Systematic Review

Impact of Omega-3 Polyunsaturated Fatty Acids on Alcohol Use and Negative Consequences: A Systematic Review

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Context: *Research suggests that alcohol consumption is associated with neuroinflammation, impacting brain regions associated with addiction and cognitive function. Long-chain omega-3 (n-3) polyunsaturated fatty acids (PUFAs), in particular docosahexaenoic acid (DHA), have been proposed to have neuroprotective effects against alcohol, reversing synaptic deficits caused by alcohol and alleviating anxiety in animal models.*

Objective: *The aim of this study was to evaluate the impact of an n-3 intervention in ameliorating behavioral changes, biochemical alterations, and the inflammatory responses induced by alcohol consumption.*

Data Sources: *A systematic review was performed using PubMed (Medline), Scopus, Web of Science, and OpenGrey databases. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed.*

Data Extraction: *A total of 3829 records were identified. The records were subject to screening against the eligibility criteria, and the data extraction and risk-of-bias assessment were carried out by 2 investigators independently.*

Data Analysis: *Twelve articles addressed n-3 PUFA interventions, and its effects on alcohol-related outcomes were finally included. Preclinical studies demonstrated that n-3 PUFAs improved behavioral, inflammation, lipid metabolism, and hepatic parameters altered by alcohol. However, clinical trials yielded inconclusive evidence.*

Conclusion: *Despite the paucity of clinical and preclinical studies, available evidence suggests that n-3 PUFAs may exert a protective influence on alcohol-related outcomes at both the behavioral and molecular levels.*

Systematic Review Registration: *PROSPERO registration no. CRD42023443095.*

Key words: n-3 PUFA, omega-3, alcohol consumption, alcohol-related adverse outcomes.

INTRODUCTION

Alcohol is a widely consumed substance in our society, with approximately 2.3 billion current drinkers worldwide. According to the World Health Organization,[1] approximately 2.5 million deaths occur annually due to diseases associated with its consumption. Chronic and excessive alcohol consumption can

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have a significant impact on different organs of the body, with the most pronounced alcohol-related diseases typically affecting the liver, the pancreas, and the brain. Recent research has highlighted the role of inflammatory processes in alcohol-mediated tissue damage, particularly in the central nervous system. In particular, alcohol triggers a long-term increase in brain proinflammatory cytokine levels, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α), as well as oxidative stress, creating a neurotoxic environment that can cause apoptosis in nerve cells.[2–5] Current research underscores the neuroimmune system as an important target of alcohol, which may contribute not only to alcohol-induced brain damage but also to cognitive, emotional, and behavioral impairments associated with its consumption.[2]

In this context, some studies have reported the potential of long-chain polyunsaturated fatty acids (PUFAs) to modulate inflammatory responses.[6–8] Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are crucial omega-3 (n-3) fatty acids, and their beneficial effect on the human body has been investigated in a large number of studies.[9] Additionally, due to their ability to cross the blood–brain barrier[10] and remain in the brain for up to 33 days[11] they are of particular interest for studying their effects on the central nervous system. Given that alcohol consumption affects both the peripheral and central levels, it would be interesting to analyze how the administration of PUFAs modifies the effect of alcohol in the body.

Previous evidence indicates that excessive alcohol consumption alters the basal level of PUFAs, resulting in an increase in omega-6 (n-6) levels and a decrease in n-3 levels. Omega-6 has a proinflammatory role, while n-3 has an anti-inflammatory role. This dysregulation has been observed in heavy drinkers with mild liver dysfunction, with a more significant effect in women.[12] The same dysregulation has also been observed in both male and female patients with hepatitis C who are heavy alcohol drinkers.[13] In this regard, patients with alcoholic hepatitis also exhibit an overabundance of proinflammatory lipid mediators and a deficiency of anti-inflammatory pro-resolving mediators of n-6 and n-3 PUFA pathways.[14] Given that the resolution of inflammation is essential to prevent tissue damage, therapies for alcoholic liver disease focus on targeting this inflammation. For example, a novel approach has demonstrated the protective effect of *Lactobacillus plantarum* ZS62 in protecting the liver by reducing inflammation and increasing antioxidant capacity in mice exposed chronically to alcohol.[15]

At the central nervous system level, alcohol consumption inhibits neural plasticity and neurogenesis, further exacerbating its deleterious effects.[16,17] DHA has been shown to prevent alcohol-induced neurodegeneration in the hippocampal region, possibly by activating the peroxisome proliferator-activated receptor-gamma (PPARγ), thereby exerting its effects through its anti-inflammatory and neuroprotective properties.[18] Animal studies involving neonatal alcohol exposure have shown that DHA can reverse alcohol-induced synaptic deficits, leading to improved long-term potentiation within the dentate gyrus in adult rats.[19] Furthermore, it should be noted that increasing evidence from preclinical and clinical research also suggests that, although DHA may provide a protective effect against cognitive and behavioral impairments associated with alcohol consumption, EPA may potentially promote it. A study carried out in adolescents found a correlation between DHA levels and the beginning of alcohol consumption among people aged 14–17 years, suggesting that elevated levels of n-3 PUFAs can increase sensitivity to alcohol.[20] Similarly, the efficacy of an n-3 supplement has been shown to reduce anxiety and cortisol levels in abstinent individuals undergoing residential treatment for alcohol addiction.[21] Furthermore, a diet rich in DHA decreases voluntary alcohol intake in alcohol-preferring rats, a strain specifically bred for alcohol consumption.[22] Furthermore, it has been found to alleviate anxiety induced by pre-exposure to alcohol in aged animals.[23]

Despite the above, there are still inconclusive data on the protective role of DHA in alcohol consumption, particularly in clinical trials.[24] This study sets out the hypothesis that consuming a diet rich in n-3 PUFAs could help alleviate behavioral consequences and/or mitigate the inflammatory response associated with alcohol consumption. Therefore, the main objective of this systematic review was to analyze the literature on the effect of an n-3 PUFA intervention on behavioral changes, biochemical alterations, and the inflammatory response caused by alcohol consumption, considering both clinical and preclinical studies.

METHODS

A systematic review of clinical trials and preclinical studies published to date was conducted from June to September 2023. As shown in Table S1, the review was conducted in accordance with the guidelines outlined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[25] and followed the recommendations set by CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies).[26] Additionally, the guidance provided in the *Cochrane Manual of Systematic Reviews* by Higgins and Green (2011)[27] was considered. The review protocol was registered in PROSPERO (CRD42023443095). The Participant, Intervention,

Comparison, Outcomes, Study design (PICOS) framework[28] was applied to explore the potential of n-3 supplementation to attenuate the effects of alcohol consumption (Table 1).

