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Neuroprotection induced by omega-3 polyunsaturated fatty acids: focus on neuropsychiatric disorders

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Abstract (100-150 words)

Existing therapies (mainly pharmacotherapy) show modest effectiveness with limited effect sizes and unsatisfactory outcomes in the treatment of neuropsychiatric disorders, such as depressive disorders and schizophrenia. More recently, nutritional treatment has become a promising area of alternative options due to the relatively low adverse effects profiles and the accessibility of the nutraceuticals and nutritional products. Omega-3 polyunsaturated fatty acids (also; ω-3-PUFAs or n-3 fatty acids), namely eicosapentaenoic acid (EPA, 20:5, ω-3) and docosahexaenoic acid (DHA, 22:6, ω-3), have shown therapeutic potential in clinical and pre-clinical research of several neuropsychiatric disorders. Here we discuss the current in vivo and in vitro evidence regarding the underlying neuroprotective mechanisms through which PUFAs exert such properties.

Keywords: Omega-3, polyunsaturated fatty acids, neuroprotection, neuropsychiatric, neuroinflammation, depression, schizophrenia, bipolar disorder
Introduction

Despite pharmacological advancements, the global burdens of unmet needs of treatment outcomes of neuropsychiatric disorders such as depressive disorders and schizophrenia, have continued to increase (Perini et al., 2019, Baxter et al., 2013, Baranne and Falissard, 2018). This has occurred alongside extreme changes in nutritional intake with an evident transition to lower-quality, calorie-dense, and nutrient-deficient diets, particularly in highly-industrialized countries (Grosso, 2021, Logan and Jacka, 2014). This dietary shift has had a profound effect on the health of individuals, and in recent years there has been increased interest in examining associations in this change with the higher incidence of neuropsychiatric disorders (Logan and Jacka, 2014, Bujtor et al., 2021). In particular, dietary Omega-3 polyunsaturated fatty acids (also; ω-3-PUFAs or n-3 fatty acids) have been of interest due to their established associations with other aspects of human health, such as in cardiovascular disease (Hu et al., 2019), and in neuropsychiatric disorders (Su et al., 2000) and their capacity to positively modulate the immune and inflammatory response (Parletta et al., 2013, Layé et al., 2018, Borsini, 2021, Su, 2008).

ω-3-PUFAs cannot be synthesized from simple precursors de novo by humans, and as such must be derived exogenously from dietary intake (Dyall and Michael-Titus, 2008, Healy-Stoffel and Levant, 2018). Major dietary sources of PUFAs include oily fish such as salmon and mackerel, microalgae, vegetable oils (e.g., avocado oil, canola oil), and seeds such as flax and hemp (Saini and Keum, 2018, Saini et al., 2021, Barta et al., 2021). Once derived from the diet, two types of naturally occurring PUFAs, the ω-6 series derived from cis-linoleic acid (LA, 18:2) and the ω-3 series derived from α-linolenic acid (ALA, 18:3), are further metabolised to generate the long-chain PUFAs (LCPUFAs): arachidonic acid (AA, 20:4, ω-6),
Eicosapentaenoic acid (EPA, 20:5, ω-3) and docosahexaenoic acid (DHA, 22:6, ω-3). ω-3-PUFAs play an essential role in nervous system functions, including synaptogenesis, cortical maturation, and myelination (Bernardi et al., 2012, Joffre et al., 2014, Wurtman, 2014). They are crucial for brain cell phospholipid (PL) membranes structure, specifically DHA which constitutes up to 15% of total brain fatty acids (Palacios-Pelaez et al., 2010, Saini and Keum, 2018), membrane fluidity and function, act as signalling molecules (Hashimoto and Hossain, 2018), are involved in neuronal structure and modulate neurotransmission and synaptic plasticity (Madore et al., 2014, Talamonti et al., 2020). Evidence suggests that ω-3-PUFAs have neuroprotective as well as anti-inflammatory properties (Su et al., 2014, Chang et al., 2019, Yu et al., 2020, Zhou et al., 2019), including the ability to prevent the reduction of neurogenesis and an increase in apoptosis (Borsini et al., 2017, Borsini et al., 2020b). This ability for ω-3-PUFAs to protect neurons from insult is relevant in the context of psychopathology. Moreover, ω-3-PUFAs deficiencies have previously been reported in people with a range of neuropsychiatric disturbances, including attention deficit hyperactivity disorder (ADHD), depressive disorders, and schizophrenia (Assisi et al., 2006, Haag, 2003, Young and Conquer, 2005, Richardson, 2006, Sinclair et al., 2007, Königs and Kiliaan, 2016, Shahidi and Ambigaipalan, 2018). According to the International Society for Nutritional Psychiatry Research (ISNPR), the expert consensus panel has agreed on using ω–3 PUFAs in MDD treatment as an important alternative therapy and for special population of depression and prevention in high-risk populations. (Guu et al., 2020)

