

Table 1. Continued.

No.	n-3 PUFAs	ALD Model	Animal	Hepatotoxicity	Mechanisms	Ref.
17	DHA	3 acute doses of ethanol every 12 h	C57BL/6 mice	↓ TG; ↓ ALT	Lipogenesis: ↓SCD1; Inflammation: ↓ IL-6, ↓TNF-α; Oxidative stress: - ROS, ↑HO-1	(Huang et al., 2013)
18	DHA	Chronic ethanol administration	mice	-TG	Oxidative stress: -CYP2E1, -MDA, ↑GSH; Lipogenesis: -SREBP-1c	(Wang et al.,2016d)
19	DHA	50 mM ethanol for 1, 1.5 or 5 h	Adult rat hepatocytes	N.A.	Oxidative stress: ↓ROS, ↓4-hydroxy-nonenal, - gluthatione peroxidase, -glutathione	(Aliche-Djoudi et al.,2013)
20	DHA/AA	Chronic ethanol feeding for 9 wks	Long-Evans rats	- AST; - ALT; ↓ TG; ↓ TC	FAs biosynthesis: ↑p-ACC; Oxidative stress: ↓CYP2E1, ↓NOS, ↓H2O2, ↓nitrite; Mitochondrial function:↓oxidized mitochondrial proteins	(Song et al.,2008)
21	EPA	50 mM ethanol for 1, 1.5, or 5 h	Adult rat hepatocytes	↑ TC	Oxidative stress: ↑ROS, ↑MDA, ↑C11 Bodipy oxidation	(Aliche-Djoudi et al.,2011)
22	ALA	Chronic ethanol feeding for 5 yrs	rhesus monkeys	N.A.	↓Steatosis, ↓Focal and diffuse cellular necrosis; ↓Fibrosis	(Pawlosky and Salem,2004)

Mature SREBP-1c (the active form) translocates to the nucleus and promotes fatty acid biosynthesis via upregulating the expression of lipogenesis-related genes, including ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and stearyl CoA desaturase-1 (SCD-1) (Han et al., 2017). ACLY and ACC are the primary enzymes responsible for acetyl-CoA synthesis and malonyl-CoA synthesis by carboxylation of acetyl-CoA, respectively. FAS catalyzes the synthesis of palmitic acid (16:0) from acetyl-CoA and malonyl-CoA. SCD-1 is responsible for fatty acid desaturation (Yin et al., 2009a; Yin et al., 2009b). Either acute or chronic alcohol exposure upregulates the expression of these lipogenic enzymes, which can be inhibited by n-3 PUFAs. Specifically, fish oil fed prior to a single dose of alcohol administration downregulates the mRNA levels of target genes, including ACC, FAS and SCD-1 (Wada et al., 2008). Endogenous n-3 PUFAs and DHA significantly decrease the expression levels of FAS and/or SCD-1 at either the transcriptional or protein levels (Huang et al., 2013; Huang et al., 2015). Additionally, endogenous n-3 PUFAs and DHA inhibit the activity of alcohol-induced SCD-1, which is responsible for the biosynthesis of palmitoleate (16:1 n-7) and oleate (18:1 n-9), major components of triglycerides, from palmitic acid (16:0) and oleic acid (18:0), respectively (Huang et al., 2015; Wada et al., 2008).

Further molecular mechanism studies have deciphered that alcohol also downregulates the factors that reduce the expression of SREBP-1c, including AMP-activated protein kinase (AMPK), sirtuin 1 (SIRT1) and adiponectin (Long and Zierath, 2006; Purohit et al., 2009). As a sensor of the cellular energy status, AMPK regulates the activity of SREBP-1c by decreasing both its mRNA and protein levels (Long and Zierath, 2006). Adiponectin is an adipose-derived bioactive adipokine that critically modulates hepatic lipid metabolism in the liver (Rogers et al., 2008; Zhong et al., 2012b). The decreased circulating level of adiponectin and impaired hepatic expression of its receptor, adipoR2, were found in ALD (Rogers et al., 2008; Xu et al., 2003). Administration of the recombinant adiponectin reduces alcoholic hepatic steatosis and inflammation in mice (Xu et al., 2003). The function of adiponectin in lipid metabolism is mediated by AMPK signaling, leading to reduced *de novo* lipogenesis and enhanced fatty acid β-oxidation (Rogers et al., 2008; Xu et al., 2003). Dietary ALA-rich flaxseed oil can ameliorate alcohol-induced adipose dysfunction, increase plasma adiponectin and hepatic adipoR2 expression, and activate the AMPK pathway.

