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Review Article

Dietary fatty acids and bioactive fatty acid metabolites in alcoholic liver disease☆

Kara H. Zirnheld [a], Dennis R. Warner [a], Jeffrey B. Warner [a, b], Josiah E. Hardesty [a], Craig J. McClain [a, b, c, d], Irina A. Kirpich [a, b, c, *]

[a] Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, University of Louisville School of Medicine, Louisville, KY, USA
[b] Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, USA
[c] University of Louisville Alcohol Center, University of Louisville School of Medicine, Louisville, KY, USA
[d] Robley Rex Veterans Medical Center, Louisville, KY, USA

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ABSTRACT

Alcoholic liver disease (ALD) comprises a spectrum of liver pathology, including steatosis, steatohepatitis, and cirrhosis. Previous work from our group and others suggests that dietary fat, both the amount and composition, plays a pivotal role in ALD development and progression; however, the impact of specific dietary fatty acids on ALD pathogenesis is not fully elucidated. Preclinical rodent models of ALD revealed the deleterious effects of omega-6 polyunsaturated fatty acids (n-6 PUFAs), specifically linoleic acid (LA), and this may be partially attributed to the increased levels of pro-inflammatory oxidized LA metabolites. There is limited understanding regarding the role of omega-3 polyunsaturated fatty acids (n-3 PUFAs, such as alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid), and bioactive n-3 PUFA-derived lipid molecules in ALD. Given that majority of n-6 and n-3 PUFAs-derived metabolites are potent endogenous signaling molecules, knowledge regarding the changes in these lipid mediators may shed new light on the mechanisms contributing to ALD pathogenesis and reveal novel therapeutic targets and biomarkers of this disease. The current review summarizes relevant scientific literature regarding the role of dietary fat, distinct fatty acids, and bioactive fatty acid metabolites in ALD, and highlights recent advances in the field.

1. Introduction

1.1. Alcoholic liver disease (ALD)

Heavy alcohol consumption results in a wide range of multi-organ pathology, including ALD. ALD is currently recognized as a major cause of alcohol-related morbidity and mortality in the United States (US) and worldwide. The clinical spectrum of ALD ranges from reversible fatty liver (steatosis) to alcoholic steatohepatitis, fibrosis, and cirrhosis which, in some cases, may progress to hepatocellular carcinoma. Patients with alcoholic steatohepatitis may develop severe acute alcoholic hepatitis (AAH), a condition associated with liver failure and high mortality. The mechanisms of ALD development and progression are not well-determined and there is no Food and Drug Administration (FDA)-approved therapy for ALD prevention or treatment. Most individuals chronically consuming >40 g of alcohol per day develop alcohol-associated liver injury, but advanced stages of ALD develop only in a subset of long-term heavy drinkers.[1,2] Sex,[3,4] genetic polymorphisms,[5] epigenetic changes,[6] drinking pattern,[7] and environmental factors (*e.g.,* smoking) are other well-known risk factors for ALD development/progression. Dietary fat is increasingly recognized as a prominent risk factor in the development of ALD, and will be discussed further.

1.2. Dietary fat in health and disease

The composition of dietary fat is known to play important roles in human health and in many chronic diseases, including alcoholic and non-alcoholic liver disease.[8–10] While there are several

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* Corresponding author. Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, University of Louisville, Louisville, KY, USA.
E-mail address: i0kirp01@louisville.edu (I.A. Kirpich).

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interventional studies examining the impact of the quantity and quality of dietary fatty acids (FAs) in clinical non-alcoholic fatty liver disease (NAFLD) (reviewed in Ref. 10), studies investigating the role of dietary fat in clinical ALD are remarkably limited. However, there is evidence from preclinical studies that highlights the importance of dietary fat, specifically the type of dietary FAs, as a key factor in ALD pathogenesis.[11] While animal models can help to identify the effects of different dietary FAs on ALD development and progression, the precise role of specific dietary fats in ALD pathogenesis in humans is yet to be elucidated. Addressing this knowledge gap through future research is vital in order to develop potential nutritional prevention and treatment strategies for ALD.

Studies from our laboratory and others have demonstrated that both alcohol and specific dietary fats play important interactive roles in the pathogenesis of ALD.[11] Evidence from rodent models demonstrated the injurious effects of omega-6 polyunsaturated fatty acids (n-6 PUFAs), specifically linoleic acid (LA), which may be partially attributed to increased levels of pro-inflammatory oxidized LA metabolites (OXLAMs) derived via the lipoxygenase (LOX) pathway.[12,13] Less is known about the role of oxidized PUFA metabolites generated through other metabolic pathways (*e.g.*, cytochrome P450 epoxygenases (CYPs)) or the lipid mediators derived from omega-3 polyunsaturated fatty acids (n-3 PUFAs) such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Given that the majority of PUFA metabolites are potent endogenous signaling molecules that function through multiple pathways, identification of changes in specific lipid mediators may shed new light on the mechanisms contributing to ALD pathogenesis/progression and reveal novel therapeutic targets and biomarkers of this disease. Because there are no FDA-approved therapies for ALD, the potential health benefits of oxylipins derived from n-3 PUFAs (*e.g.*, resolvins, protectins, and maresins) and specific PUFA-CYP-derived metabolites (PUFA epoxides) make these molecules potential candidates for new ALD therapies.[14,15]

In this review, we summarize the current knowledge on the role of dietary fat and distinct FAs in ALD pathogenesis. We also discuss the importance of various bioactive FA metabolites in the context of alcohol-associated liver injury.

2. FAs: types and dietary intake

2.1. Saturated vs. unsaturated FAs

FAs are categorized based on carbon chain length, degree of saturation, double bond position, and configuration (cis *vs.* trans). The number of carbon atoms in the hydrocarbon chain is used to define FAs as short-chain (SCFAs, 1–6 carbons), medium-chain (MCFAs, 7–12 carbons), long-chain (LCFAs, 13–21 carbons), or very-long-chain (VLCFAs, ≥22 carbons).[16,17] FAs are classified as either saturated or unsaturated depending on the absence or presence of carbon-to-carbon double bonds in their hydrocarbon chain. Saturated fatty acids (SFAs) are fully hydrogenated, and thus contain no double bonds between carbons. Saturated SCFAs include butyric acid (4:0) and caproic acid (6:0); MCFAs include caprylic acid (8:0), capric acid (10:0), and lauric acid (12:0); LCFAs include myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0); VLCFAs include behenic acid (22:0) and lignoceric acid (24:0).[18] Unsaturated fatty acids (UFAs) contain one or more double bonds in their hydrocarbon chain and can be further classified as monounsaturated fatty acids (MUFAs, one double bond, *e.g.,* palmitoleic acid (16:1) and oleic acid (18:1)) or polyunsaturated fatty acids (PUFAs, two or more double bonds).[19] PUFAs can be grouped into two families based on the position of the first double bond on the methyl (also referred to as omega) terminal end.[17] The n-3 PUFAs, *e.g.*, ALA (18:3), EPA (20:5), and DHA (22:6), contain a terminal double bond at the n-3 position. The n-6 PUFAs, *e.g.*, LA (18:2) and arachidonic acid (AA, 20:4), contain a terminal double bond at the n-6 position.