Databases

The search encompassed 4 electronic databases: Web of Science, PubMed/Medline, Scopus, and OpenGrey. Additionally, a snowball strategy was used to complete the search. To identify possible studies in electronic databases, taking as reference the PICOS question, search strategies were developed by combining structured language (Medical Subject Heading [MeSH] terms) and natural language (Table 2).

Study Eligibility Criteria

The inclusion criteria for this review were as follow: (1) randomized controlled trials (RCTs) in adults or preclinical studies in adult rats or mice, (2) interventions involving the administration of n-3 PUFAs (independent variable) regardless of the form of administration, and (3) any form of outcome related to alcohol dependence (dependent variable). Reviews, systematic reviews, meta-analyses, conference proceedings, book chapters, safety studies, nonrandomized trials, cross-sectional studies, and meeting abstracts were excluded.

Study Selection and Methodological Quality

The selection and analysis of the studies were carried out independently by 2 researchers (D.C., L.R.-R.). In cases where consensus could not be reached, another researcher (F.C.) was consulted for study inclusion. The evaluation of the methodological quality of the full-text articles was also carried out in pairs, according to the same procedure. Instruments specific to the type of article were used for the evaluation process:

- The Jadad scale was used to evaluate the methodological quality of the clinical studies.[29] This 5-item scale is structured to appraise the quality of RCTs in a straightforward manner. A study is considered high quality if it scores more than 3 points and low quality if it scores less than 3 points. These scores are determined based on the quality of double-blinding, randomization, and follow-up.[30] It is important to note that this scale addresses certain elements related to biases, thereby enhancing its reliability and external validity.[31]
- For the assessment of preclinical studies, the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool was used, as detailed by Hooijmans et al.[32] This tool comprises 10 evaluation items that cover sequence generation, baseline characteristics, allocation concealment, random housing, blinding of the investigator, random outcome assessment, blinding of outcome evaluators, incomplete outcome data, selective outcome reporting, and other sources of bias.

Table 1. PICOS criteria for inclusion of studies

Parameter	Criterion
Participant	Human and adult rodent exposure to alcohol
Intervention	n-3 PUFAs
Comparison	Control group
Outcomes	Behavioral changes, biochemical and inflammatory markers
Study design	RCT and preclinical studies

Abbreviations: PUFA, polyunsaturated fatty acid; RCT, randomized controlled trial.

Table 2. Search strategy in each database

Database	Search strategy
PubMed/ Medline	("alcohol drinking"[Title/Abstract] OR "alcohol intake"[Title/Abstract] OR "alcohol related disorders"[MeSH terms] OR "binge drinking"[MeSH terms] OR "drinking behavior"[Title/Abstract]) AND ("omega 3"[Title/Abstract] OR "fatty acid"[Title/Abstract] OR "PUFA"[Title/Abstract] OR "fatty acids, omega 3"[MeSH terms] OR "fatty acids, omega 3"[Title/Abstract)
Scopus	(TITLE-ABS-KEY ("alcohol drinking" OR "alcohol intake" OR "alcohol related disorders" OR "binge drinking" OR "drinking behavior") AND TITLE-ABS-KEY ("omega 3" OR "fatty acid" OR pufa OR "fatty acids, omega 3"))
Web of Science	((((((((((TI=(alcohol intake)) OR TI=(alcohol drinking)) OR TI=(alcohol related disorder)) OR TI=(binge drinking)) OR TI=(drinking behavior)) OR AB=(alcohol intake)) OR AB=(alcohol drinking)) OR AB=(alcohol related disorder)) OR AB=(drinking behavior)) OR AB=(binge drinking)) AND (((((((TI=(omega 3)) OR TI=(fatty acid)) OR TI=(PUFA)) OR TI=(fatty acids, omega 3)) OR AB=(fatty acids, omega 3)) OR AB=(omega 3)) OR AB=(fatty acid)) OR AB=(PUFA)
OpenGrey	Omega 3 AND alcohol

The main findings extracted from the selected articles describe changes in the ethanol-exposed groups after n-3 treatment, such as altered anxiety levels, locomotor activity, depression, or alcohol motivation. Additionally, the results include observations related to changes in hepatotoxicity, including inflammation.

Data Extraction

The information was independently extracted by 2 investigators (D.C., L.R.-R.) from each study and organized in

the following table format: (1) reference, (2) country, (3) animals/participants, (4) treatment used (n-3 treatment), (5) duration of the intervention, (6) analyzed variables, (7) data-collection tool, and (8) key findings.

RESULTS

Following the initial database search, 3829 results were identified (968 from PubMed, 1315 from Web of Science, and 1546 from CINAHL). Of these, 1323 duplicates were removed, leaving 2506 unique articles. A review of the titles and abstracts was performed to eliminate studies that were irrelevant to n-3 and alcohol consumption, resulting in a pool of 24 studies. Finally, a comprehensive review of the full-text articles was performed using inclusion and exclusion criteria, resulting in the final selection of 12 articles for review (10 preclinical and 2 clinical), as illustrated in the flowchart of Figure 1.

Data Included

Table 3 shows the main characteristics of preclinical studies included in this analysis. Among the selected studies, half of them ($n = 5$) used rats as subjects,[33–37] while the other half used mice ($n = 5$).[38–42] In all studies, n-3 PUFAs were administered in the liquid diet, except for 1 study where intragastric administration was utilized.[38] Three studies also administered n-6 PUFAs.[34,37,40] The amount of n-3 PUFAs and the duration of treatment exhibited variability in the studies, with treatment ranging from 10 days to 11 weeks. However, there was a notable challenge in the comparability of the results due to discrepancies in the way that n-3 PUFA proportions are reported. Some studies report the proportion of n-3 PUFAs in the diet,[34,37,39] while others used percentages[33,36,37,40,41] or micrograms administered.[38] And 1 study did not provide information on the amount of n-3 PUFAs administered.[35] With regard to the research focus, all studies, except for 1, were interested in evaluating the effect of n-3 in animal models treated/exposed simultaneously to alcohol. Engler et al[37] conducted the only study that specifically evaluated the effect of n-3 PUFAs on subsequent alcohol consumption.