This chapter aims to provide an overview of the current clinical, in vivo, and in vitro evidence regarding the beneficial properties of ω-3-PUFAs in reducing neuropsychiatric symptoms, and subsequently discuss the underlying neuroprotective mechanisms through which PUFAs exert such properties.
Clinical studies of ω-3-PUFAs

A growing body of literature demonstrates ω-3-PUFAs are effective in the prevention and treatment of neuropsychiatric disorders, including those that involve neuroinflammation, such as depression (Bazinet and Layé, 2014, Su, 2009). Most interestingly, ω-3-PUFAs deficiencies have previously been reported in people with a range of neuropsychiatric disturbances (Assisi et al., 2006, Haag, 2003, Young and Conquer, 2005, Richardson, 2006, Sinclair et al., 2007, Lin et al., 2010), while lower DHA levels have been found in the post-mortem orbitofrontal cortex of patients with major depressive disorder (MDD) and schizophrenia (McNamara, 2010, Hamazaki et al., 2013, Solberg et al., 2016, Cadenhead et al., 2019, Kim et al., 2016). Indeed, in our laboratory we have shown that patients with chronic hepatitis C viral infection who developed interferon (IFN)-induced depression after IFN-α intervention, were found to have lower erythrocyte ω-3-PUFAs levels, identifying both ω-3-PUFAs related genotypes and ω-3-PUFAs levels as risk factors for IFN-induced depression (Su et al., 2010).

Evidence from epidemiological studies, associates ω-3-PUFAs intake with a decreased risk of depression, in a dose-response manner. A recent systematic review and meta-analysis of 31 studies examining fish oil consumption reported a decreased risk of depression in the highest compared to the lowest intake groups for both total ω-3-PUFAs intake and fish-derived ω-3-PUFAs (Grosso et al., 2016). This was despite considerable variability of ω-3-PUFAs intake (both type and dosage) across studies, with several individual studies reporting non-significant results. The dose-response analysis demonstrated a J-shaped association, with decreased risk of depression up to 1.8g/day of ω-3-PUFAs intake, and up to 0.6g/day of EPA/DHA monotherapy intake. Although this evidence supports an association between ω-3-PUFAs and depression, the validity of findings is limited given methodological issues associated with observational study design and potential confounding factors. Cause and effect
cannot be established, and it is highly probable that the underlying characteristics of the patient populations, such as severity of illness, comorbidities, age, gender, and adjunctive medications attenuated associations (Hess and Abd-Elsayed, 2019).

Several meta-analyses of clinical studies have been conducted, examining the efficacy of ω-3-PUFAs supplementation on depressive disorders, schizophrenia, bipolar disorder, and ADHD. Studies in depressive disorders mostly demonstrate an ameliorating effect of ω-3-PUFAs on depressive symptoms (Grosso et al., 2014, Freeman et al., 2006, Lin and Su, 2007). For example, the efficacy of high-dose ω-3-PUFAs (≥2,000 mg/day) was shown to be superior to both placebo and low-dose ω-3-PUFAs (<2,000 mg/day) in patients with major depressive disorder, in an early stage of treatment (Luo et al., 2020). These results are consistent with previous findings from an 8-week double-blind, placebo-controlled trial conducted in our lab, that compared ω-3-PUFAs (6.6 g/day) with placebo, as adjunctive treatment, in 28 patients with major depressive disorder. Patients in the ω-3-PUFAs group had significantly decreased depressive symptoms as compared to placebo. Although, the study was limited by its small sample size and the potential confounding effect of adjunctive medications.