2.2. Dietary intake of total fat in the US population

The US Institute of Medicine (IOM) has established age-specific dietary macronutrient intake ranges (known as Acceptable Macronutrient Distribution Ranges (AMDRs) and determined as a percentage of total energy consumption) that are recommended to ensure adequate intake of essential nutrients and reduce the risk of chronic disease. The AMDR for total fat is 20–35% of total calories (kcal) for adult individuals.[20] Additionally, the 2015–2020 Dietary Guidelines for Americans (DGA) recommend limiting saturated fat (SF) intake to less than 10% of total kcal per day by replacing SF with unsaturated fat (USF) while maintaining total fat consumption within the AMDR.[21] For example, if an individual is consuming a 2000 kcal/day diet, total fat intake ranging from 44.4 to 77.8 g/day and SF intake of 22.2 g/day or less would meets these recommendations. DGA recommendations to substitute dietary SF with MUFAs and PUFAs are made based on observations that replacing SF with USF can reduce serum cholesterol and cardiovascular disease risk.[22–25] This recommendation should be taken with caution as a recent report showed that replacing SF with USF (specifically n-6 PUFA, LA) effectively lowers serum cholesterol but does not support the hypothesis that this translates to a lower risk of death from coronary heart disease or from all causes.[26] Moreover, replacing SF with n-6 PUFAs (without simultaneously increasing n-3 PUFAs) may increase the risk of CHD and death.[27]

According to the 2015–2016 National Health and Nutrition Examination Survey (NHANES) data, individuals 20 years and older in the US consume a daily average of ~2100 kcal with 36.0% of total kcal from fat (equivalent to 84.1 g/day), 11.8% of total kcal from SF (equivalent to 27.5 g/day), and 21.0% of total kcal from USF (equivalent to 49.0 g/day), suggesting that dietary fat consumption of US adults is slightly above the recommendations.[28] Average daily dietary fat intake per individual by age and gender in the US is shown in Fig. 1.

2.3. Dietary intake of distinct n-3 and n-6 PUFAs in the US population

The n-3 PUFA, ALA, and the n-6 PUFA, LA, are essential FAs, and therefore must be obtained through the diet.[29,30] Once ingested,

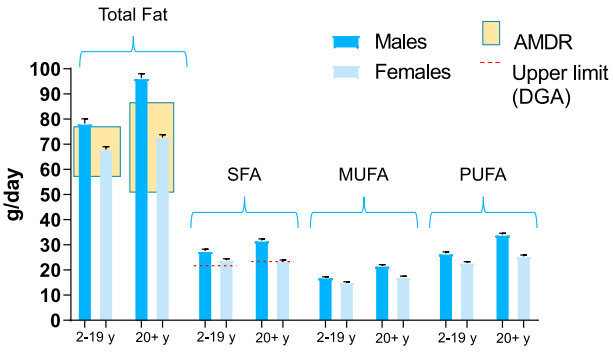

Fig. 1. Average dietary fat intake. Average daily dietary fat intake consumed per individual by age and gender in the US, based on data from the 2015–2016 NHANES.[28] AMDRs were calculated using average daily energy intakes of 1868 kcal and 2105 kcal for US individuals 2–19 and 20+ years, respectively.[28] Abbreviations: AMDR, Acceptable Macronutrient Distribution Range; DGA; Dietary Guidelines for Americans; g, grams; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; y, years.

mammalian cells can convert ALA to EPA and DHA and LA to AA (arachidonic acid) via a series of desaturation and elongation reactions. According to the Food and Nutrient Database for Dietary Studies (FNDDS), ALA and LA are typically found in high concentrations in nuts, seeds and vegetable oils (*e.g.,* flaxseed oil (53% of total fat from ALA; 14% of total fat from LA), soybean oil (8% of total fat from ALA; 52% of total fat from LA), and sunflower oil (0.5% of total fat from ALA; 66% of total fat from LA)).[31,32] From greatest to least, salad dressing, chicken and mixed chicken dishes, grain-based desserts, pizza, and yeast breads are the top sources of ALA in the American diet.[33] The top food sources of LA, in descending order, are chicken and chicken mixed dishes, grain-based desserts, salad dressing, potato/corn/other chips, and nuts/seeds and nut/seed mixed dishes.[33]

The AMDRs for ALA and LA are 0.6–1.2% and 5–10% of total kcal, respectively. Based on a 2000 kcal/day diet, ALA intake of 1.3–2.7 g/day and LA intake of 11.1–22.2 g/day meets IOM recommendations. Per 2015–2016 NHANES data, the US adults 20 years and older consume a daily mean of 0.9% of total kcal from ALA (equivalent to 1.84 g/day) and 8.2% of total kcal from LA (equivalent to 17.1 g/day); both averages are within IOM recommendations.[28] Average daily dietary n-6 and n-3 PUFAs intake per individual by age and gender in the US is shown in Fig. 2. While there are no AMDRs established for EPA and DHA, several health organizations have issued recommendations to increase dietary intake of these n-3 PUFAs due to their associated health benefits.[34] Given that fish, including sardines, herring, and mackerel, are a significant source of both EPA and DHA,[31] the American Heart Association recommends consuming oily fish twice per week to reduce risk of cardiovascular disease.[35] The 2015–2020 DGA also recommends consuming 8 or more ounces of seafood per week (providing an average of 250 mg EPA and DHA) due to its association with a reduction in cardiac death risk.[21,36] Further, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends a minimum daily intake of 500 mg EPA and DHA for cardiovascular health.[37] According to Hibbeln *et al.*,[38] for current US diets of 2000 kcal/day, a healthy dietary allowance of n-3 PUFAs was estimated to be 3.5 g/day. Additionally, these authors suggested that lowering LA intake can decrease an individual's need for n-3 PUFAs. According to the National Cancer Institute's analysis of 2005–2006 NHANES data, the top food sources of EPA and DHA in the American diet, from greatest to least, are fish, chicken, shrimp, eggs, and tuna and their associated mixed dishes.[33]

2.4. n-6/n-3 PUFA ratio

Given that n-6 and n-3 PUFAs each have specific, often opposing, physiological functions, a balanced n-6:n-3 PUFA ratio plays an important role in health and disease.[39] NHANES data indicate a LA:ALA ratio of ~9:1 for US males and females 20 years and over (Fig. 2), suggesting a highly unbalanced consumption of n-6 and n-3 PUFAs in the Western diet as compared to the n-6:n-3 PUFA ratio of ~2:1 for the traditional hunter-gatherer diet consumed by pre-agricultural humans.[28,40] The Agricultural Revolution marked a shift from a diet consisting predominantly of wild game and plants to a diet dependent on cereal grains for food production, thereby increasing the proportion of n-6 PUFAs in the diet relative to n-3 PUFAs.[40,41] The imbalance of n-6 and n-3 PUFAs in the diet has been further compounded by the advent of the vegetable oil industry and the use of grain-based feeds in agribusiness.[40] For example, during the 20th century, per capita consumption of soybean oil (52.1% LA, 7.8% ALA) in the US increased from 0.006% to 7.38% of energy.[42] Overall, these shifts in the US food composition have resulted in the imbalanced n-6:n-3 PUFA ratio associated with the modern Western diet. Increased amounts of n-6 PUFAs in the diet lead to a greater formation of biologically active and potentially pro-inflammatory metabolites, *e.g.*, eicosanoids from AA such as prostaglandins, thromboxanes, and leukotrienes. Production of these metabolites outweighs that of n-3 metabolites with predominantly anti-inflammatory properties.[43] A high n-6:n-3 PUFA ratio may create a pro-inflammatory environment contributing to the development or progression of diet-related chronic diseases including cardiovascular disease and NAFLD.[40,44]