Table 4 shows the main characteristics of the included clinical studies.[43,44] Both included studies that involved patients diagnosed with alcohol dependence. The age range for the selection of participants varied between studies, ranging from 30 to 70 years. In terms of treatment approaches, 1 study exclusively used n-3

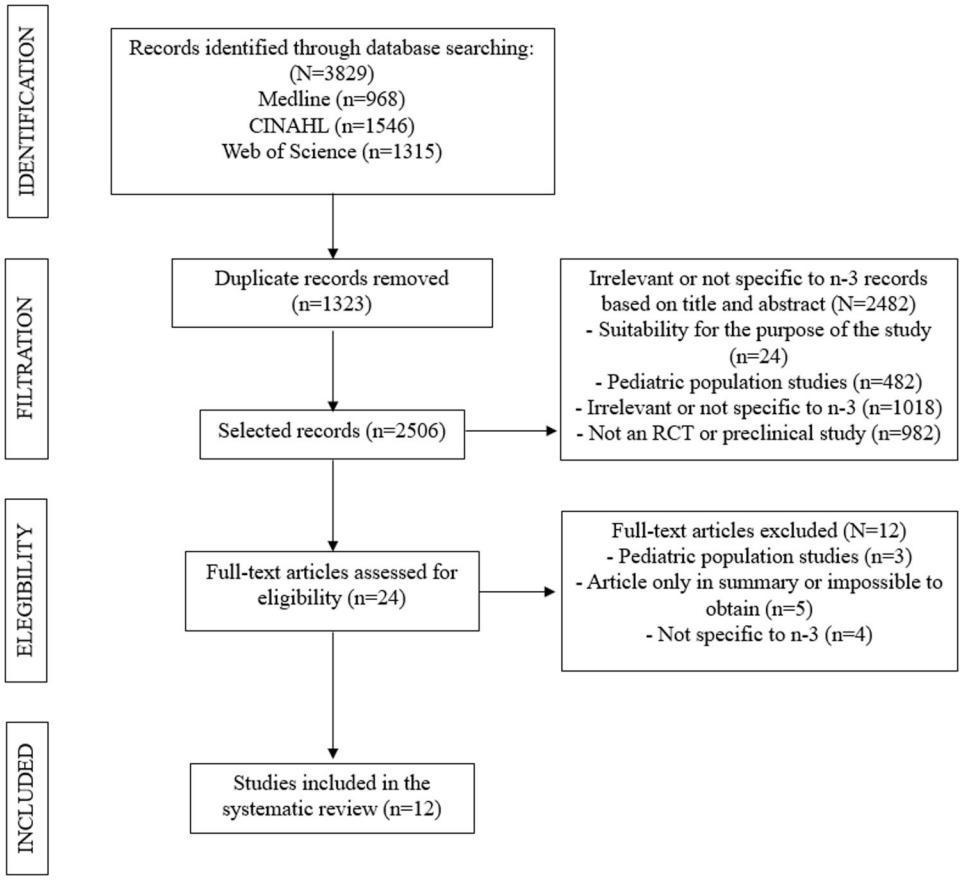

Figure 1. Flowchart of Included Studies. Abbreviation: RCT, randomized controlled trial

treatment, while the other used a combination of n-3 and n-6, with a treatment duration of 90 days.

Methodological Quality: Assessment of Bias

Figure 2A presents the evaluation of the methodological quality and the potential risk of bias in the included preclinical studies, evaluated using the SYRCLE scale. Most of these studies exhibited a questionable risk of bias, particularly in aspects related to the description of their blinding process. Only 3 studies showed a lower risk of bias for the random housing.[34,39,40] Figure 2B provides an overview of the overall scores for each evaluated item, highlighting that aspects such as incomplete outcome data and other sources of bias are not clearly elucidated.

With regard to clinical trials, Figure 3A shows the assessment of methodological quality and potential risk of bias of the included studies, evaluated using the Cochrane Collaboration scale. Figure 3B shows a graph of the overall score for each item. The study conducted by Pauluci and collaborators[43] showed a low risk of bias, while the study by Fogaça and collaborators[44] reflected an uncertain risk of bias.

Behavioral Parameters

The clinical trials reviewed primarily aimed to assess whether n-3 supplementation exerts a protective effect against the compulsion for alcohol in patients with alcohol-use disorder. No effects of treatment with n-3 were observed on behavioral parameters, such as propensity to relapse, craving, or alcohol-dependence severity.[43,44] However, Pauluci et al[43] observed a progressive reduction in the number of days in which alcohol was consumed among participants who received the intervention, 2 and 3 months after the beginning of n-3 supplementation, which disappeared at 6 months. The effect of n-3 PUFAs on anxiety and depression was also evaluated, but no differences were found.

However, only 3 preclinical studies have evaluated the effect of n-3 treatment on cognitive and/or emotional changes caused by alcohol consumption. A reduction in increased locomotor activity following alcohol withdrawal was observed after n-3 treatment in 2 of the studies.[38,39] Shi et al[38] observed that the administration of an n-3 PUFA diet for 14 weeks alleviated withdrawal symptoms induced by chronic alcohol exposure, indicated by a decrease in severity of convulsive activity, a sensitive indicator of hyperexcitability in the central nervous system during abstinence. Also, treatment decreased ethanol-induced conditioned-place preference, a protocol used to measure the rewarding properties of ethanol. Along the same line, a study by Wolstenhol et al[39] found that administration of a high–n-3 PUFA diet for 11 weeks reduced the increase in locomotor activity caused by ethanol in both inbred C57BL/6J and DBA/2J mice. However, in the study by Isaev et al,[35] no differences in locomotor activity were observed after administration of n-3 PUFAs, although the treated group did show a reduction in anxiety, as evidenced by a decrease in time spent in open arms of the elevated plus maze.

Inflammation

Only 2 studies have investigated the impact of n-3 PUFA intake on inflammatory cytokines in mice after alcohol consumption.[40,41] Using flaxseed oil as the n-3 source, Zhang et al[40] showed reduced levels of TNF-α, IL-1β, and IL-6 in both plasma and liver compared with animals treated with alcohol. Likewise, Wang et al[41] performed a study suggesting that a diet rich in n-3 PUFAs can effectively reduce levels of TNF-α and IL-1β in adipose tissue and liver after exposure to ethanol.