Despite DHA being the major ω-3-PUFAs in the brain, EPA appears to exert greater neuroprotective efficacy (Ortega et al., 2012, Reeves et al., 2017, Sublette et al., 2011, Lin and Su, 2007, Lin et al., 2012, Martins et al., 2012). A recent study demonstrated ω-3-PUFAs with EPA ≥ 60% at a dosage of ≤1 g/d demonstrate beneficial effects on depression (Liao et al., 2019), and other studies show that ω-3-PUFAs with higher proportions of EPA at > 50% (Grosso et al., 2014), >60% (Sublette et al., 2011), and >80% (Hallahan et al., 2016) of total dose are more effective in improving depression, irrespective of monotherapy or adjuvant use with DHA or antidepressants. While recent RCTs have also demonstrated EPA at dosages of 1 or 2 g/d were more effective than placebo or DHA, as a monotherapy or adjuvant, in the treatment of mild to moderate depression (Song et al., 2016). These results support the notion
that EPA is largely involved in anti-inflammatory activity while DHA plays a significant role in maintaining cell membrane integrity and fluidity (Deacon et al., 2017).

Concerning bipolar disorder, there is a paucity of data examining associations between ω-3-PUFAs treatment and symptoms. A small amount of clinical evidence demonstrates a slight beneficial effect on associated depressive symptoms in these patients when ω-3-PUFAs are added to existing psychopharmacological maintenance treatment (Stoll et al., 1999, Frangou et al., 2007). However other studies have demonstrated inconclusive results (McPhilemy et al., 2021). A recent systematic review examining ω-3-PUFAs dietary supplementation trials only reported efficacy for depressive symptoms in one of the included seven randomized controlled trials (Saunders et al., 2016). The lack of statistical significance may be attributed to included studies being underpowered owing to very small sample sizes. When examined meta-analytically, pooled estimates across 5 studies with a sample size of n=291 patients indicated strong evidence that bipolar depressive symptoms, but not manic symptoms, improved through the adjunctive use of ω-3-PUFAs (Sarris et al., 2011). These results were corroborated in another meta-analysis of n=338 patients from eight double-blind, randomized, placebo-controlled trials, examining the effect of adjunctive ω-3-PUFAs treatment on residual depression (Kishi et al., 2021). While the overall weight of the evidence does not yet support a clinical recommendation for the therapeutic use of ω-3-PUFAs in bipolar patients, meta-analytic evidence supports its adjunctive use to alleviate associated depressive symptoms.

Only 40-50% of schizophrenia patients respond to pharmacological treatments, with most experiencing prominent negative side effects, and cognitive deficits (Vancampfort et al., 2019). A growing body of evidence indicates the efficacy of ω-3-PUFAs in improving symptoms of schizophrenia, and the ability to reduce the required dosage, metabolic disturbances and other side effects of pharmacological treatments, (Berger et al., 2017, Berger
et al., 2007, Emsley et al., 2002, Qiao et al., 2018, Xu et al., 2019). Meta-analytic evidence shows ω-3-PUFAs treatment reduces patient symptoms in the prodromal phase of first-episode schizophrenia, but not in chronic states (Chen et al., 2015). While ω-3-PUFAs augmentation significantly improves psychopathology in schizophrenia patients, particularly general psychopathology, and exerts beneficial effects to positive symptoms in patients with first episode psychosis (Goh et al., 2021). With respect to the influence of the severity of psychopathology at baseline, most interestingly, patients with lower levels of ω-3-PUFAs at baseline report more severe schizophrenia symptoms in contrast to those with higher levels (Bentsen et al., 2012), and appear more responsive to ω-3-PUFAs treatment (Furukawa et al., 2015), especially in monotherapy with EPA >1g/d (Amminger et al., 2015b, Amminger et al., 2015a). Nonetheless, other studies have reported contrasting results, reporting no beneficial effects particularly in established schizophrenia (Fusar-Poli and Berger, 2012, Irving et al., 2006), and no difference in transition rates to psychosis in young males at ultra-high risk for psychotic disorders (Mechelli et al., 2017). However, given ω-3-PUFAs supplements are well tolerated with minimal negative side effects, even at high doses of up to 10g EPA/day (Schlögelhofer et al., 2014), ω-3-PUFAs treatment in early intervention for patients at ultra-high risk for psychotic disorders, or those with severe baseline schizophrenia symptoms presents as a promising therapeutic target. Further research to ascertain the efficacy of varied compositions and specific doses of ω-3-PUFAs is warranted.