Mitochondrial fatty acid β-oxidation

Acetaldehyde, an ethanol metabolite, inhibits the mitochondrial β-oxidation of fatty acid mainly via the inactivation of PPARα, a transcription factor that regulates many target genes involved in fatty acid oxidation systems in the liver, including acyl-CoA oxidase (ACO, the first enzyme of peroxisomal β-oxidation), medium-chain acyl-CoA dehydrogenase (MCAD), and carnitine palmitoyltransferase 1 (CPT1, a transport of FFA from cytoplasm into mitochondria) (de Castro and Calder, 2018; Wada et al., 2008). Impaired PPARα function during alcohol exposure leads to severe hepatic FFA overload, and, in turn, increases liver synthesis of TG, which also contributes to alcoholic hepatic steatosis (Gao and Bataller, 2011). Treatment with the PPARα agonist Wy14,643 can prevent against fatty liver in alcohol-fed mice by stimulating the rate of mitochondrial fatty acid β-oxidation (Fischer et al., 2003). Additionally, alcohol exposure also activates ACC, a rate-limiting enzyme for lipogenesis, via inhibition of AMPK, subsequently increasing the level of malonyl-CoA, a precursor in fatty acid synthesis and an inhibitor of CPT1, resulting in increased lipogenesis and decreased fatty acid β-oxidation (Viollet et al., 2009). Dietary ALA without alcohol feeding increases the rate of fatty acid oxidation and increase both the mRNA levels and activities of enzymes related to fatty acid β-oxidation, except for the activity of 3-hydroxyacyl-CoA dehydrogenase. Similar changes were observed in fish oil-fed rats (Ide, 2000). Fish oil fed prior to binge drinking can increase the expression levels of PPARα and its target genes, including ACO, CPT1, and MCAD (Wada et al., 2008). Furthermore, endogenous n-3 PUFAs increase the expression of PPARα impaired by acute alcohol exposure (Huang et al., 2015).