Parameters Related to Lipid Metabolism and Liver Function

Six studies evaluated parameters related to liver function.[33,34,36,40–42] An n-3–enriched diet was found to reduce cholesterol in rats that consumed alcohol[33] and increase cholesterol transport to the liver in rats after chronic intake of ethanol.[36] The studies by Wang et al[41,42] and Zhang et al[40] indicated a reduction in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) after n-3 administration. However, Song et al[34] found only a slight difference in ALT and AST values between the groups.

The parameters related to lipid metabolism were evaluated in 3 studies.[33,41,42] The n-3 PUFA diet normalized the expression of genes related to fat storage, fatty acid transport, triglycerides synthesis, and chylomicron uptake heightened by alcohol.[33,41,42] Similarly, n-3 PUFAs improved the alcohol-altered expression of lipolytic enzymes such as adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), as well as improved the expression of the glycoprotein CD36, a fatty acid transporter that regulates metabolism.[41,42]

Proteins Related to Neural Transmission

The expression of certain proteins, such as alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), has been examined in 1 study.[38] Research efforts have focused on exploring the activity of different proteins before and after alcohol ingestion in experimental animals, as alcohol consumption can

Table 3. Preclinical Study Characteristics

Study, year	Country	Animals	Treatment	Duration	Variables	Tools	Effects of n-3–rich diet
Shi et al, 2019[38]	China	Male C57BL/6J mice ($n = 24$)	Control Ethanol Ethanol+corn oil Ethanol+fish oil[a]	14 d	Abstinence Locomotion Alcohol preference Blood ethanol Neuron morphology in NAc AMPAR1, AMPAR2, AMPAR3, and PSD95 protein levels	HIC Circular corridor CPP test Spectrophotometry Histology Western blot	Alleviated withdrawal symptoms and alcohol dependence ↓ Psychomotor response and preference for alcohol-associated context Reversed alcohol-induced dendritic morphological changes in NAc core Inhibited expression of AMPAR1 and AMPAR3, preventing their accumulation in postsynaptic membrane in NAc
Wolstenholme et al, 2018[39]	USA	Male C57BL/6J (B6) and DBA/2J (D2) mice ($n = 60$)	B6 (low n-3 PUFA)[b] B6 (high n-3 PUFA)[c] D2 (low n-3 PUFA) D2 (high n-3 PUFA)	9–11 wk	Blood EPA, DHA, and arachidonic acid levels Locomotion Sedation Blood ethanol LORR Body weight, density, and length	HPLC/MS/MS Cylindrical apparatus for LORR Gas chromatography	↓ Ethanol-induced locomotor stimulation ↑ Ethanol metabolism and sedation in D2 mice on high–n-3 diet Body weight and length slightly lower in D2 mice on low–n-3 diet
Zhang et al, 2017[40]	China	Male C57BL/6J mice ($n = 60$)	Group PF/CO[d] (corn oil) Group AF/CO[e] (ethanol+corn oil) Group PF/FO[f] (flaxseed oil) Group AF/FO[g] (ethanol+flaxseed oil)	6 wk	Plasma AST, ALT, and LPS levels Fecal microbial 16S rRNA TNF-α, IL-1β, IL-6, and IL-10	Histology Spectrophotometry ELISA Fecal microbial 16S rRNA gene sequencing	Suppressed the elevation of AST and ALT induced by alcohol Attenuated alcohol-induced hepatic histopathological damage ↓ LPS levels ↓ Plasma and liver TNF-α, IL-1β, and IL-6 levels ↓ Proportion of Proteobacteria following chronic alcohol consumption
Wang et al, 2017[41]	China	*Fat-1* transgenic mice with C57BL/6 and their WT littermates ($n = 20$)	WT/CON[h] (wild-type/control) WT/ETH[i] (wild-type/ethanol) *Fat-1*/CON[h] (*fat-1*/control) *Fat-1*/ETH[i] (*fat-1*/ethanol) WT+n-3/ETH[j] (wild-type+n-3/ethanol)	10 d	Fatty acids in liver and plasma levels Serum ALT, AST, ALP, and total bilirubin levels Hepatic triglycerides, TNF-α, IL-1β, and MCP-1 levels *ATGL*, *Cd36*, *Fatp2*, *Pparγ*, *Fabp4*, and *Lpl* mRNA in adipose and hepatic tissues cAMP, PDE3B, GPR120, p-HSL, HSL, ATGL, PDE3B, pAMPK, and CD36 protein levels	Gas chromatography–mass spectrometry Spectrophotometry ELISA Immunofluorescence RT-PCR Western blot	↑ Levels of DHA, EPA, and total n-3 PUFAs, and ↓ ratio n-3:n-6 in the liver ↓ ALT, AST, ALP, and total bilirubin levels after ethanol exposure Ameliorated ethanol-induced adipose tissue hyperlipolysis Normalized expression of gene involved in fatty acid transport (*Pparγ*, *Cd36*, *Fabp4)* and chylomicron uptake (*Lpl*) ↓ TNF-α and IL-1β production in adipose tissue and liver after ethanol exposure Prevented alcohol-induced increase in cAMP levels by promoting PDE3B activity in adipose tissue

(continued)

