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 ω-3-PUFAs supplementation monotherapy improves both clinical symptoms and cognitive performance in children and adolescents with ADHD, especially in youth with pre-existing ω-3-PUFAs deficiency (Chang et al., 2018). Taken together, ω-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 ω-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 interleurkin-1beta (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.

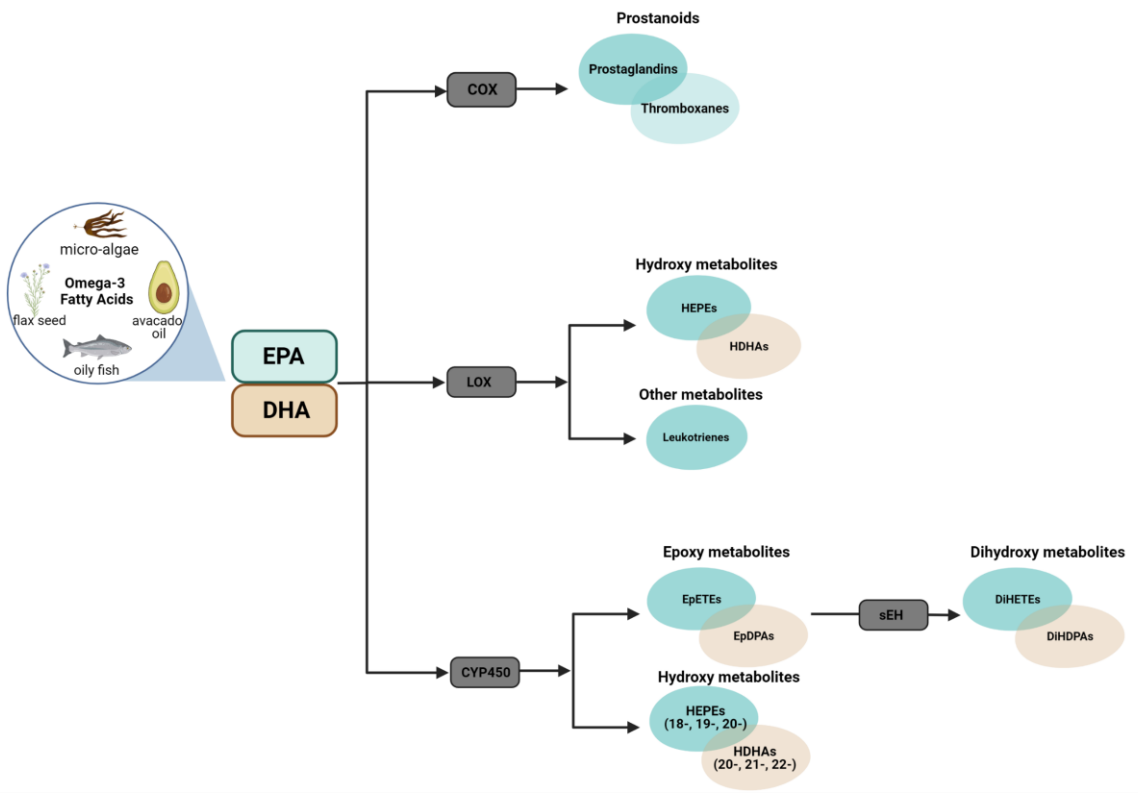

Figure 1: Key findings - overview of the current clinical, in vivo, and in vitro evidence regarding the beneficial properties of ω-3-PUFAs in reducing neuropsychiatric symptoms, and subsequently discuss the underlying neuroprotective mechanisms through which PUFAs exert such properties. Figure created 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), lipoxygenase (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).