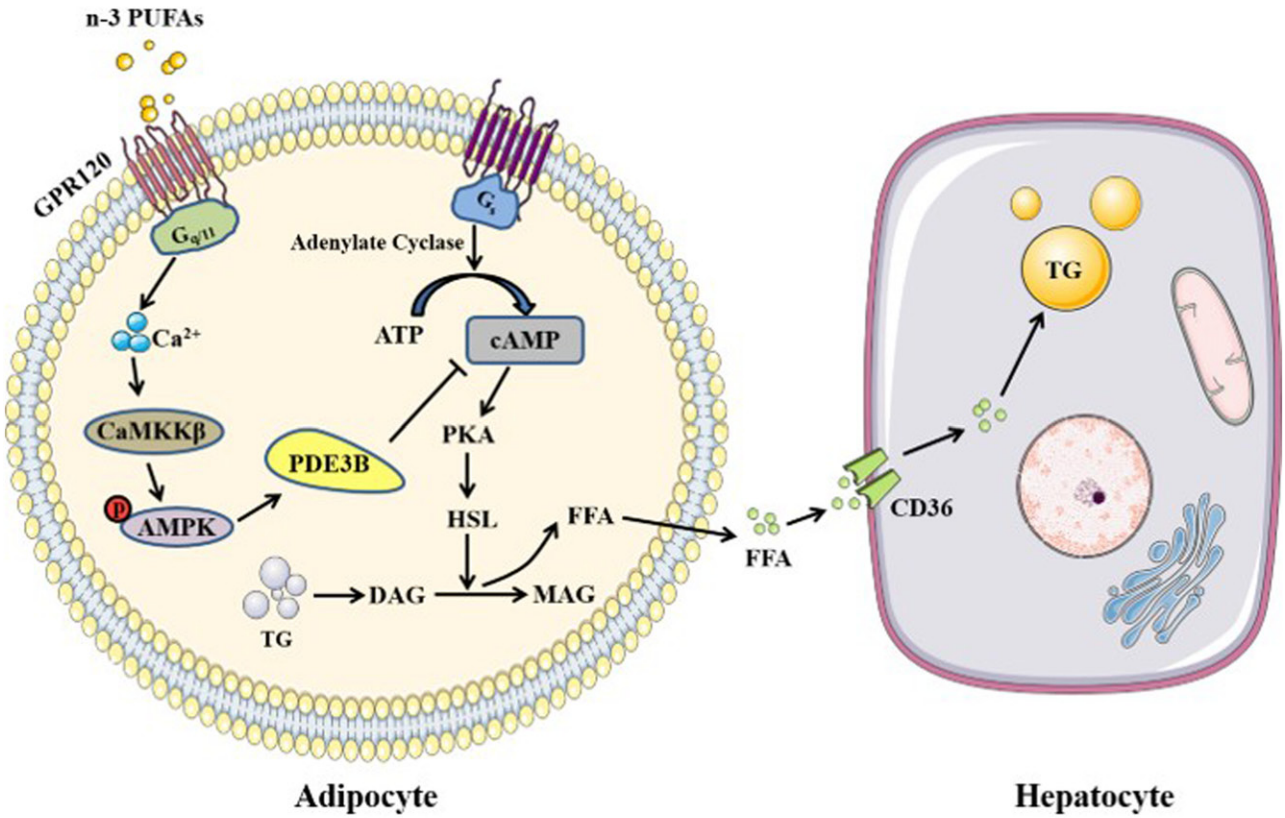

Figure 2. Schematic diagram of the molecular mechanisms underlying the inhibitory effects of n-3 PUFA on adipose lipolysis in alcoholic liver disease. Reprinted from (Wang et al.,2017a), Copyright (2017) with permission from Elsevier BV.

Lipid homeostasis at the adipose tissue-liver axis

Alcohol exposure leads to adipose tissue dysfunction by inducing adipocyte death, increasing mobilization of depot fat, and altering the secretion of adipokines, which directly and indirectly contribute to the pathogenesis of alcoholic steatosis (Wang et al., 2016b; Zhong et al., 2012b). The fatty acids released from adipose tissue are the main source of hepatic lipid accumulation in alcoholic steatosis. As a fat storage organ, adipose tissue plays a central role in lipid homeostasis of the whole body (Wang et al., 2016b). Adipose tissue dysfunction induced by alcohol exposure may increase adipocyte lipolysis, promote excess adipose-derived fatty acid influx into the liver, leading to ectopic fat deposition in the liver (Zhong et al., 2012a). This hepatic TG reverse transport from WAT after chronic alcohol exposure was identified using a deuterium tracer and mass spectrometry approach (Zhong et al., 2012a). A large body of clinical and animal studies have revealed that alcoholic hepatic steatosis hallmarked by hepatic triglyceride gain is accompanied by the loss of adipose mass (Addolorato et al., 1997, 1998). Chronic-plus-single binge ethanol feeding stimulates adipose tissue lipolysis in mice, as evidenced by the high capacity for free fatty acid (FFA) mobilizing from epididymal adipose tissue, which was remarkably ameliorated by dietary ALA-rich flaxseed oil (Wang et al., 2016b) and endogenous and exogenous n-3 PUFA enrichment (Wang et al., 2017a). Hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) are key enzymes responsible for the hydrolyzation of triacylglycerol and diacylglycerol to release fatty acids in adipocytes, respectively. Consistent with reduced fatty acid release, flaxseed oil and endogenous n-3 PUFAs inhibit the gene expression of ATGL and inactivate HSL by dephosphorylation in adipose tissue. Furthermore, n-3 PUFA enrichment can promote fat storage function of adipocytes, as evidenced by normalizing the expression of very low-density lipoprotein receptor (VLDLR) related to VLDL uptake, lipoprotein lipase (LPL) related to chylomicron uptake, and fatty acid transporter proteins (FATPs) and CD36 genes related to fatty acid transport (Wang et al., 2017a; Wang et al., 2016b). The positive effects of ALA-rich flaxseed oil on adipose tissue lipolysis might be associated with attenuating alcohol-induced ER stress (Wang et al., 2016b). The ameliorative effects of endogenous and exogenous n-3 PUFAs on alcohol-stimulated adipose lipolysis may be attributed to the increased phosphodiesterase 3B (PDE3B) activity and reduced cyclic adenosine 3',5'-monophosphate (cAMP) accumulation in adipocytes, which is associated with activating G protein-coupled receptor 120 (GPR120) and regulating Ca^{2+}/CaMKKβ/AMPK signaling in adipose tissue (Figure 2) (Wang et al., 2017a).