Table 3. Continued

Study, year	Country	Animals	Treatment	Duration	Variables	Tools	Effects of n-3–rich diet
Wang et al, 2016[42]	China	Male C57BL/6 mice ($n = 20$)	Pair-fed with corn oil group (PF/CO)[d] Alcohol-fed with corn oil group (AF/CO)[e] Pair-fed with flaxseed oil group (PF/FO)[f] Alcohol-fed with flaxseed oil group (AF/FO)[g]	10 d	Body and liver weight and eWAT Plasma, glucose, insulin, ethanol, adiponectin, ALT, and AST levels Fatty acids and triglycerides in liver and adipose tissues p-HSL, HSL, ATGL, MTTP, pAMPK, AMPK, ACC, ADIPOR2, pACC, and PPARγ protein levels mRNA of *Cd36*, *Fatp1*, *Lpl*, *Vldl-r*, *Gpat*, and *Dgat1*, *Dgat2*	Gas chromatography–mass spectrometry ELISA Spectrophotometry RT-PCR Western blot	↓ ALT and AST levels after ethanol exposure ↓ Concentration of hepatic triglycerides in alcohol-fed mice ↓ mRNA level of *Dgat2* in the liver of alcohol-fed mice Normalized upregulated ATGL, p-HSL, CD36, and MTTP protein levels induced by alcohol Improved the ethanol inhibitory effect on circulating adiponectin
Reyes-Gordillo et al, 2016[33]	USA	Female Wistar rats ($n = 20$)	Control EtOH SP+EtOH (ethanol +soy protein) Low-ω3 + EtOH (ethanol+low n-3 fish oil)	4 wk	Total serum and hepatic cholesterol, lipids and triglycerides *Sirt1*, *Pgc1*α, *Pgc1*β, *Cpt1*, *Srebp1*, *Acc*, and *c-Met* mRNA SIRT1, PGC1α, PGC1β, SREBP1, ACC, c-Met, AMPK, and pAMPK proteins	Radioactivity using [^{3}H] cholesterol Spectrophotometry RT-PCR Western blot Immunoprecipitation	↓ Serum and hepatic cholesterol and triglycerides in ethanol-fed rats ↑ Expression of lipid-oxidizing genes (*SIRT1* and *PGC1*α) and ↓that of lipogenic genes (*PGC1*β and *SREBP1*)
Song et al, 2008[34]	USA	Male Long-Evans rats ($n = 56$)	Basal diet + control[k] PUFA diet + control[l] Basal diet + EtOH[m] PUFA diet + EtOH[n]	9 wk	Plasma ALT and AST levels Oxidative/nitrosative stress (CYP2E1, NOS, hydrogen peroxide, and nitrite) Oxidatively modified mitochondrial proteins	ELISA Electrophoresis	Prevented alcohol-induced increase in the activity of CYP2E1, NOS, and the levels of hydrogen peroxide and nitrite ↓ Number and intensity of oxidized mitochondrial proteins
Isaev et al, 2001[35]	Moscow	Male albino rats (n.d.)	Group 1 (control) Group 2 (eiconol)[o] Group 3 (ethanol) Group 4 (ethanol+eiconol)	10 d	Motor, orientation, and exploratory activity Anxiety Alcohol motivation	RODEO automated device Elevated plus maze Individual cages for free choice between ethanol and water	↓ Alcohol deprivation anxiety-like behaviors as measured by EPM Corrected changes in open-field behavior in alcoholized rats caused by alcohol deprivation ↓ Alcohol deprivation effect
Marmillot et al, 2000[36]	USA	Male Wistar-Furth rats (n.d.)	Control regular (CN)[p] Ethanol regular (AN)[q] Control n-3 fatty acids (CF)[r] Ethanol n-3 fatty acids (AF)[s]	8 wk	HDL and LDL in plasma and liver	Radioactivity using [^{3}H] cholesterol	Restored the impaired function of HDL caused by alcohol intake ↑ The reverse cholesterol transport function in HDL

(continued)

Table 3. Continued

Study, year	Country	Animals	Treatment	Duration	Variables	Tools	Effects of n-3–rich diet
Engler et al, 1991[37]	USA	Male Sprague-Dawley rats ($n = 48$)	Purified diets containing n-6, n-3, and n-9 18-carbon fatty acids: SES,[t] BOR,[u] LSO[v]	7 wk	Fatty acids in platelets and thoracic aortae Blood ethanol	Gas chromatography Spectrophotometry-UV	LSO ↑ n-3 in platelets and aorta LSO ↓ n-6 fatty acids in platelets after alcohol exposure

$n = 10$.

[a]Fish oil (50 μL) contained EPA 5 mg and DHA 30 mg.

[b]Low–n-3 PUFA diet included no detectable long-chain n-3–containing EPA or DHA (from fish oil) and an n-3:n-6 ratio of 0.002.

[c]High–n-3 PUFA diet included long-chain n-3–containing EPA 14.3% and DHA 9.3% (from fish oil) and an n-3:n-6 ratio of 3.3.

[d]PF/CO diet contained casein 41.4 g/L, L-cystine 0.5 g/L, D,L-methionine 0.3 g/L, cellulose 10 g/L, maltose dextrin 115 g/L, corn oil 39.6 g/L, mineral mix 8.8 g/L, vitamin mix 2.5 g/L, choline bitartrate 0.53 g/L, and vitamin E 0.2 g/L.

[e]AF/CO diet contained casein 41.4 g/L, L-cystine 0.5 g/L, D,L-methionine 0.3 g/L, cellulose 10 g/L, maltose dextrin 44.8 g/L, corn oil 39.6 g/L, mineral mix 8.8 g/L, vitamin mix 2.5 g/L, choline bitartrate 0.53 g/L, vitamin E 0.2 g/L, and ethanol 52.6 mL/L.

[f]PF/FO diet contained casein 41.4 g/L, L-cystine 0.5 g/L, D,L-methionine 0.3 g/L, cellulose 10 g/L, maltose dextrin 115 g/L, flaxseed oil 39.6 g/L, mineral mix 8.8 g/L, vitamin mix 2.5 g/L, choline bitartrate 0.53 g/L, and vitamin E 0.2 g/L.

[g]AF/FO diet contained casein 41.4 g/L, L-cystine 0.5 g/L, D,L-methionine 0.3 g/L, cellulose 10 g/L, maltose dextrin 44.8 g/L, flaxseed oil 39.6 g/L, mineral mix 8.8 g/L, vitamin mix 2.5 g/L, choline bitartrate 0.53 g/L, vitamin E 0.2 g/L, and ethanol 52.6 mL/L.

[h]WT/CON and *fat-1*/CON diets included casein 41.4 g/L, L-cystine 0.5 g/L, D,L-methionine 0.3 g/L, cellulose 10 g/L, maltose dextrin 115 g/L, corn oil 39.6 g/L, mineral mix 8.8 g/L, vitamin mix 2.5 g/L, choline bitartrate 0.53 g/L, and vitamin E 0.2 g/L.

[i]WT/ETH and *fat-1*/ETH diets contained casein 41.4 g/L, L-cystine 0.5 g/L, D,L-methionine 0.3 g/L, cellulose 10 g/L, maltose dextrin 44.8 g/L, corn oil 39.6 g/L, mineral mix 8.8 g/L, vitamin mix 2.5 g/L, choline bitartrate 0.53 g/L, vitamin E 0.2 g/L, and ethanol 52.6 mL/L.

[j]WT+n-3/ETH diet contained casein 41.4 g/L, L-cystine 0.5 g/L, D,L-methionine 0.3 g/L, cellulose 10 g/L, maltose dextrin 44.8 g/L, corn oil 18.8 g/L, EPA (58% EPA, 15% DHA) 9.9 g/L, DHA (11% EPA, 48% DHA) 9.9 g/L, mineral mix 8.8 g/L, vitamin mix 2.5 g/L, choline bitartrate 0.53 g/L, vitamin E 0.2 g/L, and ethanol 52.6 mL/L.