2.5. Diets promoting an optimal n-6:n-3 PUFA ratio

Along with the Western diet, several other dietary patterns with distinct nutrient compositions are followed today, including the Mediterranean and Paleolithic diets. The Mediterranean diet is

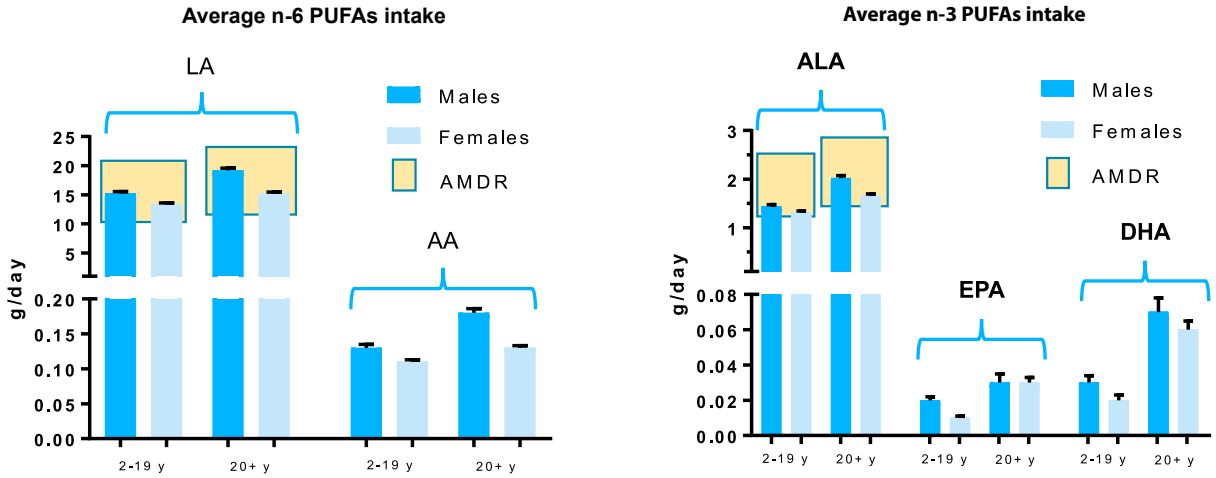

Fig. 2. Dietary intake of distinct n-3 and n-6 PUFAs. Average daily n-3 and n-6 PUFAs intake per individual by age and gender in the US, based on data from the 2015–2016 NHANES.[28] AMDRs calculated using average daily energy intakes of 1868 kcal and 2105 kcal for US individuals 2–19 and 20+ years, respectively.[28] Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; AMDR, Acceptable Macronutrient Distribution Range; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; g, grams; kcal, calories; LA, linoleic acid; n-3 PUFAs, omega-3 polyunsaturated fatty acids; n-6 PUFAs, omega-6 polyunsaturated fatty acids; y, years.

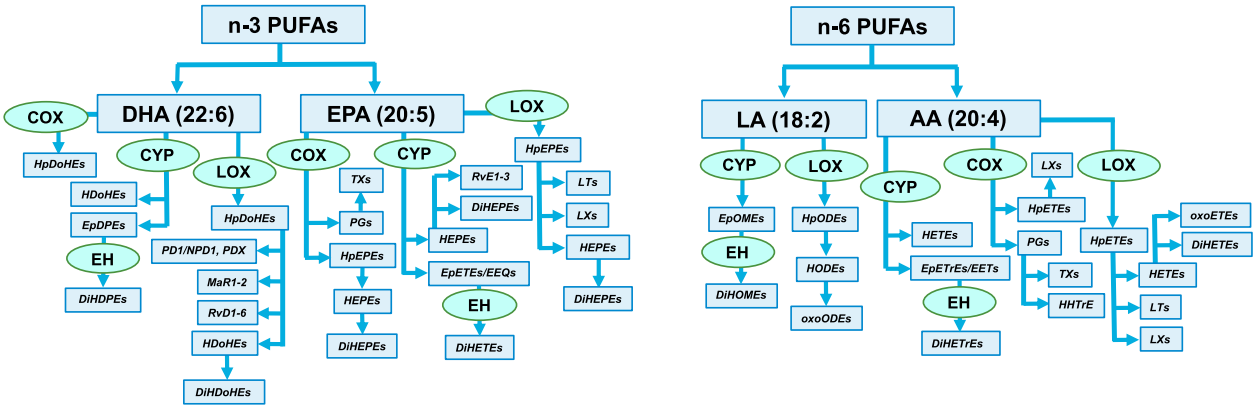

Fig. 3. n-3 and n-6 PUFAs-derived metabolites. Schematic diagram exhibits key enzymes and pathways of n-3 and n-6 PUFAs metabolism with production of various metabolites/oxylipins from parental FAs, such as docosanoids from DHA, eicosanoids from EPA and AA; and octadecanoids from LA. Enzymes are indicated in ovals and major products are boxed. Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; CYP, cytochrome P450; DHA, docosahexaenoic acid; DiHDoHEs, dihydroxy-docosahexaenoic acids; DiHDPEs, dihydroxy-docosapentaenoic acids; DiHEPEs, dihydroxy-eicosapentaenoic acids; DiHETEs, dihydroxy-eicosatetraenoic acids; DiHETrEs, dihydroxy-eicosatrienoic acids; DiHOMEs, dihydroxy-octadecenoic acids; EH, epoxide hydrolase; EPA, eicosapentaenoic acid; EpDPEs, epoxy-docosapentaenoic acids; EpETEs/EEQs, epoxy-eicosatetraenoic acids; EpETrEs/EETs, epoxy-eicosatrienoic acids; EpOMEs, epoxy-octadecenoic acids; HDoHEs, hydroxy-docosahexaenoic acids; HEPEs, hydroxy-eicosapentaenoic acids; HETEs, hydroxy-eicosatetraenoic acids; HHTrE, hydroxy-heptadecatrienoic acid; HODEs, hydroxy-octadecadienoic acids; HpDoHEs, hydroperoxy-docosahexaenoic acids; HpEPEs, hydroperoxy-eicosapentaenoic acids; HpETEs, hydroperoxy-eicosatetraenoic acids; HpODEs, hydroperoxy-octadecadienoic acids; LA, linoleic acid; LOX, lipoxygenase; LTs, leukotrienes; LXs, lipoxins; MaR, maresins; n-3 PUFAs, omega-3 polyunsaturated fatty acids; n-6 PUFAs, omega-6 polyunsaturated fatty acids; oxoETEs, oxo-eicosatetraenoic acids; oxoODEs, oxo-octadecadienoic acids; PD, protectins; PGs, prostaglandins; Rv, resolvins; TXs, thromboxanes.