Interestingly, chronic alcohol challenge also upregulates the expression levels of the genes related to fatty acid uptake in the liver, including CD36, FATP2 and FATP5. These alterations can be normalized by dietary flaxseed oil and endogenous n-3 PUFAs, indicating that n-3 PUFAs inhibit the entry of fatty acid into the liver (Wang et al., 2017a; Wang et al., 2016b). Additionally, dietary flaxseed oil

downregulates alcohol-induced expression of diacylglycerol acyltransferase 2 (DGAT2), a key enzyme responsible for triglyceride synthesis, and upregulates microsomal triglyceride transfer protein (MTP), an essential lipid transfer protein that is required for the assembly and secretion of VLDL in the liver, indicating that the protective effect of n-3 PUFAs against alcoholic steatosis may also be attributed to the inhibition of triglyceride synthesis and increase in VLDL export in the liver (Wang et al., 2016b).

n-3 PUFAs reduce alcohol-induced oxidative stress in the liver

Convincing evidence has demonstrated that alcohol-induced oxidative stress plays a central role in the pathogenesis and progression of ALD (Albano, 2008). Oxidative stress-related alcohol toxicity is mainly caused by excess generation of ROS, including superoxide, hydroxyl radical and hydrogen peroxide, which is toxic to hepatocytes because it causes oxidative modification of cellular macromolecules, leading to DNA damage, protein denaturation, and lipid peroxidation of the cellular membrane (Sugimoto and Takei, 2017; Wu and Cederbaum, 2009). Alcohol-provoked ROS are mainly generated by the ethanol-metabolizing cytochrome P450 2E1 (CYP2E1) in hepatocytes. Either acute or chronic alcohol exposure induces CYP2E1 by protein stabilization via impeding ubiquitin-mediated proteasomal degradation of CYP2E1. CYP2E1 catalyzes the conversion of ethanol to acetaldehyde and causes overproduction of ROS (Beckman et al., 1990; Jimenez-Lopez and Cederbaum, 2005). Excess ROS are physiologically counterbalanced by endogenous nonenzymatic antioxidants, including glutathione (GSH), enzymatic antioxidants, including superoxide dismutase (SOD) and other redox molecules, including heme oxygenase-1 (HO-1), during alcohol consumption (Wang et al., 2017b). Alcohol can also upregulate the expression of inducible nitric oxide synthase (iNOS), which plays a key role in alcohol-dependent hepatotoxicity and mitochondrial dysfunction (Venkatraman et al., 2004).

The impact of n-3 PUFAs in alcohol-induced oxidative stress has been controversial. Song *et al.* reported that DHA/AA treatment normalized alcohol-induced oxidative/nitrosative stress, as evidenced by the decreased hepatic levels of CYP2E1 and iNOS, mitochondrial hydrogen peroxide, and oxidatively modified mitochondrial proteins in chronic alcohol-fed rats (Song et al., 2008). Dietary ALA-rich flaxseed oil also ameliorates oxidative stress induced by chronic alcohol exposure, as evidenced by normalizing the alterations of MDA, GSH and SOD in the liver (Wang et al., 2016a). Consistently, endogenous n-3 PUFA enrichment inhibits the expression of hepatic CYP2E1 and increases the protein level of HO-1, a strong antioxidant with anti-inflammatory effects, in the liver (Huang et al., 2015). By contrast, several studies have shown that fish oil intensifies alcohol-induced oxidative stress (Marmillot et al., 2000; Nanji et al., 2004; Nanji et al., 1994a; Wada et al., 2008). The most plausible explanation would appear to be that n-3 PUFA oxidation *ex vivo* may occur in these studies with opposite results.