[k]Basal diet+control contained protein 22.4%, carbohydrates 66.2%, total fat 11.4%, and 0.3% of both 18:2n-6 and 18:3n-3.

[l]PUFA diet+control contained protein 22.4%, carbohydrates 66.2%, total fat 11.4%, 0.3% of 18:2n-6 and 18:3n-3, and 0.5% of 20:4n-6 and 22:6n-3.

[m]Basal diet+EtOH contained protein 22.4%, carbohydrates 30.2%, total fat 11.4%, 0.3% of both 18:2n-6 and 18:3n-3, and ethanol 36%.

[n]PUFA diet+EtOH contained protein 22.4%, carbohydrates 30.2%, total fat 11.4%, 0.3% of 18:2n-6 and 18:3n-3, 0.5% of 20:4n-6 and 22:6n-3, and ethanol 36%.

[o]Eiconol consisted of fatty acids from n-3 and n-6 families (ratio of 8:1) along with vitamins A, D, and E.

[p]Control normal fat (CN) diet contained olive oil, cod-liver oil, and corn oil in a ratio of 67:8:25; 47 g/L of casein; and 61.36 g/L of dextrin maltose.

[q]Alcohol-normal fat (AN) diet included olive oil, cod-liver oil, and corn oil in a ratio of 67:8:25, along with 50 g/L of ethanol, 47 g/L of casein, and 11.36 g/L of dextrin maltose.

[r]Control n3-FA fat (CF) diet included a mixture of olive oil, cod-liver oil, and menhaden fish in a ratio of 67:8:25.

[s]Alcohol-n3FA fat (AF) diet contained a mixture of olive oil, cod-liver oil, and menhaden fish in a ratio of 67:8:25 along with 50 g/L of ethanol.

[t]Sesame oil (SES) rich in 18:1n-9 (35%).

[u]Borage oil (BOR) rich in 18:3n-6 (24 %).

[v]Linseed/safflower oil (LSO) rich in 18:3n-3 (25%).

Abbreviations: ACC, acetyl-CoA carboxylase; ADIPOR2, adiponectin receptor; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMPAR1, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 1; AMPAR2, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 2; AMPAR3, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 3; AMPK, AMP-activated protein kinase; AST, aspartate aminotransferase; ATGL, adipose triglyceride lipase; BOR, borage oil; cAMP, cyclic adenosine 3′,5′-monophosphate; *Cd36*, fatty acid translocase; c-Met, receptor for hepatocyte growth factor; CPP, conditioned place preference; CPT1, carnitine palmitoyltransferase 1; CYP2E1, cytochrome P450 2E1; *Dgat1*, diacylglycerol acyltransferase 1; *Dgat2*, diacylglycerol acyltransferase 2; DHA, docosahexaenoic acid; ELISA, enzyme-linked immunosorbent assay; EPA, eicosapentaenoic acid; EtOH or ETH, ethanol; eWAT, epididymal white adipose tissue; *Fabp4*, fatty acid binding protein 4; *Fatp1*, fatty acid transporter protein 1; *Fatp2*, fatty acid transporter protein 2; *Gpat*, glycerol-3-phosphate acyltransferase; GPR120, G protein–coupled receptor 120; HDL, high-density lipoprotein; HIC, handling induced convulsion; HPLC/MS/MS, high-performance liquid chromatography with tandem mass spectrometry; HSL, hormone-sensitive lipase; IL-1β, interleukin-1beta; IL-6, interleukin-6; IL-10, interleukin-10; LC, long-chain; LDL, low-density lipoprotein; LORR, loss of righting reflex; LPL, lipoprotein lipase; LPS, lipopolysaccharide; LSO, linseed/safflower oil; MCP-1, monocyte chemoattractant protein-1; mRNA, messenger RNA; MTTP, microsomal triglyceride transfer protein; NAc, nucleus accumbens; n.d., not defined; NOS, nitric oxide synthase; pACC, phosphorylated acetyl-CoA carboxylase; pAMPK, phosphorylated AMP activated protein kinase; PDE3B, phosphodiesterase 3B; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PGC1β, peroxisome proliferator-activated receptor gamma coactivator 1-beta; p-HSL, phosphor-hormone-sensitive lipase; PPARγ, peroxisome proliferator-activated receptor-gamma; PSD95, postsynaptic density protein-95; PUFA, polyunsaturated fatty acid; RT-PCR, reverse transcription–polymerase chain reaction; SES, sesame oil; SIRT1, sirtuin 1; SREBP1, sterol regulatory element-binding protein 1; TNF-α, tumor necrosis factor-alpha; UV, ultraviolet; *Vldl-r*, very low-density lipoprotein receptor; WT, wild-type; 16S rRNA, 16S ribosomal RNA.

Table 4. Clinical study characteristics

Study, year	Country	Participants	Treatment	Intervention	Variables	Tools	Results
Pauluci et al, 2022[43]	Brazil	AUD patients OG = 59 CG = 52	Omega-3	90 d, 3 capsules/d (each capsule contains 1 g of fish oil, with 12% DHA and 18% EPA)	Timeline follow back Depression and anxiety Obsessive-compulsive drinking Craving Alcohol dependence	Self-reported Beck scales OCDS PACS SADD	No differences between groups Reduction in number of days of alcohol consumption in OG
Fogaça et al, 2011[44]	Brazil	Volunteers with dependent of alcohol according to DSM-IV OG = 12 CG = 11 NG = 11 NOG = 9	Omega-3 + omega-6	90 d 1 g *Borago officinalis* oil (omega 6: 120 mg GLA and 400 mg EPA + DHA) + 1 g fish oil (omega 3: 160 mg EPA and 240 mg DHA)	Drinking days Obsessive-compulsive drinking Alcohol dependence	Self-reported OCDS SADD	No differences between groups

$n = 2$.

Abbreviations: AUD, alcohol-use disorder; CG, control group; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; OG, omega-3 group; NG, naltrexone group; NOG, naltrexone + omega-3 group; OCDS, Obsessive-Compulsive Drinking Scale; PACS, Penn Alcohol Craving Scale; SADD, Short Alcohol Dependence Data.

