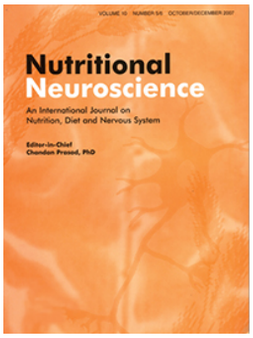

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Omega-3 fatty acids and anxiety: A systematic review of the possible mechanisms at play

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Introduction: Anxiety is prevalent, costly, and associated with significant adverse outcomes. The importance of nutrition is underestimated in the management of mental health disorders. In particular, omega-3 fatty acids (ω-3 FAs) are a critical component for healthy development and have been shown to reduce anxiety symptoms.
Objective: This paper reviews the current state of the research to identify potential mechanisms underlying the relationship between ω-3 FAs and anxiety reduction.
Method: Studies were identified using PubMed, PsycINFO, and CINAHL databases.
Results: Of the 197 full-text studies screened, six met criteria for inclusion. Four mechanisms were identified based on primary outcomes reported by each study, *Inflammatory Response, Brain-Derived Neurotrophic Factor (BDNF), Cortisol,* and *Cardiovascular Activity.*
Conclusion: *Five key recommendations* are provided to guide future research examining ω-3 FAs and anxiety. They include: (1) standardization of dosage and duration of ω-3 supplementation, (2) more rigorous measurement of variables, (3) effective blinding of participants, (4) designing experiments that test mediation, and (5) increasing sample diversity.

Keywords: Anxiety, Brain-derived neurotrophic factor, Inflammation, Omega-3 fatty acids

Anxiety disorders are generally defined as the presence of excessive or uncontrollable fear in response to a real or imagined stimuli and is associated with prolonged distress and functional impairment.[1] These disorders affect one in 14 individuals globally[2] and can adversely impact an individuals' quality of life, social relationships, occupational and academic pursuits, and pose a fourfold increased risk of developing a comorbid psychological condition.[3,4] Cognitive behavioral therapy (CBT) and psychotropic medications are efficacious treatments for anxiety disorders.[5] These treatment approaches are successful in up to 70% of individuals, though the remaining subset prematurely discontinues treatment or fails to achieve symptom reduction.[6] Interventions can be costly, time-consuming,[7] and difficult to tolerate (e.g., exposure exercises, side effects).[8,9] Given these limitations, therapeutic lifestyle interventions are being explored as potential treatment options for anxiety.[10]

Nutritional supplementation with ω-3 fatty acids

There has been a recent upsurge in examining the role of nutrition in the development, management, and prevention of mental health disorders because nutrition can influence important domains associated with psychological health including inflammation, the gut microbiome, immune responses, and cognitive abilities.[11,12] Omega-3 fatty acids (ω-3 FAs or Ω-3 FAs), in particular, are an essential component of the human diet, necessary for healthy brain function including central nervous system development.[13] Ω-3 FAs have also been linked with recovery from neurological damage. For example, supplementation of ω-3 FAs has been found to help with neuroprotection, neuroinflammation, and neuroregeneration in individuals who have experienced traumatic brain injuries.[14]

Ω-3 FAs are long chain polyunsaturated fatty acids (PUFA). The three main ω-3 FAs important to human functioning are α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALA is an essential fatty acid meaning it cannot be synthesized by mammals. ALA can be metabolized into EPA and DHA, although this conversion is inefficient.[15,16] Thus, humans must acquire these fatty acids from diet. ALA can be acquired from plant-based oils (e.g., seeds, nuts, leafy vegetables) and EPA and DHA from marine-based oils (e.g., fish, algae).

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Evidence of the relationship between ω-3 FAs and anxiety

Collectively, observational and experimental studies have demonstrated that ω-3 supplementation can ameliorate anxiety symptoms. High ω-3 intake is associated with reduced likelihood of meeting diagnostic criteria for an anxiety disorder[17,18] and lower levels of self-reported anxiety symptom severity.[19,20] In randomized control trials (RCTs), ω-3 supplementation is associated with reduced anxiety symptoms and related behaviors.[21–23] Accordingly, given the anxiolytic properties of ω-3 FAs, supplementation of ω-3 FAs holds significant promise as novel class of treatment for anxiety.