n-3 PUFAs attenuate alcohol-induced hepatic inflammation

Accumulated lipid causes hepatocytes to be susceptible to further injury, which may result in inflammation in the liver. It is well recognized that hepatic inflammation plays an extremely important role in the progression of ALD, especially the progression from hepatic steatosis to alcoholic hepatitis, which is featured by persistent liver inflammation (Chayanupatkul and Liangpunsakul, 2014; Purohit et al., 2004). Either acute or chronic alcohol exposure disrupts intestinal barrier function, leading to the leakage of bacterial endotoxin, a trigger of inflammation, from the gut lumen into the portal vein (Zhong et al., 2010). After binding to lipopolysaccharide binding protein (LBP), gut-derived endotoxin can be recognized by Toll-like receptor 4 (TLR4) by the aid of its coreceptor, CD14, resulting in the activation of Kupffer cells in the liver and the TLR4-mediated cascade (Hritz et al., 2008; Yoon et al., 2012). Both TLR-4-knockout mice (Hritz et al., 2008) and CD14-deficiency mice (Yin et al., 2001) have been shown to be resistant to hepatic inflammation and liver injury induced by alcohol exposure. Activation of the TLR4-mediated cascade requires the recruitment of adaptor molecules, such as myeloid differentiation factor 88 (MyD88), leading to nuclear factor κB (NF-κB) activation. In past decades, the anti-inflammatory properties of n-3 PUFAs have been well established by competitively inhibiting the metabolism of arachidonic acid, regulating the expression of several pro-inflammatory genes, and producing a family of anti-inflammatory lipid mediators (Yates et al., 2014). ALA-rich flaxseed oil has been shown to inhibit endotoxin-triggered inflammation induced by chronic-plus-single-binge ethanol feeding in mice via blocking the TLR4/MyD88/NF-κB cascades in the liver (Wang et al., 2016a).

NF-κB is a pivotal transcription factor that regulates the expression of many pro-inflammatory genes, including cytokines (e.g., TNF-α, IL-1β and IL-6), chemokines (e.g., MCP-1) and eicosanoid metabolism enzymes (e.g., cyclooxygenase-2 and COX-2) (Neuman, 2003). A close correlation between the circulating inflammatory cytokine levels and severity of alcoholic hepatitis (Fujimoto et al., 2000) and alcoholic cirrhosis (Hanck et al., 1998) has been established in clinical studies. The roles of cytokines in ALD have also been well summarized previously (Kawaratani et al., 2013; Neuman, 2003; Neuman et al., 2015). TNF-α, released by the activated Kupffer cells and recruited monocytes, is one of the most pivotal cytokines in ALD (Honchel et al., 1992; Khoruts et al., 1991; Song et al., 2004; Tilg et al., 2003). Pentoxifylline, a phosphodiesterase inhibitor that decreases the transcription of the TNF-α promoter, has been used for the treatment of alcoholic hepatitis in clinical trials (Chayanupatkul and Liangpunsakul, 2014). Higher circulating and hepatic levels of IL-6 were found in alcohol-fed animals (Horiguchi et al., 2008; Huang et al., 2013). Interestingly, opposite results were obtained regarding the role of IL-6 in animal models of alcoholic steatosis and liver damage. IL-6-deficient mice exhibited more susceptibility to alcoholic liver injury (El-Assal et al., 2004; Hong et al., 2002; Zhang et al., 2010). Treatment with IL-6 has beneficial effects on fatty liver diseases *in vivo* (Hong

et al., 2004). Obviously, the role of IL-6 in ALD remains obscure and inconclusive. Furthermore, as an important chemokine, MCP-1 is fundamentally implicated in regulating the recruitment of monocytes/macrophages to the sites of injury. MCP-1 deficiency protects against alcohol-induced liver injury (Mandrekar et al., 2011). Studies have demonstrated that flaxseed oil (Wang et al., 2016a; Zhang et al., 2017), fish oil (Chen et al., 2016), endogenous n-3 PUFAs (Huang et al., 2015; Wang et al., 2017a) and DHA (Huang et al., 2013) decreased the hepatic production of inflammatory cytokines, including TNF-α, IL-1β and IL-6, and the chemokine MCP-1 in alcohol-fed animals. By contrast, several studies have indicated that fish oil accentuated alcohol-induced inflammation, as evidenced by the increased TNF-α and COX-2 in the liver (Donohue et al., 2004; Wada et al., 2008). These outcomes may be associated with the poor quality of fish oil used.