In patients with ADHD, ω-3-PUFAs supplementation monotherapy improves both clinical symptoms (Manor et al., 2012, Perera et al., 2012) and cognitive performance (Sinn et al., 2008, Vaisman et al., 2008). Meta-analytic evidence from seven RCT’s in n=534 patients with ADHD showed children and adolescents with ADHD have lower blood levels of DHA, EPA, and total ω-3-PUFA, while supplementation with ω-3-PUFAs improved ADHD clinical symptom scores, and pooled data from three RCTs, totalling n=214 randomized youth with
ADHD showed improved cognitive measures associated with attention (Chang et al., 2018). Furthermore, we have previously shown that children with ADHD have greater EFA deficiency, a clinical syndrome arising from insufficient fatty acid levels with symptoms such as dry and scaly skin, eczema, and dry eyes (Chang et al., 2016). EFA deficiency correlates negatively with plasma DHA levels (Stevens et al., 1995) as well as positively correlating with ADHD symptoms in these patients (Chang et al., 2016). Taken together, this evidence indicates omega-3-PUFAs supplementation monotherapy improves both clinical symptoms and cognitive performance in children and adolescents with ADHD, especially in youth with pre-existing omega-3-PUFAs deficiency (Chang et al., 2018). Taken together, omega-3-PUFAs is a plausible treatment option for ADHD. However, significant heterogeneity exists in the literature in terms of the most effective dose, ranging from 2.7-640 mg of DHA and 80 to 650 mg of EPA, with one study using EPA (560 mg) as the sole source of n-3 PUFAs supplementation. Most interestingly, only studies with EPA doses of >500 mg improved hyperactivity symptoms, thus EPA supplementation dosage >500 mg should be specifically considered when treating youth with ADHD, especially those that present with severe hyperactivity/impulsivity symptoms.

More recently, neuroimaging studies have been used to investigate the role of omega-3-PUFAs intake in human brain structure and function to overcome methodological limitations associated with epidemiological and clinical studies (McNamara et al., 2008). However, most of these studies have been conducted in healthy middle-aged or elderly adults without psychiatric illness. There are comparably fewer studies that have looked at the effects directly in populations of patients with neuropsychiatric disorders. Findings from one study in patients with schizophrenia demonstrated 26-weeks of adjunctive fish oil supplementation significantly attenuated reductions in cortical thickness in the parieto-occipital regions of first-episode antipsychotic-treated schizophrenia patients (Pawełczyk et al., 2018). While another in patients with MDD found an increase in white matter integrity in the corpus callosum, cingulum, and
bilateral anterior corona radiate of MDD patients, following 6-weeks of supplementation with fish oil (Chhetry et al., 2016). In this study fish oil also decreased patients' depressive symptoms alongside an increase in white matter integrity in the left corticospinal tract and superior longitudinal fasciculus. Most interestingly, the results of our recent study comparing EPA and DHA in functional MRI revealed the specific brain effects of cognitive and emotion controls by EPA, but not DHA, over dorsolateral prefrontal cortex, and underpin personalized medicine with anti-inflammatory nutraceuticals toward depression treatments (Tu et al., 2020). While limited, these findings support an association between ω-3-PUFAs intake and structural integrity in the brain, reinforcing the need for further neuroimaging studies specifically focussed on evaluating neuropsychiatric disorders.