Although research is in the early stages with human subjects, the literature demonstrates favorable associations between ω-3 FAs and anxiety. Utilizing ω-3 FAs to treat anxiety is an innovative and potentially cost-effective therapeutic option. Yet, the mechanisms underlying why omega-3 FAs may ameliorate anxiety are poorly understood and have received little empirical attention. However, two mediating mechanisms, the inflammatory response and brain-derived neurotrophic factor, have been hypothesized to potentially play a role and will be reviewed below.

Potential mechanisms

Inflammatory response

The inflammatory response is pertinent to the pathophysiology of anxiety.[24] The presence of anxiety is associated with increased production of proinflammatory cytokines including tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6).[25] Both TNF-α and IL-6 are proteins that regulate immune and inflammatory reactions to injury or infection.[26] While an increase in anxiety is associated with an increase in inflammatory cytokine production, it is generally supported that ω-3 FA supplementation leads to decreased inflammatory cytokine production.[27,28] A meta-analysis of 68 RCTs showed that ω-3 supplementation was associated with reduced TNF-α and IL-6 in healthy adults and adults with chronic illness.[29] As both ω-3 FAs and inflammation are involved in anxiety, this overlap may be a pathway by which ω-3 FAs can be used to ameliorate anxiety symptoms. It is possible that ω-3 FAs inhibit or reduce the inflammatory processes associated with anxiety, and thus, may act as a mechanism explaining the relationship between ω-3 FAs and emotional functioning.[30]

Brain-derived neurotrophic factor (BDNF)

A second possible mechanism of ω-3 FA function is through brain-derived neurotrophic factor (BDNF). BDNF is a type of protein that regulates nervous system functions by encouraging neuronal survival, differentiation, and growth[31] and is expressed within the central and peripheral nervous system. It is important to note there is mixed evidence as to whether circulating BDNF blood levels are associated with BDNF within the brain. Specifically, unmodified BDNF, which are larger molecules, has difficulty crossing the blood-brain barrier (BBB) when administered peripherally (e.g., veins in the arms, hands). However, pre-clinical research suggests that BDNF can cross the BBB via synthesis of a chimeric peptide making BDNF transportable to the brain and shown to act as a neuroprotective factor against brain ischemia.[32,33] BDNF is also involved with neuronal synaptic formation and plasticity,[34] both of which have been shown to promote healthy brain function.

The neurotrophin theory of depression posits that BDNF mediates the development of stress induced depressive symptoms through an inverse relationship.[35,36] When BDNF is low, it fails to stimulate synaptic growth of serotonergic neurons in the brain,[37] and low levels of serotonin are associated with anxiety and depression.[38] Thus, it is hypothesized that BDNF may reduce depression and anxiety by stimulating synaptic growth of serotonergic neurons in the brain.[37,39] Ω-3 supplementation may demonstrate anti-depressant effects given that it is associated with increased levels of BDNF and serotonin transmission.[40] Supplementation of EPA and DHA in animal studies is associated with increased BDNF levels in the brain.[40–43] Studies have also demonstrated that ω-3 FA supplementation can normalize BDNF brain levels after an experimental traumatic brain injury in rats.[44] Although this relationship has not been rigorously tested in individuals with anxiety, research does suggest a positive relationship between ω-3 levels and BDNF in patients with anxiety symptoms.[45] Thus, examining BDNF as a mediator in the relationship between ω-3 FAs and anxiety is warranted.

Current aim

Detecting the mechanisms of action can provide empirical support for the development of targeted therapeutic interventions. This systematic review examines the current literature and identifies potential mechanisms responsible for the association between ω-3 FAs and anxiety. We offer recommendations to move this area of research forward and to implement ω-3 FAs in the most effective manner.

Method

Study eligibility

Studies were included in the review if they met six criteria: (i) examining any possible mechanism underlying the relationship between anxiety symptoms or disorders and ω-3 FAs; (ii) one or more components of ω-3 FAs (DHA, ALA, or EPA) were consumed

by participants via pill or diet and measured via blood or self-report diet; (iii) assessment of anxiety symptoms, anxiety disorders, or anxiety-related disorders via self- or clinician-report; (iv) the study was peer-reviewed original research; (v) included human subjects; and (vi) was written in English. Given the limited literature, no date constraints were applied.