n-3 PUFAs maintain intestinal permeability and modulate gut microbiota

Many studies have demonstrated that an imbalanced intestinal homeostasis is implicated in the pathogenesis and progression of ALD by promoting liver injury and inflammation (Rao et al., 2004). Selective permeability of the intestinal barrier made the essential nutrients influx into the circulation and block the passage of toxic compounds, such as endotoxin (Farhadi et al., 2003). Alcohol and its metabolites, particularly acetaldehyde, causes imbalanced intestinal homeostasis by destroying the integrity of the intestinal epithelial cell layer (Lippai et al., 2014) and disrupting intestinal barrier function, leading to alcohol-induced endotoxemia and bacterial translocation (Zhong et al., 2010). Numerous clinical and animal studies have supported that alcohol exposure causes increased circulating levels of endotoxin derived from Gram-negative gut bacteria, which is closely correlated with the severity of alcohol-induced liver injury (Jokelainen et al., 2001; Szabo and Bala, 2010; Wang et al., 2015). Supplementation with either fish oil (Lakshman et al., 1988) or flaxseed oil (Wang et al., 2016a; Zhang et al., 2017) has shown to reverse the elevated plasma endotoxin induced by chronic alcohol exposure. Furthermore, the alcohol-induced expression of bacterial 16S rRNA in the liver, a marker of bacterial translocation, was notably downregulated by dietary flaxseed oil (Wang et al., 2016a).

The elevated intestinal permeability to macromolecules and gut microbiota dysbiosis contribute to alcoholic endotoxemia (Zhong et al., 2010). Intestinal permeability is mainly maintained by the epithelium and apical junction complex between epithelial cells, including adherens junctions and tight junctions (Laukoetter et al., 2006). Acetaldehyde induces tyrosine phosphorylation of tight junction-associated proteins, including zonula occludens-1 (ZO-1), occludins and claudins, resulting in enhanced intestinal permeability to endotoxin (Basuroy et al., 2005). A recent study by Marmillot *et al.* indicated that fish oil can reverse the increased intestinal permeability caused by chronic alcohol exposure, as measured by urinary lactulose/mannitol ratio (Chen et al., 2016). Flaxseed oil downregulated the transcriptional expression of ZO-1, occludin and claudin-4 in the ileum tissue of mice with chronic-plus-single-binge ethanol feeding (Wang et al., 2016a). Accumulating evidences has indicated that gut microbiota dysbiosis also plays a crucial role in ALD. The impaired gut microbiota homeostasis by alcohol exposure induces the overgrowth of pathogenic bacteria, particularly Gram-negative bacteria, in the gut, generating excessive endotoxins (Szabo, 2015). Reducing the overgrowth of gut Gram-negative bacteria by the oral administration of probiotics, e.g., *Lactobacillus rhamnosus* GG (Wang et al., 2012), and antibiotics, polymyxin B and neomycin (Adachi et al., 1995), protect against alcohol-induced endotoxemia and liver injury. Zhang *et al.* conducted fecal metagenomic analysis using microbial 16S rRNA and revealed that chronic alcohol exposure caused an obvious difference in fecal bacterial species over pair-fed feeding, and dietary flaxseed oil markedly decreased the gut proportion of *Proteobacteria* and decreased the proportion of *Porphyromonadaceae* in chronic alcohol exposure (Zhang et al., 2017). The other study revealed that the partial substitution of olive oil with fish oil in diets notably decreased the gut proportion of *Escherichia coli* and increased the number of *Bifidobacterium* in the feces of rats under chronic ethanol feeding (Chen et al., 2016).

Perspective of n-3 PUFAs for the treatment of ALD

Despite severe public health concerns and the heavy economic burden of ALD, few satisfactory therapeutic modalities are available for the management of ALD, except for alcohol abstinence (Altamirano and Bataller, 2011). Thus, the efficacious and safe nutritional or therapeutic approaches are essential to prevent the onset and progression of ALD. Over the recent decade, increasing attention has been paid to understand the association between n-3 PUFAs and ALD. In the present review, we comprehensively summarized the current findings of n-3 PUFAs and n-3 PUFA-rich oils in the cited literatures that were specifically used to treat ALD. Although still controversial, n-3 PUFAs derived from both plants and seafoods may be useful in alleviating alcoholic steatosis and alcohol-induced liver injury through multiple mechanisms, including decreased *de novo* lipogenesis and lipid mobilization from adipose tissue, enhanced fatty acid β-oxidation, reduced hepatic inflammation and oxidative stress, and promoted intestinal homeostasis, positively suggesting that n-3 PUFAs might be promising for the management of ALD. However, additional studies considering the following issues will be needed to determine the role of n-3 PUFAs in ALD. (*i*) The oxidation of n-3 PUFAs prior to feeding was rarely considered. There is evidence that oxidized n-3 PUFAs can increase oxidative stress and inflammation in the liver, leading to exacerbated alcohol-induced liver injury. It is unclear whether the observed results in these studies are derived from n-3 PUFAs or its oxidation product. (*ii*) Fish oil, containing abundant long-chain n-3 PUFAs, is commonly used in animal studies, but the content and type of n-3 PUFAs are greatly varied in different commercial fish oil product. In addition to n-3 PUFAs, other compositions, including SFAs, MUFAs, n-6 PUFAs,