In summary, while the clinical evidence discussed demonstrates an advantage of ω-3-PUFAs treatment across a range of neuropsychiatric disorders, further research is needed to ascertain the efficacy of varied compositions and doses of ω-3-PUFAs specific to each psychopathology, and indeed in specific subgroups of patients non-responsive to traditional methods of treatment within each disorder itself. Moreover, clinical studies cannot elucidate the exact molecular (neuroprotective and anti-inflammatory) mechanisms underlying the evident effects of ω-3-PUFAs treatment on neuropsychiatric disorders. As such evidence obtained from rodent in vivo models, as well as cultured cellular in vitro and ex-vivo rodent models, may provide crucial information about such molecular pathways, and will be discussed next.

In vivo studies of ω-3-PUFAs

An extensive body of evidence from rodent in vivo studies exists, demonstrating associations between ω-3-PUFAs intake and brain development (Bos et al., 2016), neural
maturation (Denis et al., 2013), resilience (Hashimoto, 2019), and degeneration (Denis et al., 2015). Studies that examined ω-3-PUFAs deficiency during rodent development report evidence of delays in neurogenesis (Beltz et al., 2007, Janssen et al., 2015), reduced nerve growth factor, and brain-derived neurotrophic factor (BDNF) expression (Rao et al., 2007, Ikemoto et al., 2000) synaptogenesis and plasticity (Cao et al., 2009), compromised forebrain white matter microstructural integrity (McNamara et al., 2018), and astrocyte-mediated glucose uptake and metabolism (Salem et al., 2001), as well as reduced hippocampal neuronal size (Ahmad et al., 2002). Most interestingly, both single- and multi-generational exposure to dietary ω-3-PUFAs deprivation has been shown to induce depressive and anxious symptoms in rats (Rao et al., 2007).

In one study, maternal ω-3-PUFAs deficiency during pregnancy and lactation imprinted long-term changes of brain development in adult offspring, impacting neurogenesis and apoptosis, as well as reducing BDNF levels, especially mRNA expressions of BDNF transcripts IV and IX (Fan et al., 2016). These negative effects were not reversed in offspring by ω-3-PUFAs supplementation after weaning (Fan et al., 2016). Furthermore, ω-3-PUFAs lifelong deprivation is associated with increased depressive symptoms and aggression (DeMar et al., 2006), as well as impaired cognitive function (Bach et al., 2014, Fedorova et al., 2009).

Similarly, other studies have shown that a diet enriched with ω-3-PUFAs exerts neuroprotective effects in rodents, increasing dendritic spine density (Calon et al., 2004), and increasing neuronal and white matter resilience to insults arising from inflammation (Orr et al., 2013), lipid peroxidation (Wu et al., 2016) and, glutamate excitotoxicity (Hennebelle et al., 2014). Specifically, in relation to neuropsychiatric disorders, ω-3-PUFAs treatment in rodents exerts a robust effect similar to that achieved through the use of anti-depressant medications, significantly reducing depressive-like behaviours (Huang et al., 2008), through the suppression of neuroinflammation (Shi et al., 2017). Indeed, in a rat neuroinflammation model,
intracerebroventricular administration of interleukin-1 beta (IL-1β) evoked depressive symptoms and the release of various stress- and inflammation-related mediators. While in contrast, supplementation with ethyl-EPA attenuated rat stress and anxiety behaviour (Song et al., 2003). Moreover, in another study rats supplemented with EPA (12%) and DHA (18%) and then administered a forced swim test (FST), spent more time swimming, and demonstrated increased serotoninergic neurotransmission and sensitivity of hippocampal serotonin 5-HT1A receptor, a subtype of serotonin receptor located in presynaptic and postsynaptic regions activated by antidepressant medications (Carabelli et al., 2015). Such an increase in 5-HT1A receptor signalling by ω-3-PUFAs has been shown to elevate the expression of BDNF, which in turn is thought to enhance neurogenesis (Vines et al., 2012). Lastly, 0.5% EPA and 1% DHA, when co-administered in rats, increased plasma serotonin and hippocampal c-AMP response element-binding (CREB) protein. This transcription factor is down-regulated in stress- and depression-related pathology (Tang et al., 2015). Taken together, findings from in vivo studies show that ω-3-PUFAs can improve detrimental changes in neurogenesis and depressive-like behaviours observed in mice exposed to a stress-related challenges (See Figure 1).