characterized by a high intake of plant foods, *e.g.,* vegetables, fruits, nuts, seeds, and cereals; olive oil as the primary fat; moderate consumption of fish; red meat in small amounts; and alcohol primarily in the form of wine consumed with meals.[43] The health benefits of the Mediterranean diet, including its potent anti-inflammatory properties, are well-studied.[44–47] Further, a study by Ambring *et al.*[48] noted a 45% decrease in the serum phospholipid n-6:n-3 PUFA ratio of individuals consuming a Mediterranean-inspired diet for four weeks compared to an ordinary Swedish diet (5% and 1% of energy from n-6 and n-3 PUFAs, respectively), suggesting adoption of a Mediterranean-style eating pattern would reduce the imbalance of n-6 and n-3 PUFAs consumption in the industrialized world. The Paleolithic diet shares similarities with the Mediterranean diet in that both emphasize intake of plant foods and limit added sugar; however, the Paleolithic diet does not include grains, dairy products, or alcohol as is found in the Mediterranean diet.[49] The Paleolithic diet is modeled after that of pre-agricultural hunter-gatherers that foraged and hunted for food in the environment and is high in lean protein, PUFAs (especially n-3 PUFAs), MUFAs, fiber, vitamins, minerals, and antioxidants.[50] Consumption of a Paleolithic diet is thought to be beneficial, as this diet reflects the eating pattern that has evolutionarily programmed human metabolism and physiology.[51] Modeling intake to more closely resemble the diet of our ancient ancestors helps correct the disproportional intake of n-6 and n-3 PUFAs that characterizes the modern Western diet. Several health benefits have been demonstrated with consumption of a Paleolithic diet, including reductions in systemic inflammation and liver fat.[52,53]

3. FA-derived bioactive metabolites

Given that various FAs have distinct biochemical properties, they can exert differential metabolic and physiological effects acting as unaltered biochemical molecules or FA-derived bioactive metabolites. Recent advances in analytical technology, including liquid chromatography and mass spectrometry, have led to the identification of a variety of lipid mediators derived from n-6 and n-3 PUFAs with potentially pro-inflammatory or anti-inflammatory, pro-resolving, and anti-fibrotic activities, respectively.[54,55]

3.1. Oxidized PUFA metabolites and oxylipins

Oxidized PUFA metabolites (oxylipins) are formed through four major routes: (i) cyclooxygenases (COXs), (ii) LOXs, (iii) CYPs, and (iv) non-enzymatic autoxidation, particularly during oxidative stress. In some instances, the same metabolite can be produced from more than one pathway. Many of these compounds can be further metabolized via multiple routes; for example, hydrolysis of epoxides (epoxy-FAs, CYP metabolites) to their corresponding dihydroxy-FAs through the action of soluble epoxide hydrolase (sEH).[56] Diverse n-6 and n-3 PUFAs-derived oxylipins are shown in Fig. 3. The biological effects of distinct metabolites depend on the target tissue, concentrations, presence of other mediators, receptors, the nature of downstream signaling, and other factors. For example, OXLAMs induce mitochondrial dysfunction and apoptosis.[57] The n-3 PUFAs, EPA and DHA, are precursors to numerous metabolites, many of which (*e.g.*, resolvins, protectins, and maresins) possess beneficial health properties.[14,58,59]

3.2. PUFA peroxidation-derived products

Oxidative degradation of PUFAs via peroxidation generates a variety of electrophilic aldehydes and ketones called lipoxidation products.[60] The most abundant aldehyde produced by oxidation of n-6 PUFAs, such as AA and LA, is 4-hydroxy-2-nonenal (4-HNE). The group of lipoxidation products also includes 4-oxo-nonenal, malondialdehyde, and acrolein. Lipoxidation products can be produced via several metabolic pathways including enzymatic and non-enzymatic oxidation of PUFAs, and they can also be derived from sources other than PUFAs. Acrolein, for example, can be produced from the peroxidation of PUFAs as well as from the metabolism of amino acids and polyamines. PUFA lipoxidation products exert a variety of biological effects. In general, they are considered as toxic compounds due to their ability to modify deoxyribonucleic acid (DNA) and proteins; and they are also associated with the

onset of several pathological conditions such as endothelial dysfunction and inflammation.[61]

3.3. PUFA-derived endocannabinoids

Endocannabinoids are bioactive lipid mediators derived from dietary precursors, n-3 and n-6 PUFAs, through multiple distinct pathways.[62,63] The endocannabinoid system is widely distributed throughout the body, including the liver, and consists of endocannabinoids, enzymes for their biosynthesis and degradation, and their receptors.[64] The best characterized endocannabinoids, 2-arachidonoylglycerol (2-AG) and *N*-arachidonoyl-ethanolamine (anandamide, AEA), are synthesized from membrane-bound AA and function as endogenous ligands for cannabinoid receptors (CB1 and CB2).[63,65–67] These lipid mediators, along with their receptors, have been shown to play a role in the pathogenesis of various diseases, including NAFLD and ALD. Both ethanol and a high-fat diet can increase production of 2-AG and AEA, respectively, and activity of the CB1 receptor.[68,69] CB1 activation leads to increased lipogenesis and decreased FA oxidation, promoting hepatic fat deposition.[68] Preclinical animal and human studies have shown that endocannabinoid production and their associated receptor activity can be modulated by dietary intake,[62,67] pointing to dietary PUFA manipulation as a potential therapy for various pathologies of the liver.

3.4. Bacterial production of unique bioactive PUFA metabolites

Over the past decade, the gut microbiota have emerged as a key player in host health and disease. The role of gut microbial metabolites as critical signaling molecules that may function via host receptors has become increasingly recognized, focusing tremendous attention to the diet-gut microbiota-host homeostasis axis. In general, FA metabolites are endogenously generated in the host; however, accumulating evidence indicates that intestinal bacteria can also metabolize dietary lipids and generate bioactive lipid mediators.[70–72] For example, many species of lactic acid bacteria, *e.g.*, *Lactobacillus plantarum*, possess enzymes involved in the metabolism of PUFAs, generating multiple PUFA-derived species such as hydroxy- and oxo-conjugated and partially saturated *trans*-FAs.[71] Many of these unique lipid species exert specific physiological functions. For instance, 10-hydroxy-cis-12-octadecenoic acid, a gut microbial metabolite of LA, attenuated intestinal epithelial barrier dysfunction and ameliorated intestinal inflammation in dextran sulfate sodium (DSS)-induced colitis in mice by suppressing the up-regulation of tumor necrosis factor receptor 2 (TNFR2).[73] Given that alcohol consumption resulted in alterations in gut microbial composition and function,[74,75] including decreased abundance of *Lactobacillus* species,[76] investigating unique PUFA-derived bacterial products may provide new insight into the complex interactions between the gut microbiota and host metabolism.