cholesterol and antioxidants, are also present in fish oil. Thus, the contents of n-3 PUFAs and other compositions are suggested to be considered before planning future studies investigating the effects of n-PUFAs in ALD. (*iii*) The *fat-1* mouse is a well-controlled model to explore the impacts of n-3 PUFAs in ALD. However, transgenic animals may not well mimic human dietary exposure to n-3 PUFAs, which can be resolved by arranging an additional group of WT mice fed high-quality n-3 PUFAs. (*iv*) Although almost all current studies have been conducted to understand the effects of n-3 PUFAs on hepatic steatosis and liver injury induced by alcohol, the potential impacts of n-3 PUFAs on alcoholic fibrosis and cirrhosis remain largely unclear. (*v*) The benefits of n-3 PUFAs in ALD eventually remains to be evaluated in a randomized, placebo-controlled clinical trial.

Conclusion

In this review, we summarized the current findings from recent studies determining the role of n-3 PUFAs in ALD and their underlying mechanisms. Although the results remain controversial, increasing evidence indicates the beneficial effects of n-3 PUFAs in alcohol-related liver diseases. The oxidation of n-3 PUFAs prior to feeding might mainly contribute to the conflicting conclusions. These findings suggest that supplementation with n-3 PUFAs may offer a new prevention/treatment option in the management of ALD, but more effort is necessary to explore the impact of n-3 PUFAs in nutritional and clinical studies.

Abbreviations

AA	arachidonic acid
ACC	acetyl-CoA carboxylase
ACLY	ATP-citrate lyase
ACO	acyl-CoA oxidase
ALA	α-linolenic acid
ALD	alcoholic liver disease
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMPK	AMP-activated protein kinase
AST	aspartate aminotransferase
ATGL	adipose triglyceride lipase
cAMP	cyclic adenosine 3',5'-monophosphate
COX	cyclooxygenases
CPT1	carnitine palmitoyltransferase 1
CYP2E1	cytochrome P450 2E1
DGAT2	diacylglycerol acyltransferase 2
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
DTA	docosatetraenoic acid
EPA	eicosapentaenoic acid
ER	endoplasmic reticulum
ETA	eicosatetraenoic acid
FAS	fatty acid synthase
FATPs	fatty acid transporter proteins
GLA,γ	linolenic acid
GPR120	G protein-coupled receptor 120
GSH	glutathione;
H&E	haematoxylin-eosin;
HO-1	heme oxygenase-1;
HSL	hormone-sensitive lipase;
IL-6	interleukin-6
iNOS	nitric oxide synthase
LA	linoleic acid
LBP	lipopolysaccharide binding protein
LOX	lipoxygenases
LXR	liver X receptor
LXREs	LXR response elements
MCP-1	monocyte chemotactic protein-1
MTP	microsomal triglyceride transfer protein
MUFA	monounsaturated fatty acids
MyD88	myeloid differentiation factor 88
n-3 PUFAs	omega-3 polyunsaturated fatty acid
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NF-κB	transcription factor nuclear factor κB
PDE3B	phosphodiesterase 3B
PPAR	peroxisome proliferator-activated receptor
ROS	reactive oxygen species
RXR	retinoid X receptor
SCD-1	stearyl CoA desaturase-1
SDA	stearidonic acid
SFA	saturated fatty acids
SIRT1	sirtuin 1
SOD	superoxide dismutase
SREBP	sterol regulatory element-binding protein
TB	total bilirubin
TC	total cholesterol
TG	triacylglycerol
TLR4	Toll-like receptor-4
TNF-α	tumor necrosis factor-α
VLDLR	very low-density lipoprotein receptor
WT	wild type;
ZO-1	zonula occludens-1

Disclosure statement

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