Literature search strategy

Data collection and extraction were in concordance with the PRISMA criteria.[46] A keyword-based search was conducted in PubMed, PsycINFO, and CINAHL to obtain relevant results. First, medical subject headings (MeSH) terms associated with anxiety ("anxiety" or "anxiety disorders") were searched and combined with omega-3 terms ("fatty acids, omega-3″, or "fatty acids"). Next, keywords related to anxiety (anxiet* or anxious* or anxiety disorders or "obsessive compulsive" or "panic" or "social phobia" or "post traumatic" or "agoraphobia" or "phobia" or GAD or PTSD or OCD or SAD) were combined with keywords related to omega-3 FAs (omega* or Lcn* or "DHA" or "ALA" or "EPA" or eicosapentaenoic acid or alpha-linolenic acid or docosahexaenoic acid or fatty acid) to obtain hit results. A backward search of reference lists was also done to identify any additional relevant articles.

Study selection

The first author (ARP) conducted the literature search in November 2017. Two raters (ARP and HS) independently screened article titles and abstracts for study appropriateness and duplicate records were eliminated. The remaining studies were then evaluated based on the inclusion criteria. Discrepancies were resolved through discussion.

Data extraction

For each study included in the systematic review, two reviewers independently extracted demographic data (i.e., age, sex, and ethnicity) and the instrument used to measure anxiety. Type, dosage and measurement of ω-3 FAs administered (i.e., DHA, ALA, or EPA), as well as the duration of time it was dispensed, was extracted. Lastly, potential mechanisms were identified.

Assessment of risk of bias

We utilized the Cochrane Collaboration Risk of Bias Tool[47] to evaluate the quality of each study with respect to five domains of bias: selection, performance, detection, attrition, and reporting. Each assessor independently reviewed and categorized each study in all papers for low, high, or unclear risk on each domain.

Data synthesis

Due to the small number of studies, a narrative synthesis of the data is presented to elucidate the potential mechanisms explaining the relationship between ω-3 FAs and anxiety. Guidelines to conduct the synthesis is based on the Cochran Consumers and Communication Review Group.[48]

Description of studies

Results of the search

A total of 1,311 citations were identified; of these 393 duplicates were removed. An additional 721 studies were deemed irrelevant based on title and abstract review and excluded for the following reasons: (1) not investigating ω-3 FAs or anxiety ($n = 458$); (2) examining anxiety but not ω-3 FAs ($n = 167$); (3) examining ω-3 FAs but not anxiety ($n = 78$); and (4) non-peer reviewed article ($n = 18$). The full text of the remaining 197 studies were screened based on the eligibility criteria. Reasons for excluding studies at the full-text screening phase were: non-human participants ($n = 75$), not assessing anxiety or potential mechanism ($n = 68$), review papers ($n = 35$), participants had a personality or developmental disorder ($n = 5$), non-peer reviewed papers ($n = 6$), and one duplicate and one non-English paper that were not excluded in the initial round of reviews. Six studies met inclusion criteria.

Included studies

Participants

A total of 469 participants were enrolled across the six studies with 8–196 participants per study. The average age was 29.57 years, approximately 79% of participants were male and 100% were over the age of 18. Three studies were conducted in Japan, two in the United States, and one in Israel. Characteristics from each study are presented in Table 1.

Study intervention characteristics

All six studies supplemented participants with ω-3 FAs across time, with duration ranging from 3 to 12 weeks ($M = 9.3$ weeks). Five studies administered a combination of EPA and DHA supplements to participants. The average mass of EPA supplemented was 841 mg while the average mass of DHA was 1176 mg. One study supplemented participants with 45 mg of ALA and 180 mg of linoleic acid. Four of the six studies had a placebo condition.

Quality of included studies

Generally, there was low risk of bias in the five domains across the six studies. Two studies were rated high risk in performance bias; in one, participants were not blinded to their condition;[54] in the other, 66% of participants guessed their treatment condition.[49] See Table 2 for complete details.