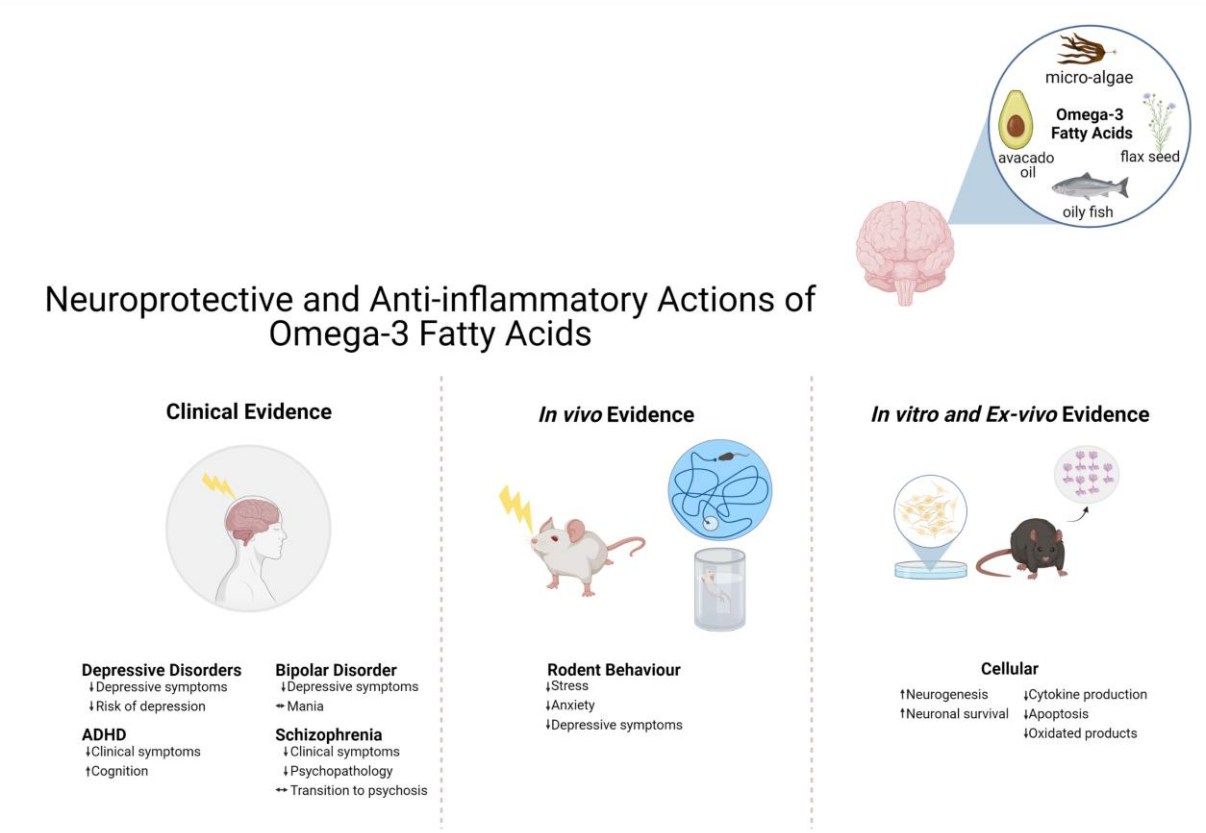

Figure 2: ω-3 PUFAs enzymatic synthesis pathways

ω-3 PUFAs (EPA/DHA) are metabolised by COX, LOX and CYP450 enzymes. COX and LOX enzymes convertω-3 PUFAs intoprostanoids, hydroxy fatty acids and leukotrienes, while CYP450 monooxygenases convertω-3 PUFAs into epoxy and hydroxy fatty acids. Epoxy fatty acids are thenmetabolised 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.

References

AHMAD, A., et al. 2002. A decrease in cell size accompanies a loss of docosahexaenoate in the rat hippocampus. *Nutr. Neurosci.,* 5**,** 103-113.

AMMINGER, G., et al. 2015a. Predictors of treatment response in young people at ultra-high risk for psychosis who received long-chain omega-3 fatty acids. *Translational Psychiatry,* 5**,** e495-e495.

AMMINGER, G. P., et al. 2015b. Longer-term outcome in the prevention of psychotic disorders by the Vienna omega-3 study. *Nature Communications,* 6**,** 7934.

ANACKER, C., et al. 2013. Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. *Neuropsychopharmacology,* 38**,** 872-883.

ANACKER, C., et al. 2011. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. *Mol. Psychiatry,* 16**,** 738-750.

ANTONIETTA AJMONE-CAT, M., et al. 2012. Docosahexaenoic acid modulates inflammatory and antineurogenic functions of activated microglial cells. *J. Neurosci. Res.,* 90**,** 575-587.

ASSISI, A., et al. 2006. Fish oil and mental health: the role of n-3 long-chain polyunsaturated fatty acids in cognitive development and neurological disorders. *Int. Clin. Psychopharmacol.,* 21**,** 319-336.

ASTARITA, G., et al. 2015. Targeted lipidomic strategies for oxygenated metabolites of polyunsaturated fatty acids. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids,* 1851**,** 456-468.

BACH, S. A., et al. 2014. Dietary omega-3 deficiency reduces BDNF content and activation NMDA receptor and Fyn in dorsal hippocampus: implications on persistence of long-term memory in rats. *Nutr. Neurosci.,* 17**,** 186-192.

BARANNE, M. & FALISSARD, B. 2018. Global burden of mental disorders among children aged 5–14 years. *Child and adolescent psychiatry and mental health,* 12**,** 19.