4. The link between dietary fat and alcohol-induced liver disease: evidence from human studies

Evidence of a link between the diet (specifically dietary fat), alcohol consumption, and ALD came from the early epidemiologic (population-level) studies demonstrating significant correlations between rates of alcoholic cirrhosis mortality and the products of dietary animal fat and alcohol consumption.[8,77] Using epidemiological data from 17 countries, a study by Nanji and French[8] investigated the relationship between estimated alcoholic cirrhosis mortality, dietary cholesterol, and SF and USF. This study revealed that the percentage of deviations from expected cirrhosis mortality was negatively correlated with dietary cholesterol ($r = -0.86$, $P = 0.001$) and SFAs ($r = -0.80$, $P = 0.001$). A positive correlation was observed between PUFA consumption and percentage of deviations from expected cirrhosis mortality ($r = 0.55$, $P = 0.001$). The authors suggested that a diet high in cholesterol and SF protected against the development of alcoholic cirrhosis while polyunsaturated fats promoted cirrhosis. The same authors reported significant correlation between cirrhosis mortality and alcohol consumption ($r = 0.64$, $P < 0.01$), cirrhosis mortality and pork consumption ($r = 0.40$, $P < 0.05$), and cirrhosis mortality with both alcohol and pork consumption ($r = 0.98$, $P < 0.001$).[9] The authors indicated that the observed correlations do not necessarily imply a causal relationship and should be further investigated. It was unclear how pork consumption enhanced cirrhosis, but the authors speculated that pork contained more LA than other fat sources, *e.g.,* beef. To the best of our knowledge, there is a lack of studies to date that examine the causal relationship between different types of dietary fat and distinct dietary FAs in patients with ALD, or that investigate potential benefits of n-3 PUFA supplementation in clinical ALD, as it has been demonstrated in NAFLD.[78] Among a very few studies, a double-blind, placebo-controlled pilot study conducted by Fogaça *et al.*[79] examined effects of n-3 PUFA supplementation on alcohol dependence. In this trial, EPA+DHA dietary supplementation for 3 months did not reduce the amount of alcohol consumption, craving, or alcohol dependence severity in alcohol-dependent subjects as compared to placebo group. However, liver injury markers were not evaluated in this study.

5. Dietary FAs in alcohol-associated liver injury: evidence from preclinical animal models of ALD

The evidence regarding the effects of distinct dietary FAs on alcohol-induced liver pathology is predominantly derived from preclinical animal models. Our recent work provided a systematic review of the studies examining the role of dietary fat in ALD.[11] Here, we briefly summarize previous knowledge and highlight recent advances in the field.

5.1. Experimental Lieber-DeCarli (LDC) liquid diet utilized for animal models of ALD

The LDC liquid diet containing ethanol (EtOH) is the most commonly used experimental animal model for EtOH-induced multi-organ pathology, including the liver. There are several LDC modifications and customized formulations utilized by different research groups that vary in nutritional profile, some of them are shown in Table 1. The recent most widely used model, the so-called 10+1 or acute-on-chronic EtOH administration, suggests using an LDC formulation with corn oil, olive oil, and safflower oil as the major dietary fat sources (F1258SP from Bio-Serv, Flemington, NJ, USA).[80] Several studies from our group[12,81] and others[82] utilized a formulation with high levels of LA derived from corn oil as the major lipid source (*e.g.,* L10056 from Research Diets, New Brunswick, NJ, USA). A diet supplemented with beef fat and MCT oil (*e.g.,* L10058 from Research Diets) served as a control diet for the high LA formulation. It is important to note that all LDC formulations have a very high (>30:1) n-6:n-3 PUFA ratio. Notably, the percent of energy derived from LA in the L10056 diet is ~2.5-fold higher than that in the F1258SP diet (22.3% and 8.6%, respectively).

5.2. Dietary fat and the role of n-6 PUFA, LA, in preclinical models

Some of the early experimental evidence recognizing the importance of dietary fat in ALD demonstrated that dietary SF

Table 1
LDC liquid diet formulations commonly used in preclinical animal models of ALD.

	BioServ F1258SP 10+1 model	BioServ F1341SP low fat	Dyets 710260 regular	Research Diets L10056A
Kcal/L				
Protein	151	150	176	170
Carbohydrate	135	370	101	70
Fat	359	125	350	400
Ethanol	355	355	358	350
Kcal/L				
Corn Oil	77	27	75	356
Olive Oil	257	90	251	–
Safflower Oil	24	8	24	–
Soybean Oil	–	–	–	40
*g/L (standardized values)**				
Total SFAs	5.88	2.05	5.74	6.55
Total MUFAs	23.85	8.35	23.29	12.19
Total PUFAs	9.80	3.40	9.61	25.24
C18:2 Linoleic	9.51	3.30	9.32	24.51
C18:3 Linolenic	0.265	0.093	0.259	0.742
C18:2/C18:3 (n-6:n-3 PUFA ratio)	35.9	35.6	36.1	33.0

*When estimating fatty acid profiles for each diet, the following procedure was used to ensure standardized analysis: (i) each source of oil in the diet (kcal/L) was divided by 9 kcal/g (fat provides 9 kcal/g) to determine total grams of oil per liter of diet; (ii) total grams of oil per liter were multiplied by the respective percentage according to Dubois *et al.*[32] to determine grams of fatty acid provided from each source of oil; (iii) the sum of the grams of fatty acid provided from each source of oil in the diet was taken to determine total grams of individual fatty acids in the diet. Abbreviations: ALD, alcoholic liver disease; g, grams; Kcal, calories; MUFAs, monounsaturated fatty acids; n-3 PUFAs, omega-3 polyunsaturated fatty acids; n-6 PUFAs, omega-6 polyunsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

attenuated and dietary USF (specifically rich in corn oil, 56% LA), promoted alcohol-induced liver damage in rodents.[83,84] Moreover, dietary LA was required for the development of experimental EtOH-induced liver injury, and the severity of ALD was correlated with the amount of LA in the diet.[85] Further research showed that dietary SF, *e.g.,* palm oil (~44% palmitic acid (C16:0) and ~39% oleic acid (C18:1)) or MCT oil (predominantly octanoic (C8:0) and decanoic (C10:0) acids) reversed the established experimental ALD and beef tallow and MCT oil improved EtOH-induced liver pathology in a dose-dependent manner.[82,86] The deleterious effects of dietary USF in comparison to the protective effects of dietary SF are partially mediated through induction of lipid peroxidation and oxidative stress,[82,84,87–89] elevated endotoxin levels, and associated increased production of pro-inflammatory cytokines.[86,89,90] A decrease in liver and intestinal hepatocyte nuclear factor-alpha, a master transcriptional regulator of lipid metabolism, was associated with increased hepatic fat accumulation and disruption of intestinal barrier integrity in rats fed EtOH and dietary USF (corn oil) but not SF (MCTs).[91,92] Compared to EtOH and dietary corn oil, animals fed EtOH and dietary SF, *e.g.,* cocoa butter (rich in palmitic acid (C16:0) and stearic acid (C18:0)) or MCTs, had attenuated liver injury, reduced hepatic macrophage activation, neutrophil infiltration, and pro-inflammatory cytokine expression.[93] In addition, dietary cocoa butter or MCTs prevented endotoxemia observed in mice fed EtOH and a corn oil-rich diet. On a mechanistic level, MCTs prevented EtOH-induced down-regulation of intestinal tight junction (TJ) proteins, while cocoa butter normalized EtOH-increased hepatic endotoxin levels via up-regulation of an endotoxin detoxifying enzyme, argininosuccinate synthase 1.[93] The beneficial effects of dietary SF in comparison to dietary USF in experimental ALD was also attributed to the modulation of the hepatic SIRT1-SREBP-1-histone H3 axis, resulting in suppression of genes encoding lipogenic enzymes and the induction of adiponectin, an adipocyte hormone which plays a beneficial role in ALD.[94–96]

5.3. Dietary n-3 PUFA supplementation and endogenous n-3 PUFA enrichment in experimental ALD

While the damaging effects of dietary n-6 PUFAs, specifically LA, in ALD are well-documented (reviewed in Ref. 11), studies examining effects of dietary n-3 PUFAs are controversial. Protection,[97–99] exacerbation,[100,101] or no effects of n-3 PUFA supplementation on EtOH-induced hepatic steatosis and/or liver injury have been reported.[102] This controversy may be attributed to differences in experimental design, *e.g.,* doses, duration, and quality of n-3 PUFA supplementation; n-6:n-3 PUFA ratio in the experimental diets; and/or the nature of the animal models (*e.g.,* acute, chronic, or acute-on-chronic EtOH administration). Nevertheless, a growing body of evidence demonstrates the beneficial effects of dietary n-3 PUFAs or endogenous n-3 PUFA enrichment on EtOH-associated liver injury in preclinical animal models of ALD.[103] For example, a recent study[104] demonstrated that dietary flaxseed oil (a rich source of ALA) attenuated EtOH-induced hepatic steatosis in mice, reduced endoplasmic reticulum (ER) stress, and normalized lipid metabolism in adipose tissue.