**In vitro and ex-vivo studies of ω-3-PUFAs**

*In vitro* studies have demonstrated that enrichment with ω-3-PUFAs attenuates the production of pro-inflammatory cytokines (Chen et al., 2018a), oxidated products (Shi et al., 2018), apoptosis (Chen et al., 2018b) and exerts a protective effect on neuronal survival (Evbuomwan et al., 2022) and neurogenesis (Belayev et al., 2018). In microglial cell lines or primary culture microglial cells, ω-3-PUFAs has been shown to suppress lipopolysaccharide (LPS) and IFN-γ induced, cytokine production of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and IL-6, and decrease oxidative stress, nitric oxide (NO)
production, and inducible nitric oxide synthase (iNOS) expression (Corsi et al., 2015, Inoue et al., 2017, Fourrier et al., 2017, De Smedt-Peyrusse et al., 2008, Lu et al., 2010). Furthermore, DHA has been shown to prevent LPS-induced mitogen-activated protein kinase (MAPK) phosphorylation, the pathway which plays an essential role in the expression of inflammatory molecules, and induce peroxisome proliferator-activated receptor (PPAR)-γ nuclear translocation, known to mediate anti-inflammatory effects on the brain (Antonietta Ajmone-Cat et al., 2012).

One possible explanation for such beneficial findings of ω-3-PUFAs is that at the cellular level, DHA is mainly incorporated into cell membranes and lipid bodies (LBs), which are dynamic organelles that contribute to the maintenance of neural cell homeostasis (Tremblay et al., 2016). The mechanism by which DHA has been shown to attenuate the inflammatory response in LPS-stimulated microglial cells is through the remodelling of LBs and altering their interplay with mitochondria and other associated organelles, modifying their neuroprotective and anti-inflammatory capabilities (Tremblay et al., 2016). Concerning the treatment of microglial cells with EPA, a reduction in the production of TNF-α, IL-6 and NO has been demonstrated through inhibiting NFκB phosphorylation via sirtuin-1 (SIRT-1), an important regulator to modulate transcription, apoptosis, cell survival, DNA repair, inflammation, and oxidative stress (Inoue et al., 2017).

Other studies have examined the effect of ω-3-PUFAs treatment on neurogenesis and apoptosis, in both ex-vivo and in vitro studies utilising hippocampal neurons derived from embryonic rats and studies in immortalised human hippocampal cell lines. In ex-vivo studies, DHA supplementation in culture increases neuron populations, with longer neurite length per neuron and significantly more dendritic branches while in contrast, hippocampal cultures obtained from n-3 fatty acid-deficient animals contained lower DHA levels and a neuronal population with shorter neurite length per neuron (Calderon and Kim, 2004). In a separate
study, a similar treatment model demonstrated a significant increase in neurons in cells receiving DHA supplementation as compared to control, as well as increased differentiation of neural stem cells into neurons by promoting cell cycle exit and suppressing apoptosis (Kawakita et al., 2006). Indeed, in a study from our laboratory using an immortalised human hippocampal progenitor cell line, we demonstrated that treatment with EPA and DHA can prevent a reduction in neurogenesis caused by IL-1β, similarly to the effects of treatment with the antidepressants, sertraline, and venlafaxine (Borsini et al., 2017). Most interestingly, EPA and DHA also reversed the IL-1β-induced increase in the production of the kynurenine metabolite, as well as mRNA levels of indolamine-2,3-dioxygenase (IDO); while DHA and sertraline reverted the IL-1β-induced increase in quinolinic acid and mRNA levels of kynurenine 3-monooxygenase (KMO)(Borsini et al., 2017). This demonstrates that both EPA and DHA have a similar neuroprotective effect in human hippocampal neurogenesis to monoaminergic antidepressants in the reduction caused by IL-1β, and that this effect is mediated by regulation of the kynurenine pathway.