Table 1 Study characteristics

Author (Year)	*N*	Age M (SD)	Omega-3 Administered	Control/ Placebo	Length of Intervention	Anxiety Measure	Proposed mechanism	Study findings
Giles (2015)[49]	*n* = 36 intervention; *n* = 36 control	20.64*	1680 mg EPA, 1120 mg DHA	2800 mg/ day olive oil	5 weeks	State Trait Inventory for Cognitive and Somatic Anxiety	IL-1β / salivary cortisol	ω-3s had no effect on salivary cortisol or IL-1β
Kiecolt-Glaser (2011)[50]	*n* = 34 intervention; *n* = 34 placebo	23.65 (1.87)	2085 mg EPA, 348 mg DHA	Mixture of palm, olive, soy, canola, and coco butter oils	12 weeks	Beck Anxiety Inventory	IL-6, TNF-α	ω-3 FAs decreased IL-6, and anxiety symptoms
Matsumura (2012)[51]	8	36.6 (17.6)	147 mg EPA, 1470 mg DHA	No control condition	12 weeks	The Clinician-Administered PTSD Scale	Cardiovascular activity	Unknown if ω-3 FAs effected physiological factors
Matsuoka (2011)[52]	15	34.0 (18.0)	147 mg EPA, 1470 mg DHA	No control condition	12 weeks	Psychiatrist assessed PTSD by structured clinical interview	BDNF	ω-3 FAs increased BDNF and decreased PTSD symptoms
Matsuoka (2015)[53]	*n* = 53 intervention; *n* = 57 placebo	39.5*	147 mg EPA, 1470 mg DHA, 0.3% alpha-tocopherol	rapeseed oil (47%), soybean oil (25%), olive oil (25%), fish oil (3%), 0.3% α-tocopherol	12 weeks	The Clinician-Administered PTSD Scale	BDNF	ω-3 FAs had no effect on BDNF or PTSD symptoms
Yehuda (2005)[54]	*n* = 88 intervention; *n* = 38 placebo; *n* = 70 control	23*	225 mg ALA, LA in ratio of 1:4	Mineral oil	3 weeks	SMART test (test anxiety)	Salivary cortisol	ω-3/ω-6 FAs reduced cortisol levels and improved test anxiety symptoms

Note. ALA = α-linolenic acid; BDNF = brain-derived neurotrophic factor; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; IL-6 = interleukin-6; IL-1β = interleukin 1-beta; LA = linoleic acid; PTSD = post-traumatic stress disorder; ω-3 FA = omega-3 fatty acids; ω-6 FAs = omega-6 fatty acids; TNF-α = tumor necrosis factor alpha.
* = SD unavailable.

Results

The aim of this systematic review was to elucidate potential mechanisms underlying the association between ω-3 FAs and anxiety. Of the six studies reviewed, four potential mechanisms were identified. For expected mechanisms, two studies reported on *Inflammatory Response*, while a separate two studies examined *Brain-Derived Neurotrophic Factor*. The literature review also generated two additional mechanisms that were not anticipated. Two studies examined

Table 2 Risk of bias

	Selection Bias		Performance Bias	Detection Bias	Attrition Bias	Reporting Bias
	Random Sequence Generation	Allocation Concealment	Blinding of Participants	Blinding of Outcomes	Incomplete Outcome Data	Selective Reporting
Giles (2015)						
Kiecolt-Glaser (2011)						
Matsumura (2012)						
Matsuoka (2011)						
Matsuoka (2015)						
Yehuda (2005)						

Note: Each study was independently reviewed by an assessor and categorized for the risk as "low risk", "high risk", or "unclear risk" for each study on each domain.
Low risk =
High risk =
Unclear risk =

Cortisol, and one study examined *Cardiovascular Activity*, both of which are reviewed below as possible mediators explaining the relationship between ω-3 FAs and anxiety. The results below are organized according to mechanism examined.

Inflammatory response

The two studies in the present review assessed interleukin-beta (IL-1β), IL-6, and TNF-α. A study by Giles[49] tested the effects of ω-3 FAs on the proinflammatory cytokine IL-1β and anxiety symptoms in response to a stressful task among 73 young adults. Participants were randomized to receive either a ω-3 or an olive oil capsule daily for five weeks. For both conditions, IL-1β increased during the stressful task only versus the non-stressful tasks. The authors concluded that ω-3 supplementation did not affect stress-induced change in inflammatory markers.

Kiecolt-Glaser[50] examined whether a 12-week supplementation of ω-3 would decrease IL-6 and anxiety symptoms in a sample of 68 medical students. Participants received a ω-3 supplement (treatment) or a pill with a mixture of oils (control). Participants who received the ω-3 supplement demonstrated a significant 14% reduction in stimulated IL-6 and a significant 20% decrease in anxiety scores. While there was a comparable reduction in stimulated TNF-α, the effect was not statistically significant.