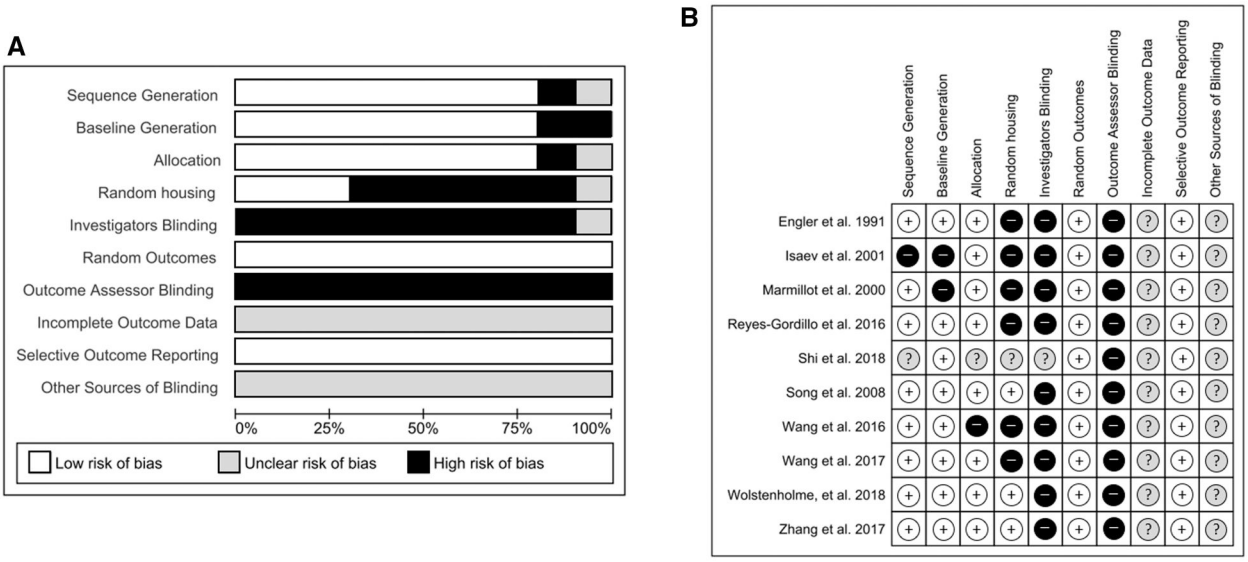

Figure 2. Methodological Quality Assessment of Preclinical Studies ($n = 10$). (A) Risk-of-bias graph. (B) Risk-of-bias summary (+ = yes; – = no; ? = unknown)

lead to changes in AMPAR expression. In mice treated with n-3–rich fish oil, a decrease in the expression of AMPARs and postsynaptic density protein 95 (PSD-95) has been observed.[38]

DISCUSSION

The objective of this systematic review was to identify clinical and preclinical studies that investigated the impact of a diet rich in n-3 fatty acids in mitigating behavioral changes, biochemical alterations, and the inflammatory response resulting from alcohol consumption. The evaluation included both human subjects and animal models of alcohol use. The findings indicate that n-3 fatty acids may exert a protective effect on certain neurobiological and behavioral responses associated with alcohol.

Preclinical research on the effects of n-3 regarding alcohol-related adverse effects shows promise, albeit being somewhat limited. According to Shi et al,[38] it has been observed that n-3 supplementation decreases

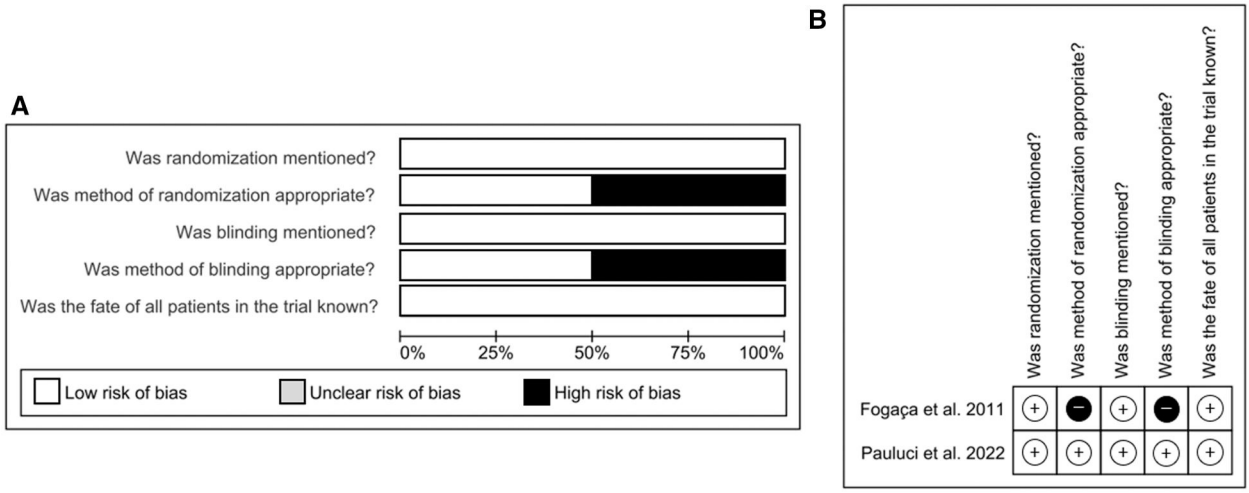

Figure 3. Methodological Quality Assessment of Clinical Trials ($n = 2$). (A) Risk-of-bias graph. (B) Risk-of-bias summary (+ = yes; − = no)

withdrawal-induced locomotor activity and mitigates alcohol-associated place preference in alcohol-exposed mice. Furthermore, it appears to counteract alcohol-induced alterations in the morphology of the nucleus accumbens. Moreover, a diet abundant in n-3 has been observed to impact alcohol metabolism and alcohol-induced locomotor activity.[39] Conversely, a DHA-rich diet reduces voluntary alcohol intake in selectively bred alcohol-preferring P rats.[22] Studies involving animal models with neonatal exposure to alcohol indicate that DHA can reverse alcohol-induced synaptic deficits, thereby enhancing alcohol-related potentiation. DHA has also demonstrated the capacity to reverse alcohol-induced synaptic deficits, thereby improving long-term potentiation in the dentate gyrus of adult rats.[19] Additionally, in prejuvenile stage, it has been demonstrated to mitigate the anxiety associated with prenatal alcohol exposure.[23] Indeed, the efficacy of an n-3 dietary supplement in reducing anxiety and cortisol levels has been observed in abstinent individuals.[21] However, it is worth noting that n-3 may elevate the risk of psychosis in individuals with a predisposition to mental health issues, potentially linked to the metabolic consequences of chronic inadequate nutrition within the population.[45]