We have also examined the effect of ω-3-PUFAs treatment in the prevention of cortisol-induced reduction in human hippocampal neurogenesis, used here as a model of stress-induced depression. In particular, we exposed the same human hippocampal progenitor cell line to pre-treatment, with either EPA or DHA, followed by treatment with cortisol, which can elicit a detrimental effect on cell proliferation as well as obstruct early hippocampal progenitor cell differentiation into neurons (Anacker et al., 2013, Anacker et al., 2011). While this association was previously studied ex vivo in mixed cortical cultures from postnatal rats, with results demonstrating DHA treatment attenuated cortical culture cell death, the authors did not also examine the effects of EPA treatment (Pusceddu et al., 2016). In contrast, our study provided the first evidence demonstrating that treatment with both EPA and DHA could prevent cortisol-induced reduction in human hippocampal neurogenesis. Additionally, we identified novel
molecular mechanisms underlying these effects. Using transcriptomic analyses, we showed that both EPA and DHA regulated pathways, such as nuclear factor (erythroid-derived 2)-like 2 (Nrf2), Signal transducer and activator of transcription 3 (STAT3), IFN, and IL-1 signalling, involved in oxidative stress and the immune. Furthermore, DHA also regulated pathways involved in cell development and neuronal formation such as cAMP-response element-binding protein (CREB) signalling (Borsini et al., 2020b).

Taken together, findings from in vivo, in vitro and ex-vivo studies unequivocally demonstrate the ability of cytokines to detrimentally affect neurogenesis, a key mechanism implicated in the pathogenesis of neuropsychiatric disorders, and crucial for pharmacological efficacy in neuropsychiatric disorders (Park, 2019, de Oliveira et al., 2020, Planchez et al., 2020). These findings also highlight the crucial neuroprotective and anti-inflammatory properties of ω-3-PUFAs and demonstrate their ability to exert effects similar to antidepressant medication (see Figure 1). Such that, they protect neurones from inflammatory insults which results in increased cell proliferation and neurogenesis and a reduction in apoptosis. Furthermore, they do so without the negative side effects that commonly accompany pharmacological treatments (Borsini et al., 2018, Borsini et al., 2020a). However, the exact molecular mechanisms by which ω-3-PUFAs exert their anti-inflammatory effects remains to be fully understood.
Figure 1: Key findings - overview of the current clinical, in vivo, and in vitro evidence regarding the beneficial properties of ω-3-PUFAs in reducing neuropsychiatric symptoms, and subsequently discuss the underlying neuroprotective mechanisms through which PUFAs exert such properties. Figure created by the author with BioRender.

One possible molecular mechanism underlying the effect of ω-3-PUFAs on neurogenesis is the production of ω-3-derived metabolites, derived from cyclooxygenase (COX), lipooxygenase (LOX) and cytochrome P450 (CYP450) enzymes, which exhibit potent immune regulatory activities (Gabbs et al., 2015, Astarita et al., 2015). Existing studies in pre-clinical models of depression have predominantly focussed on specialised pro-resolving LOX derived metabolites like resolvins, protectins, and maresins (Ishikawa et al., 2017, Deyama et al., 2018, Giacobbe et al., 2020). In a recent study from our laboratory, we conducted both in vitro and clinical investigations providing the first evidence for the relevance of LOX- and CYP450-derived EPA/DHA bioactive lipid metabolites as neuroprotective molecular targets.
for human hippocampal neurogenesis and depression (Borsini et al., 2021). In particular, we found that pre-treating human hippocampal neurons with either EPA or DHA prevented a reduction in neurogenesis and increase in apoptosis after exposure to pro-inflammatory cytokine (IL1β, IL6, and IFN-α) insults (Borsini et al., 2021).