The use of transgenic *fat-1* mice is an innovative and effective tool to examine the role of n-3 PUFAs in different pathologies, as it eliminates dietary modulation and supplementation as confounding factors. These mice express the *fat-1* gene that encodes a n-3 PUFA desaturase and catalyzes the endogenous conversion of n-6 to n-3 PUFAs, resulting in an increase in n-3 PUFAs and subsequent decrease in the n-6:n-3 PUFA ratio. Acute EtOH-induced liver injury was attenuated in *fat-1* mice, possibly through the down-regulation of hepatic lipogenesis, inflammatory responses, and oxidative stress.[105] A recent study by Wang *et al.*[103] demonstrated that both endogenous n-3 PUFA enrichment in *fat-1* mice as well as dietary DHA and EPA supplementation in wild-type mice alleviated acute-on-chronic EtOH-induced liver steatosis, liver injury, and inflammation confirmed by reduced hepatic triglycerides, decreased plasma levels of alanine- and aspartate-aminotransferase (ALT and AST, respectively), and diminished tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and monocyte chemoattractant protein-1 (MCP-1) protein levels in the liver. The authors suggested that inhibition of EtOH-induced adipose lipolysis via the PDE3B-AMPK axis underlined the beneficial effects of n-3 PUFAs on EtOH-induced liver steatosis and injury in their experimental models. Our unpublished data (manuscript under revision) showed that liver injury caused by chronic EtOH administration and single dose of lipopolysaccharide (LPS) challenge was attenuated in *fat-1* mice compared to wild-type littermates. Improvement of liver

injury was associated with beneficial changes in the intestinal homeostasis and the gut microbiota.

5.4. Dietary lipids, EtOH consumption, and the gut-liver axis

The evidence from preclinical animal studies underscores the importance of different types of dietary fat in EtOH-associated alterations in the gut-liver axis. Compared to a diet enriched in saturated MCFAs, a diet high in the n-6 PUFA, LA, exacerbated EtOH-induced intestinal inflammation, permeability, and endotoxemia associated with liver injury in mice.[81,106] A growing body of studies suggests that dietary FAs influence the gut microbiota composition, which, in turn, may affect host metabolic health.[107–109] Moreover, different types of dietary fat can differentially modulate EtOH-mediated changes in the gut microbiota. Compared to a MCT-rich diet, a diet high in n-6 PUFAs combined with excessive alcohol intake resulted in gut microbiota alterations, specifically a prominent reduction in Bacteroidetes and an increase in the Gram-negative Proteobacteria and the Gram-positive Actinobacteria phyla.[110] EtOH and a diet rich in oleic and linoleic acids resulted in reduced proportion of Firmicutes, increased numbers of Bacteroidetes, and reduced proportion of *Lactobacillus* species in experimental mice, while mice fed EtOH and a diet supplemented in palmitic and stearic acids maintained intestinal eubiosis.[111] Further, endogenous n-3 PUFA enrichment in experimental mice,[112] or n-3 PUFA supplementation in humans can alter gut microbial composition,[113] for example, by increasing several beneficial taxa, including the butyrate-producing *Roseburia*, *Bifidobacterium* and *Lactobacillus*.[114] We recently observed that EtOH administration can differentially modulate gut microbiota in transgenic *fat-1* mice (mice that are enriched in n-3 PUFAs) compared to wild-type littermates.[115] For example, Lactobacillaceae and Erysipelotrichaceae were depleted in EtOH-fed mice compared to control pair-fed mice in wild-type but not *fat-1* animals. In addition, Porphyromonadaceae: Barnesiella and Lachnospiraceae were differentially enriched in EtOH-fed *fat-1*, but not in EtOH-fed wild-type mice in response to LPS administration that was associated with improved liver injury in *fat-1* mice.

It is important to note that there are significant challenges to evaluate the effects of dietary FAs on EtOH-induced liver injury as they relate to changes in the gut microbiota in humans. Most studies have been conducted in mice and have yet to be tested in human populations. One of the few published studies on the liver injury-diet-microbiota axis demonstrated that a diet rich in fermented milk products, vegetables, cereals, and coffee/tea was associated with increased microbial diversity and reduced hospitalization in cirrhotic patients.[116] Although there are no studies testing the effects of specific dietary fats on ALD in humans, we can hypothesize that different types of dietary FAs may differentially affect gut microbiota, liver injury associated with EtOH consumption, and individual susceptibility to ALD in human population. Of note, evidence from clinical studies suggests that the gut microbiota can influence the severity of alcoholic hepatitis (AH). It has been reported that patients with severe AH (sAH) had elevated abundance of *Bifidobacteria* and *Streptococci*, and less *Atopobium* compared to patients with no AH (noAH).[117] *Streptococci and Enterobacteria* were positively correlated with AH severity. This study[117] has also elegantly demonstrated that intestinal microbiota contributes to individual susceptibility to ALD, that the degree of susceptibility is transmissible from patients to mice, and that this susceptibility could be reversed with normalization of the microbiota. The authors demonstrated that mice which received microbiota from a patient with sAH developed more severe alcohol-induced liver injury, inflammation, greater intestinal permeability and higher translocation of bacteria than mice which received microbiota from noAH patients.

6. Alcohol consumption and PUFA-derived bioactive metabolites: relevance to ALD

Bioactive lipid mediators play a crucial role in numerous pathological conditions and in the induction and resolution of inflammation.[14] Alterations in PUFA-derived lipid metabolites have been linked to the pathogenesis of numerous diseases, including ALD and NAFLD.[118–124] Both alcohol consumption and dietary factors can modify the pattern and the levels of PUFA-derived bioactive metabolites that, in turn, may affect physiological and pathological processes as well as general health.

6.1. Clinical studies

Barden *et al.*[125] conducted a study in which otherwise healthy men with regular moderate to high alcohol consumption were randomized to drink red wine (RW, 375 mL/day), the equivalent volume of dealcoholized red wine (DRW), or water for 4 weeks in a 3-period crossover study. Significantly elevated levels of plasma 20-HETE (a pro-inflammatory mediator) and anti-inflammatory specialized pro-resolving mediators (SPMs), including 18-HEPE (EPA metabolite) and RvD1 and 17R-RvD1 (DHA metabolites) were observed after consumption of RW compared with DRW and water. Levels of total plasma EETs and their sEH metabolites, DHETs, were unaffected by consumption of RW. The authors concluded that this increase could be due to alcohol-mediated activation of key enzymes involved in SPM synthesis. Interestingly, in a similarly designed study performed by the same research group, RW (300 mL/day for men and 230 mL/day for women) did not affect plasma levels of any SPMs in patients with type 2 diabetes mellitus.[126] The authors suggested that the differences between these two studies might be attributed to lower alcohol intake and the possible influence of concurrent treatment in patients with diabetes.