Overall, these studies partially support inflammation as a mediator. Support was found for certain inflammatory markers (e.g., IL-6) but not for others (e.g., TNF-α, IL-1β). However, it is important to note that the Giles study[49] had insufficient blinding of treatment conditions (83% of participants in the ω-3 condition and 44% of the control condition correctly guessed their treatment), and participants had high ω-3 intake at baseline (ceiling effect), thus the null findings must be evaluated in the context of this limitation.

Brain-derived neurotrophic factor (BDNF)

Two studies conducted by the same research group assessed BDNF in an initial pilot study[52] and a subsequent RCT.[53] The aims of the open-label pilot study were to examine whether a 12-week ω-3 FA supplementation would increase BDNF levels, and to assess whether the changes in BDNF were associated with improved post-traumatic stress symptoms (PTSS) among 15 adults in an intensive care unit (ICU) who experienced an accidental injury. The results of this study indicate that the supplementation of ω-3 FAs significantly increased serum levels of BDNF and that BDNF may be associated with decreased PTSS at follow-up.

In a follow-up RCT with 110 injured adult patients who were treated in the ICU, participants were randomly assigned to an ω-3 supplement or a placebo for 12 weeks. Participants in both conditions had increased BDNF at post-assessment with no group differences at the 12-week follow-up. There was no association between changes in BDNF and PTSS.

Overall, although the pilot study supported BDNF as a possible mediator between ω-3 FAs and anxiety, the effect was not replicated in the larger controlled study.

Hypothalamic-pituitary-adrenal (HPA) axis activity: cortisol

The hypothalamic-pituitary-adrenal (HPA)-axis is triggered by stress, and anxiety disorders are characterized by a hyperactive HPA-axis response, resulting in an excess secretion of cortisol.[55] Little research has examined the impact of ω-3 FAs on cortisol levels. Some research demonstrates that ω-3 FAs are associated with reduced perceived stress and cortisol within a sample of abstinent alcoholics[56] and healthy men.[57] These findings suggest that ω-3 FAs influence the stress response, and in turn anxiety.

Cortisol was measured within two studies to test HPA-axis activity as a potential mediator of the association between ω-3 FAs and anxiety. Yehuda[54] administered a mixture of a ω-3/ω-6 FAs to 88 undergraduate students for three weeks to assess the effects on test anxiety and salivary cortisol. Cortisol levels decreased and behavioral symptoms of test anxiety (i.e., concentration, fatigue) improved among the intervention group, with no improvements shown in the placebo group post-assessment. In contrast, Giles[49] (also described in the *Inflammatory Response* section) studied the effects of ω-3 FAs on salivary cortisol levels and anxiety symptoms among 73 young adults. Participants were randomized to receive either an ω-3 capsule or an olive oil capsule daily for five weeks. While cortisol increased during stressful tasks across groups, there were no between group differences on anxiety measures.

Findings for salivary cortisol demonstrate mixed results. Yehuda[54] found support for cortisol as a mediator between ω-3 FAs and anxiety, whereas Giles[49] did not. However, it bears repeating that there was insufficient blinding in the Giles study[49] and in the Yehuda study,[54] participants were not blinded to their condition, limiting the utility of these findings.

Cardiovascular activity

Anxiety is associated with lower heart rate variability (HRV; the variation in time between heartbeats) due to impairment in vagal nerve activity.[58] Over time, low HRV may subsequently lead to higher inflammation[58] and increased incidence of cardiovascular disease.[59,60] In contrast, the consumption of ω-3 FAs can potentially improve HRV[61] and reduce risk of cardiovascular disease and associated conditions (e.g., high blood pressure)[62,63] Thus, it is plausible that

cardiovascular factors mediate the relationship between ω-3 FAs and anxiety.

Matsumura[51] analyzed physiological stress reactivity data (e.g., heart rate, skin conductance, and blood pressure) as a possible mechanism explaining the relationship between ω-3 FAs and clinician assessed PTSD. In this study, eight adults who experienced an injury requiring hospitalization were administered ω-3 FA supplements. After 12-weeks of supplementation, subjects participated in an experiment where they listened to startle tones and a trauma script related to their individual injury. Physiological stress data was recorded during the experiment to assess response to a trauma related cue. After the experiment, one participant met criteria for PTSD and demonstrated a larger reactivity to the startle tones (e.g., increased heart rate, blood pressure, skin conductance) as compared to the seven participants without PTSD. However, there were no differences in physiological reactivity to the personalized trauma script in the participant with PTSD compared to the other participants. This study did not support cardiovascular activity as a potential mediator between ω-3 FAs and anxiety. Results should be interpreted with caution as this study included only eight participants, lacked a comparison group, and PTSD symptoms were not assessed at baseline.