Most interestingly, we found these effects were mediated by the lipoxygenase (LOX) and cytochrome P450 (CYP450) EPA/DHA metabolites, 5-hydroxyeicosapentaenoic acid (HEPE), 4-hydroxydocosahexaenoic acid (HDHA), 18-HEPE, 20-HDHA, 17(18)-epoxyeicosatetraenoic acid (EpETE) and 19(20)-epoxydocosapentaenoic acid (EpDPA) (see Figure 2), which we detected for the first time in supernatant of human hippocampal neurones (Borsini et al., 2021). In fact, co-treatment with these metabolites prevented cytokines-induced reduction in neurogenesis and apoptosis. Moreover, co-treatment with 17(18)-EpETE and 19(20)-EpDPA and the soluble epoxide hydroxylase (sEH) inhibitor, TPPU (which prevents their conversion into dihydroxyeicosatetraenoic acid (DiHETE)/ dihydroxydocosapentaenoic acid (DiHDPA) metabolites) further enhanced their neurogenic and anti-apoptotic effects (Borsini et al., 2021).
Figure 2: ω-3 PUFAs enzymatic synthesis pathways

ω-3 PUFAs (EPA/DHA) are metabolised by COX, LOX and CYP450 enzymes. COX and LOX enzymes convert ω-3 PUFAs into prostanoids, hydroxy fatty acids and leukotrienes, while CYP450 monooxygenases convert ω-3 PUFAs into epoxy and hydroxy fatty acids. Epoxy fatty acids are then metabolised via the sEH enzyme to the corresponding dihydroxy metabolites. **Legend:** eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA); soluble epoxide hydrolase (sEH); cyclooxygenase (COX); lipoxygenase (LOX); cytochrome P450 (CYP450); hydroxyeicosapentaenoic acids (HEPEs); hydroxydocosahexaenoic acids (HDHAs); epoxyeicosatetraenoic acids (EpETEs); epoxydocosapentaenoic acids (EpDPAs); dihydroxyeicosatetraenoic acids (DiHETEs); dihydroxydocosapentaenoic acids (DiHDPAs). Figure created by the author with BioRender.

Of importance, we were then able to replicate these findings in a clinical sample of n=22 patients with a DSM-IV MDD. Treatment with either EPA (3.0 g/day) or DHA (1.4 g/day) for 12 weeks, with the same LOX and CYP450 lipid metabolites, increased in the plasma of these patients, following treatment with their precursor, EPA or DHA, demonstrated higher levels of these metabolites were correlated with less severe depressive symptoms (Borsini et al., 2021). Only one other observational study has examined similar LOX and CYP450-derived lipid classes (Hennebelle et al., 2017). Results also showed negative correlations between
metabolites levels and depressive symptoms; however, the cohort consisted of patients with seasonal depression, and only few lipid species were identified, produced naturally rather than during PUFAs treatment. Nevertheless, the magnitude of the effects of PUFAs administration were the same in both studies, thus supporting the notion of clinical relevance.

Overall, these crucial findings confirm and extend previous evidence for the antidepressant, anti-inflammatory, and neuroprotective properties of both EPA and DHA and identifies LOX-derived 5-HEPE and 4-HDHA, and CYP450-derived 18-HEPE, 20-HDHA, 17(18)-EpETE and 19(20)-EpDPA as among the mediators of these effect of ω-3 PUFA. In addition, results from the clinical studies supports the relevance of LOX and CYP450 hydroxy and epoxy PUFA derivatives and the enzymes involved in their metabolism, especially in the context of depression. As such, it highlights the importance of sEH inhibitors as a promising therapeutic strategy for patients with depressive symptoms, at least in the subgroup of them presenting with increased inflammation.

Conclusion

In conclusion, clinical, in vivo, in vitro and ex-vivo studies demonstrate a clear potential therapeutic benefit for the role of ω-3 PUFA in the treatment and prevention of neuropsychiatric disorders, owing to its neuroprotective and anti-inflammatory properties. However, to date there is still a paucity of data surrounding the efficacy of varied compositions (EPA and/or DHA) and doses of ω-3-PUFAs specific to each psychopathology, and indeed in specific subgroups of patients non-responsive to traditional methods of treatment within each disorder itself. Additionally, while promising advancements have been made, the exact molecular mechanisms by which ω-3-PUFAs exert their anti-inflammatory effects still, remain to be fully understood. As such, further research is needed to elucidate specific clinical doses
and combinations of ω-3-PUFA, as well as further investigation into relevant metabolites and the enzymes associated with their metabolism, in order to develop specific formulations that are effective in the prevention and treatment of patients across a range of neuropsychiatric disorders.
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