Previous studies have highlighted the role of n-3 in preventing withdrawal symptoms and relapse to substance abuse. One primary hypothesis suggests that it achieves this effect through its anti-inflammatory function and its impact on synaptic remodeling.[46] An imbalance in the levels of PUFAs has been observed in individuals with impaired liver function, specifically an increase in n-6 (proinflammatory) and a reduction in n-3 (anti-inflammatory) PUFAs, particularly affecting women.[12] With regard to the outcomes of 2 studies mentioned in this review, the administration of n-3 PUFAs effectively reduced TNF-α and IL-1β levels in the plasma, liver, and adipose tissue of animals exposed to alcohol.[40,41] Taken together, these results suggest that n-3 PUFAs may alleviate physiological and behavioral changes induced by alcohol consumption.

Clinical and preclinical studies have demonstrated that alcohol consumption is associated not only with a modified fatty acid profile[47,48] but also with a phenomenon called intestinal dysbiosis.[49,50] This imbalance in the composition of the gut microbiome typically results in a decrease in Bacteroidetes and an increase in Proteobacteria, often accompanied by elevated levels of serum endotoxins and impaired permeability.[51,52] Consequently, transferring fecal microbiota from individuals with alcohol-use disorder or alcohol-exposed mice into healthy mice has been shown to induce symptoms related to withdrawal-induced depression and anxiety.[50,53] A study conducted in this context demonstrated that mice exposed to opioids and then experiencing withdrawal exhibited diminished richness in their intestinal microbiota and a tendency towards anxiety symptoms. However, a diet supplemented with n-3 PUFAs improved the intestinal bacterial profile and reduced opioid-seeking behaviors in the absence of drug availability, potentially alleviating anxiety.[54] Several studies conducted in animals exposed to alcohol indicate that diets enriched with n-3 can help mitigate intestinal microbiota disruption.[40,55] Therefore, it may be relevant to investigate the potential mediating role of the microbiota in the effect of n-3 PUFAs on the behavioral and emotional effects of alcohol.

The impact of n-3 PUFAs has also been explored in relation to substance addictions, including those to cocaine, nicotine, and opioids. For example, n-3 PUFA supplementation has been shown to reduce tobacco craving and decrease daily cigarette consumption in regular smokers.[56] Additionally, a cross-sectional study

revealed that smokers generally consume fewer n-3–rich foods and have lower levels of DHA and EPA compared with nonsmokers, suggesting that increasing n-3 intake could be a valuable strategy for reducing tobacco use.[57] In the case of cocaine addiction, research indicates that individuals with a history of aggression often exhibit lower levels of n-3 PUFAs and may be at a higher risk of relapse. Consequently, n-3 PUFA supplementation could help improve behavior, reduce impulsivity, and mitigate aggressive tendencies in individuals affected by cocaine use.[58] Similarly, a preclinical study has demonstrated that supplementation with n-3 PUFAs following chronic cocaine administration yielded favorable outcomes, including a reduction in opioid-seeking behaviors and anxiety, a restoration of DHA levels and glutamatergic activity in the striatum disrupted by opioid use, and an enhancement of microbial richness and phylogenetic diversity of the gut microbiota.[59] Collectively, these findings suggest that n-3 PUFAs may play a pivotal role in the mitigation of addictive behaviors and the promotion of mental and physical well-being in individuals struggling with substance use.

Limitations

The current systematic review is not without limitations. Some of the selected studies used both n-3 PUFAs and n-6 PUFAs, demonstrating varied outcomes regarding alcohol compulsion, dependence, and alcohol deprivation.[34,35,39,44] In this regard, it seems plausible that the effects of n-3 may be masked by the presence of n-6. This is of significance in contemporary society, as modern diets often have an imbalance in the n-6:n-3 ratio.[24]

It is important to consider the source of the n-3 PUFAs used, as some studies utilized vegetable oils derived from flaxseed oil,[37,40,42] while others used fish oil.[33,36,38,39,43] In terms of plant-based n-3 PUFAs, they consist of alpha-linolenic acid (ALA). This PUFA within the n-3 fatty acid group requires conversion into EPA and DHA to become biologically effective. Nevertheless, the conversion process is restricted in humans.[60] Consequently, EPA and DHA sourced from animals are frequently regarded as more effective in reaping the associated benefits of n-3.

Finally, it is important to note the differences in the effects of PUFAs observed between preclinical and clinical studies. Only 2 human studies were included after applying the selection criteria, making it difficult to draw definitive conclusions. In both studies, no significant differences were found when using n-3 PUFAs alone or in combination with n-6 PUFAs compared with controls.[43,44] The observed discrepancies can be attributed to the inherent differences between preclinical studies, conducted under controlled conditions with homogeneous subjects, and clinical trials, which are subject to factors such as genetic variability, lifestyle, comorbidities, and treatment adherence.[61–63] Furthermore, ethical constraints in clinical trials may limit the dosage or duration of supplementation.[64,65] These differences highlight the challenges associated with translating preclinical benefits into clinical practice and emphasize the necessity for more robust and diverse clinical research.

CONCLUSION

This study highlights the potential benefits of n-3 PUFAs in the treatment of alcohol-related disorders, both in clinical settings and in preclinical models. Specifically, DHA, a type of n-3 PUFA, may alleviate the negative effects of alcohol on organs such as the brain, liver, and intestine; reduce inflammation; and deter behaviors linked to drug-seeking and relapse. Additionally, n-3 supplements are known to be safe, well tolerated, and to have minimal adverse effects.

While the results are promising, more research is needed to optimize the effectiveness of n-3 PUFAs in mitigating alcohol-induced brain damage and the associated cognitive and behavioral disorders. This will entail an investigation of variables such as dosage, administration method, exposure duration, and specific types of PUFAs. Understanding the underlying mechanisms of PUFAs' benefits is crucial, with dietary animal models potentially playing a pivotal role. Conducting comprehensive studies will help clarify the conditions and mechanisms for effectively reducing alcohol-related damage and improving outcomes for individuals with alcohol-use disorders.

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Supplementary Material

Supplementary Material is available at *Nutrition Reviews* online.

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Conflicts of Interest

None declared.

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