Summary and future directions

The present review examined the literature for potential mechanisms underlying the relationship between ω-3 FAs and anxiety. Based on the six studies that met inclusion criteria for the systematic review, four potential mechanisms were identified: *Inflammatory Response*, *BDNF, Cortisol,* and *Cardiovascular Activity*. Three of the six studies demonstrated a reduction in anxiety as a result of ω-3 supplementation. Across these studies, IL-6, neuroendocrine protein BDNF, and cortisol were revealed as potential mechanisms by which ω-3 FAs influence anxiety. However, when considering all studies, findings for each mechanism were mixed, and this review only included six studies with significant heterogeneity. Therefore, to better establish which mechanisms are responsible for the link between ω-3 FAs and anxiety, this paper offers *five key recommendations* for future research in the area, listed below.

Standardize ω-3 dosage and duration, as well as the comparison condition in intervention studies

Dosages of ω-3 fatty acids

There were variations in the type of ω-3 FAs administered to participants (for specific dosage breakdown see Table 1). However, with the exception of the Yehuda study,[54] all supplements administered ranged from 1617 to 2800 mgs of ω-3 FAs, which is higher than a typical over the counter ω-3 supplement, containing approximately 300 mg of EPA and DHA combined.[64]

In our review, two studies administered supplements with higher EPA than DHA content. Giles[49] administered 560 mg more EPA than DHA, whereas Kiecolt-Glaser[50] administered a ratio three times larger with 1737 mg more EPA than DHA. Studies administering ω-3 supplements vary in the proportions of EPA and DHA content, although this ratio appears to be important for optimal effects. One meta-analysis found beneficial effects for depressive symptoms when EPA supplementation was 200–2200 mg more than DHA.[65] Hence, the higher EPA ratio in Kiecolt-Glaser's study[50] may explain why ω-3 supplementation resulted in fewer anxiety symptoms. Precise dosages and ratios of each ω-3 are important to assess;[66] however, specific guidelines do not exist. Since each ω-3 (i.e., ALA, EPA, DHA) has a different biochemical make-up, each may have different implications on mediators (e.g., cytokines).[67] Therefore, creating benchmark ω-3 recommendations will facilitate standardization across research designs.

In addition to increasing consistency of the dosage and ratio of EPA and DHA of ω-3 FAs across studies, ω-3 FA supplement storage is also important to monitor. Both researchers and participants should store ω-3 FA supplements in a cool dry environment and not in direct sunlight. Fish oil can also oxidize and become rancid and toxic if proper precautions are not taken. One way to ensure freshness of ω-3 FAs is to add vitamin E to the supplements.[68]

Duration of ω-3 fatty acids

Across the six studies, the duration of ω-3 supplementation varied from 3 to 12 weeks. The length of supplementation can significantly impact treatment effects, and consequently, whether underlying processes of change can be measured. For instance, a meta-analysis of 68 RCTs found that ω-3 supplementation longer than 8 weeks resulted in greater reductions of IL-6 and supplementation longer than 12 weeks demonstrated even greater reductions in TNF-α.[29] Another meta-analysis examining the impact of ω-3 supplementation in patients with chronic heart failure found greater reductions in IL-6 and TNF-α in trials longer than 4 months compared to trials less than 4 months.[69] In the current report, of the two studies that analyzed inflammatory markers,[49,50] the lengthier 12-week study was effective in reducing IL-6, whereas the shorter five-week study did not reduce IL-1β. It is unclear whether the shorter duration of supplementation, lack of participant blinding, or some other factor was responsible for lack of results. Based on previous meta-analyses,

a minimum duration of ω-3 supplementation between 8 and 12 weeks may be necessary to affect systemic inflammation. Further research is needed to establish optimal length of ω-3 supplementation to reduce anxiety, as these parameters are unknown.

Comparison condition

Four of the six studies reviewed included a control group. However, all comparison conditions received various oil supplements rather than a non-active placebo, which may have resulted in "active" comparison groups rather than true placebo controls. For instance, olive oil has been associated with less cognitive decline[70] and fewer depressive symptoms.[71] Studies that use a placebo would strengthen the research by helping us understand the true impact of ω-3FAs on emotional functioning.