A recent paper by Gao *et al.*[127] evaluated serum and fecal oxylipin profiles in patients with alcohol use disorder (AUD) and patients with AH compared to non-alcoholic controls. The study identified several EtOH-mediated oxylipin changes and associations of these alterations with clinical parameters such as intestinal permeability and the degree of liver steatosis and fibrosis. Compared to controls, researchers noted significantly elevated 5-HETE, 5,6-DiHETrE, and 4-HDoHE levels in AUD patients that were associated with increased total gut permeability. 9,10-DiHHex, 8,9-EpETrE, and 20-HETE were positively correlated with steatosis in AUD patients. 20-HETE was also positively correlated with the grade of steatosis and polymorphonuclear neutrophil infiltration on liver biopsy and negatively correlated with 90-days survival in AH patients.

Elevated levels of bioactive OXLAMS, formed enzymatically from LA, primarily via the actions of 12/15-LOX or non-enzymatically via free radical-mediated oxidation in response to oxidative stress, are associated with ALD in humans and rodents.[12,119,128] Elevated plasma 9- and 13-HODE levels in patients with alcoholic cirrhosis were observed in parallel with an increase in hepatic expression of 15-LOX-1 and 15-LOX-2 mRNA. Notably, the plasma content of HODEs in patients with ALD was more than 46 times higher than that in healthy subjects and more than 4 times higher than in NAFLD patients.[119] In addition, this study also reported that plasma levels of 5-, 12-, and 15-HETEs (oxidized metabolites of AA) were also increased in patients with ALD.

6.2. Preclinical animal studies

6.2.1. EtOH and saturated and unsaturated dietary fat induce unique patterns of hepatic n-6 and n-3 PUFAs-derived oxylipins

In our recent study,[129] we identified diet-specific changes in the hepatic n-6 and n-3 PUFAs-derived oxylipins in response to chronic-binge EtOH administration in mice, which are summarized in Fig. 4. We found that EtOH+USF but not EtOH+SF caused elevated plasma ALT levels, hepatic steatosis, and inflammation. These pathologies were associated with increased levels of bioactive lipid metabolites generally involved in pro-inflammatory responses, including 13-HODE; 9,10- and 12,13-DiHOMEs; 5-, 8-, 9-, 11-, 15-HETEs; and 8,9- and 11,12-DiHETrEs in parallel with an increase in pro-resolving mediators, such as lipoxin A4. 18-HEPE and 5,6-DiHETE were elevated by EtOH in both SF and USF-fed groups and 17,18-EpETE was elevated exclusively in EtOH+SF-fed animals. These results suggested that dietary fat may significantly affect the pattern of EtOH-induced alterations in PUFA-derived metabolites; therefore, dietary composition needs to be taken into account when interpreting the results.

6.2.2. OXLAMs: mechanistic insight into ALD pathogenesis

EtOH-induced OXLAM levels, specifically 9- and 13-HODEs, were observed in several experimental animal models of ALD in parallel with hepatic steatosis, oxidative stress and hepatocyte damage.[12,13,128,130] Increases in HODEs were found in obese mice compared with lean mice, with a higher magnitude of change due to the interaction of obesity and alcohol.[123] The loss of *arachidonate 15-lipoxygenase* (*Alox15*) expression, the major enzymatic pathway of OXLAM production, did not appreciably alter OXLAM levels in