Implement more stringent measurement of variables, including: typical dietary patterns (including ω-3 intake) and anxiety symptoms

Dietary habits

Diet is largely variable across individuals and can provide ω-3 FAs, therefore, it is critical to assess typical dietary patterns of participants. Of the studies reviewed in this paper, one study collected self-report diet retrospectively for the previous 3-months,[50] while another collected weekly reports to capture consumption of foods high in ω-3 and ω-6.[49] The remaining four studies excluded individuals if they had a nutritional supplement regimen (e.g., ω-3 supplementation for three months prior to the study) or regular consumption of fish (e.g., more than 2 or 4 times per week).

Dietary sources contain different amounts of each type of ω-3.[64,72] As such, recording dietary habits via food frequency questionnaires or food diaries would allow researchers to understand the impact of participants' dietary ω-3 consumption beyond the study supplementation. However, under-reporting is a common limitation of self-report diet surveys[73] and may be a barrier to receiving accurate dietary information from participants if nutrition is not measured objectively. Objective measures such as assessing ω-3s in blood samples[74] would improve the accuracy of measurement of each type of ω-3 and control for recall biases.

Furthermore, it is necessary to record both baseline and post-intervention levels of ω-3 FAs to assess the impact of the intervention.[75] The exclusion of participants whom regularly consume fish high in ω-3 FAs may be needed as there will likely be undetectable changes in ω-3 FA levels.

There also may be particular issues surrounding DHA intake that are important to consider. As aforementioned, DHA plays a critical role in the development and function of a healthy brain, such as cognitive functioning and memory.[76] Although DHA is abundant in the human brain, it is difficult for certain forms of DHA to successfully transport across the blood-brain barrier. There are two types of DHA that are supplied to the brain, nonesterified-DHA (NE-DHA), or free DHA, and Lysophosohatidycholine-DHA (LPC-DHA) and different mechanisms facilitate the uptake of each DHA through the blood-brain barrier. Some research indicates that fatty acid transport protein 1 and fatty acid binding-protein 5 play a significant role in transporting NE-DHA to the brain.[77,78] However, other research posits that NE-DHA does not increase brain DHA and only administration of LPC-DHA increases brain DHA via a protein of the major facilitator superfamily, Mfsd2a.[79,80] Overall, DHA uptake is a complex system with no straightforward mechanism by which all DHA is transmitted to the brain. Hence, reporting the type of DHA being administered and the use of objective markers to assess DHA levels may be important. Furthermore, research studies that have objectively measured DHA in humans are assessing DHA levels within the blood; it is uncertain as to how much DHA is transferred to the brain. However, consistency in measurement across studies and conditions should help to minimize measurement error.

In addition to implementing more stringent measures of ω-3 levels within research trials, it is important to acknowledge whether taking ω-3 FA supplements is similar to eating fresh fish. One study examining patients with hyperlipidemia found that those eating fresh fish demonstrated larger reductions in cholesterol levels than those who were taking the fish oil supplements over the course of two months.[81] A review examining the role of ω-3 FAs and cognitive decline concluded that longitudinal observations of fish intake yielded beneficial results on cognitive health in older health adults. However, if patients had an established cognitive disorder, such as Alzheimer's disease, supplementation was not helpful.[82] Consuming ω-3 FAs within various oils and supplements demonstrate beneficial effects. For instance, krill oil and sardine oil, rich in ω-3 FAs, have shown to promote working memory function in elderly populations.[83] Therefore, any ingestion of ω-3 FAs are likely to have some beneficial effects compared to no ω-3 FA dietary intake.

Anxiety symptoms

Anxiety measures across studies ranged from self-report of general anxiety symptoms to domain specific anxiety (e.g., test anxiety), as well as clinical diagnostic interviews. Anxiety symptom severity may modify the effectiveness of ω-3 FAs and be a determinant for the specific patient populations for which ω-3 FAs would

be recommended. For instance, in individuals with depression, ω-3 supplementation has shown to be more beneficial in patients with more severe depression compared to individuals with mild depression.[84]

Additionally, the Diagnostic and Statistical Manual of Mental Disorders-V has changed the original classification of Anxiety Disorders to three separate categories: (1) Anxiety Disorders, (2) Obsessive-Compulsive Disorders, and (3) Trauma and Stressor-Related Disorders.[85] Three of the six studies reviewed examine participants with PTSD, which is in category three. While the new classifications have overlapping symptoms,[86] including neurological activity,[87] and respond to similar treatments (e.g., SSRIs), they also have distinct features. Thus, anxiety conditions should be examined to test whether ω-3 supplementation effects are consistent across diagnostic classifications.

Employ effective blinding of participants

There was insufficient blinding of treatment conditions in the Giles study[49] and no blinding in the Yehuda study.[54] The Cochrane Collaboration Risk of Bias Tool classifies the non-blinding of participants as "high risk" and indicates that improper blinding may significantly alter the results of the study.[47] There are protective measures to take when administering ω-3 supplements. One common method to successfully blind participants is to mask the fishy flavor of the ω-3 supplement by adding a citrus flavor to all supplements administered.[88] Another method is to add small amounts of fish oil to placebo capsules and inform all participants to expect a fishy flavor.[89] Attempting to ensure that all participants have a similar experience in the trial can reduce the number of variables, namely expectancy effects, which can influence study outcomes.

Design experiments that test mediating mechanisms

In the present review, our goal was to identify potential mechanisms that may mediate the relationship between ω-3 FAs and anxiety. However, none of the reviewed studies used statistical mediation to test mechanistic associations. Matsumura[51] proposed a mediation hypothesis: that ω-3 FAs prevent PTSD through cardiovascular activity. Yet, they did not statistically test cardiovascular activity as a mediator. The other studies reviewed here also measured variables that are potential mediating mechanisms between ω-3 FAs and anxiety, but none tested whether there was an indirect effect of ω-3 FAs on anxiety through the proposed mechanism. To properly test mediation, the independent variable must be manipulated (or measured) prior to measuring the mediating mechanism and outcome variables.[90] We recommended that future studies are designed to temporally manipulate the predictor (ω-3 FAs), followed by measuring the mediator, and outcome (anxiety), and to statistically test for mediation. Studies such as these will produce findings that can clearly be interpreted as causal, and will reveal the magnitude of an indirect effect of ω-3 FAs on anxiety through the mediator. Uncovering mediating mechanisms with greater precision will provide a basis for designing interventions to target the mediator with confidence.

Investigate ω-3 supplementation with more diverse samples

Within the six studies reviewed, 79% of the subjects were male, predominately white, in their 30′s, and sex differences were not reported. It is recommended that sex be accounted for when investigating the effects of ω-3 supplementation. It appears that there are higher concentrations of DHA[91] and lower concentrations of EPA[92] in the blood of females compared to males. These differences suggest that females and males may metabolize fatty acids differently as dietary fish intake was similar in men and women.[92] Due to sex differences in baseline DHA and EPA levels, it may be necessary for researchers to have sex specific dosing in order to detect beneficial effects on anxiety and potential mediators at play.

Age and body mass index also influence the impact of ω-3 supplementation on cardiovascular disease risk factors.[93] For example, ω-3 FAs more significantly lowered IL-6 in older participants compared to younger participants.[29] These findings imply that larger and more diverse samples are needed to examine demographic variables and better understand for whom ω-3s are most effective.

Three of the six studies were conducted in Japan, a region associated with high rates of seafood (and thus ω-3 FA) consumption. A diet high in ω-3 FAs may have caused participants to have high ω-3 FAs prior to participation, and produced a ceiling effect, as Japanese individuals can have up to a three times higher proportion of ω-3 concentrations than Americans.[94] Two of these studies tried to control for this by excluding individuals who consumed fish more than four times per week, and only one study assessed pre-treatment baseline ω-3 levels via blood. The predominant type of fatty acid a person consumes regularly depends on geographical region.[95] For instance, dietary intake of ω-3 FAs will vary based on coastal or non-coastal location, with coastal residents consuming more than twice as much ω-3 FAs than non-coastal residents.[96] It is important to

consider geographical location when conducting and evaluating studies investigating diet.

Conclusions

Growing evidence suggests that ω-3 FAs play an important role in the prevention and treatment of anxiety. However, research is only now starting to move beyond correlational designs to explore potential mechanisms that explain this relationship. The present review was conducted to examine the pathways through which ω-3 FAs may impact anxiety. Based on this report, only a handful of such studies exist, and none directly tested mechanisms underlying this relationship. If ω-3 FAs are going to be utilized as a therapeutic option for anxiety, understanding the mechanisms by which they operate is crucial to designing the most effective treatment strategies. Five key recommendations for future empirical efforts were provided.

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