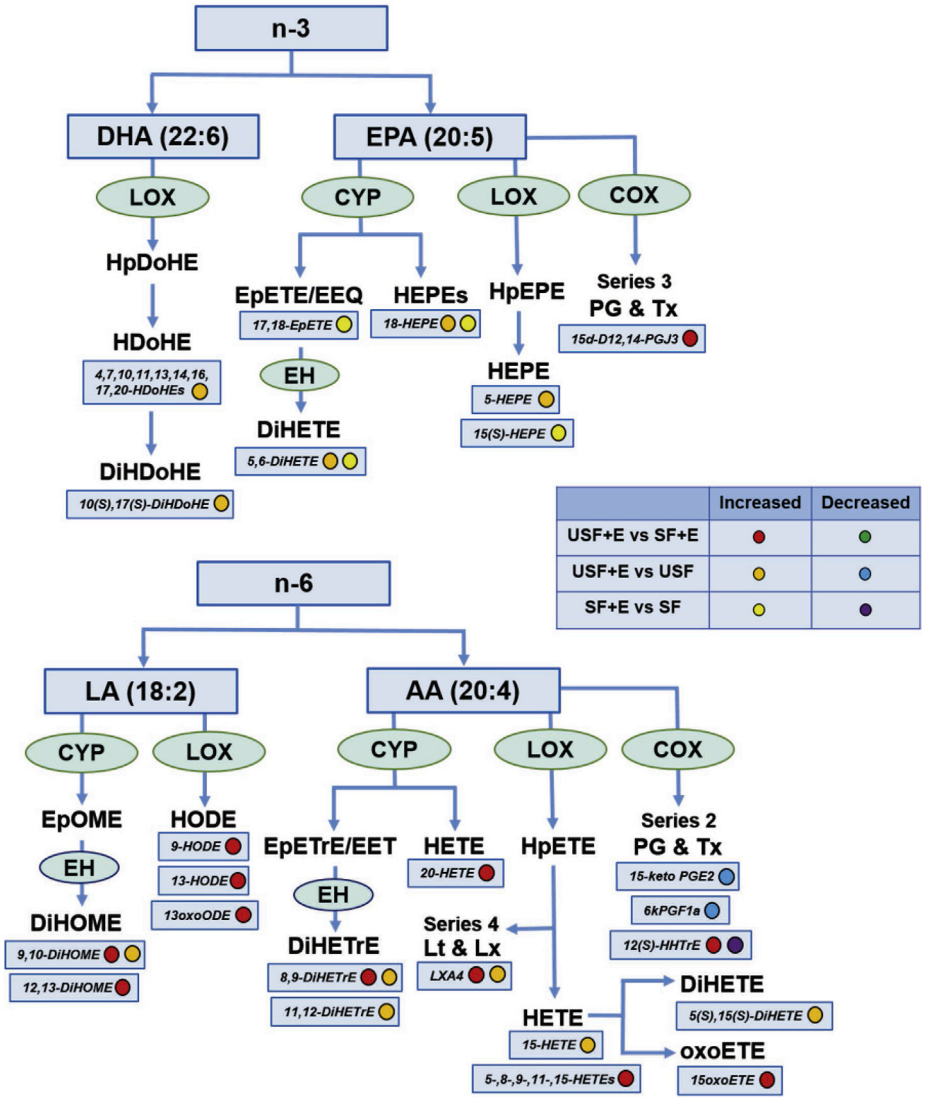

Fig. 4. EtOH-mediated alterations in liver oxylipins. Different types of dietary fat cause distinct changes in n-3 and n-6 PUFAs-derived metabolites in response to EtOH exposure. The mice were fed control or EtOH-containing diets *ad libitum* for 10 days followed by a single EtOH binge. The diet contained either SF (enriched in beef tallow: medium chain triglycerides) or USF (enriched in corn oil). Two way ANOVA, $P < 0.05$, N = 6 animals per group. Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; CYP, cytochrome P450; DHA, docosahexaenoic acid; DiHDoHE, dihydroxy-docosahexaenoic acid; DiHETE, dihydroxy-eicosatetraenoic acid; DiHETrE, dihydroxy-eicosatrienoic acid; DiHOME, dihydroxy-octadecenoic acid; E, ethanol; EH, epoxide hydrolase; EPA, eicosapentaenoic acid; EpETE/EEQ, epoxy-eicosatetraenoic acid; EpETrE/EET, epoxy-eicosatrienoic acid; EpOME, epoxy-octadecenoic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HHTrE, hydroxy-heptadecatrienoic acid; HODE, hydroxy-octadecadienoic acid; HpEPE, hydroperoxy-eicosapentaenoic acid; HpETE, hydroperoxy-eicosatetraenoic acid; HpDoHE, hydroperoxy-docosahexaenoic acid; LA, linoleic acid; LOX, lipoxygenase; Lt, leukotriene; Lx, lipoxin; n-3 PUFAs, omega-3 polyunsaturated fatty acids; n-6 PUFAs, omega-6 polyunsaturated fatty acids; oxoETE, oxo-eicosatetraenoic acid; PG, prostaglandin; SF, saturated fat; Tx, thromboxane; USF, unsaturated fat.

mice, while modestly attenuating chronic-binge EtOH-induced liver injury with no effect on hepatic steatosis.[12] This observation suggests that non-enzymatic LA oxidation via free radical-mediated oxidation in response to oxidative stress, rather than through the 12/15-LO pathway of LA oxidation, is likely the main mechanism contributing to the EtOH-mediated increase in OXLAM production in this model. In another study by Zhang *et al*.,[13] *Alox15* genetic ablation and pharmacological inhibition attenuated chronic EtOH-induced liver injury and steatosis. On mechanistic levels, OXLAMs affect multiple cells, tissues, and organs, including the liver, by acting through their receptors, *e.g.,* transient receptor potential vanilloid 1 (TRPV1).[131] Our recent study[128] demonstrated that TRPV1 deficiency protected against chronic-binge alcohol induced hepatic inflammation and injury with no effects on hepatic steatosis, suggesting that OXLAM/TRPV1 interactions may contribute to the progression from simple steatosis to steatohepatitis. Interestingly, distinct OXLAMs may exhibit differential effects, *e.g.,* 9-HODE was primarily pro-inflammatory while 13-HODE was mainly neutral or had some anti-inflammatory activity in LPS-stimulated RAW264.7 monocyte/macrophage cell line.[12] Exposure of 13-HODE to Hepa-1c1c7 cells induced oxidative stress, ER stress, apoptosis, and also altered proteins related to lipid metabolism.[13]

6.2.3. Acrolein as a potential pathogenic mediator of ALD

Acrolein, a PUFA-derived endogenous metabolite and an environmental and dietary pollutant, has recently been identified as a pathogenic mediator of ALD. A study by Chen *et al*.[132] demonstrated that alcohol consumption resulted in hepatic accumulation of acrolein protein adducts associated with hepatic steatosis, ER stress, and liver injury. The authors also showed that direct exposure to acrolein *in vitro* mimicked the *in vivo* effects of alcohol and hydralazine, a known acrolein scavenger, protected against alcohol-induced ER stress and liver injury. Acrolein exposure can also affect the intestinal epithelium via a decrease/redistribution of TJ proteins and ER stress-mediated epithelial cell death resulting in barrier dysfunction.[133] Importantly, the urinary acrolein metabolite, 3-hydroxypropylmercapturic acid (HPMA), was higher in patients with severe AAH compared with healthy controls or non-severe AAH.[134] Strong positive correlation of hepatocyte cell death markers with HPMA, combined with pro-inflammatory cytokines in AAH patients, further support the pathogenic role of acrolein in ALD in humans.

6.2.4. Endogenous endocannabinoids and ALD

There is evidence demonstrating an involvement of the endocannabinoid system in ALD pathogenesis. EtOH feeding increased hepatic expression of CB1 receptor and upregulated the endocannabinoid 2-AG[68] Further, global or hepatocyte-specific CB1 knockout mice are resistant to EtOH-induced steatosis.[68] It has been reported that CB2 knockout mice exhibited more pronounced liver damage after chronic EtOH challenge, including hepatic stellate cell activation and collagen production.[135] In addition, CB2 receptor displays beneficial effects on alcohol-induced inflammation by inhibiting M1 polarization and favors the transition to an M2 phenotype in Kupffer cells.[136] These data indicate differential effects of CB1 and CB2 receptors in ALD, suggesting a protective role of CB2 receptor, and identify CB2 agonists as promising therapeutic drugs for ALD management.

7. Conclusions

ALD has emerged as a leading cause of chronic liver disease and a rapidly increasing indicator for liver transplantation in the most severe cases. The treatment options are limited with no FDA-approved therapy. Furthermore, mechanisms of disease development and progression are not fully understood. Growing evidence suggests that the composition of dietary fat may play important roles in ALD pathogenesis. Preclinical animal models have been instrumental in demonstrating the deleterious or beneficial effects of distinct dietary PUFAs in ALD. While it is well-documented that dietary enrichment in the n-6 PUFA, LA, results in exacerbation of liver injury in experimental ALD, the effects of other PUFAs, specifically n-3 PUFAs, are not well established. Given that the Western diet is heavily reliant upon n-6 PUFA-rich sources at the expense of n-3 PUFAs, it is essential to investigate the role of modulation/decrease of the n-6:n-3 PUFA ratio as a potential dietary preventative/therapeutic approach in different pathological conditions, including alcohol-induced multi-organ pathology. Many of the PUFA-mediated biological effects can be attributed to the PUFA-derived bioactive lipid mediators (*e.g.*, oxylipins, peroxidation-derived products, endocannabinoids, and unique bioactive PUFA metabolites of bacterial origin). Multiple n-6 PUFA-derived products, such as oxidized LA metabolites, are known for their pro-apoptotic and pro-inflammatory properties, while n-3 PUFA-derived metabolites collectively termed specialized pro-resolving mediators, which include resolvins, protectins, and maresins, have been shown to limit the inflammatory response in numerous experimental models. The evidence from preclinical animal studies underscores the importance of different types of dietary fat in EtOH-associated alterations in the gut-liver axis, specifically in the gut microbiome, which can influence individual susceptibility to ALD, and affect disease severity. Given the lack of translational studies, future research is required to determine the role of dietary PUFAs and their bioactive metabolites in clinical ALD. An understanding of how dietary lipids contribute to ALD pathogenesis will enhance our knowledge regarding the molecular mechanisms of ALD development and progression, may help to identify novel dietary intervention strategies for ALD prevention/treatment, and may help to explain why only some people who drink heavily develop clinically relevant ALD.

Authors' contributions

K. H. Zirnheld and I. A. Kirpich reviewed the literature and wrote the manuscript. All the authors discussed and critically reviewed the content, edited, and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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