BARTA, D. G., et al. 2021. Microalgae as sources of omega-3 polyunsaturated fatty acids: Biotechnological aspects. *Algal Research,* 58**,** 102410.

BAXTER, A. J., et al. 2013. Global epidemiology of mental disorders: what are we missing? *PLoS One,* 8**,** e65514.

BAZINET, R. P. & LAYÉ, S. 2014. Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nature Reviews Neuroscience,* 15**,** 771-785.

BELAYEV, L., et al. 2018. Docosanoids promote neurogenesis and angiogenesis, blood-brain barrier integrity, penumbra protection, and neurobehavioral recovery after experimental ischemic stroke. *Mol. Neurobiol.,* 55**,** 7090-7106.

BELTZ, B. S., et al. 2007. Omega-3 fatty acids upregulate adult neurogenesis. *Neurosci. Lett.,* 415**,** 154-158.

BENTSEN, H., et al. 2012. Clinical and biochemical validation of two endophenotypes of schizophrenia defined by levels of polyunsaturated fatty acids in red blood cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 87**,** 35-41.

BERGER, G. E., et al. 2007. Ethyl-eicosapentaenoic acid in first-episode psychosis: a randomized, placebo-controlled trial. *The Journal of clinical psychiatry,* 68**,** 0-0.

BERGER, M., et al. 2017. Omega-6 to omega-3 polyunsaturated fatty acid ratio and subsequent mood disorders in young people with at-risk mental states: a 7-year longitudinal study. *Translational psychiatry,* 7**,** e1220-e1220.

BERNARDI, J. R., et al. 2012. Fetal and neonatal levels of omega-3: effects on neurodevelopment, nutrition, and growth. *The Scientific World Journal,* 2012.

BORSINI, A. 2021. Depression: What neuroinflammation has to do with it and how nutrition can play a beneficial role. *Psychoneuroendocrinology,* 131**,** 105550.

BORSINI, A., et al. 2017. Rescue of IL-1β-induced reduction of human neurogenesis by omega-3 fatty acids and antidepressants. *Brain, behavior, and immunity,* 65**,** 230-238.
BORSINI, A., et al. 2018. Interferon-alpha reduces human hippocampal neurogenesis and increases apoptosis via activation of distinct STAT1-dependent mechanisms. *Int. J. Neuropsychopharmacol.,* 21**,** 187-200.
BORSINI, A., et al. 2020a. Pro- and anti-inflammatory properties of interleukin (IL6) in vitro: relevance for major depression and for human hippocampal neurogenesis. *The international journal of neuropsychopharmacology*.
BORSINI, A., et al. 2021. Omega-3 polyunsaturated fatty acids protect against inflammation through production of LOX and CYP450 lipid mediators: relevance for major depression and for human hippocampal neurogenesis. *Mol. Psychiatry***,** 1-16.
BORSINI, A., et al. 2020b. The role of omega-3 fatty acids in preventing glucocorticoid-induced reduction in human hippocampal neurogenesis and increase in apoptosis. *Transl Psychiatry,* 10**,** 219.
BOS, D. J., et al. 2016. Effects of omega-3 polyunsaturated fatty acids on human brain morphology and function: what is the evidence? *Eur. Neuropsychopharmacol.,* 26**,** 546-561.
BUJTOR, M., et al. 2021. Associations of Dietary Intake on Biological Markers of Inflammation in Children and Adolescents: A Systematic Review. *Nutrients,* 13**,** 356.
CADENHEAD, K. S., et al. 2019. Metabolic abnormalities and low dietary Omega 3 are associated with symptom severity and worse functioning prior to the onset of psychosis: Findings from the North American Prodrome Longitudinal Studies Consortium. *Schizophr. Res.,* 204**,** 96-103.
CALDERON, F. & KIM, H. Y. 2004. Docosahexaenoic acid promotes neurite growth in hippocampal neurons. *J. Neurochem.,* 90**,** 979-988.
CALON, F., et al. 2004. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron,* 43**,** 633-645.
CAO, D., et al. 2009. Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function. *J. Neurochem.,* 111**,** 510-521.
CARABELLI, B., et al. 2015. The Antidepressant-Like Effect of Fish Oil: Possible Role of Ventral Hippocampal 5-HT 1A Post-synaptic Receptor. *Mol. Neurobiol.,* 52**,** 206-215.
CHANG, J. P.-C., et al. 2016. Delay Aversion, Temporal Processing, and N-3 Fatty Acids Intake in Children With Attention-Deficit/Hyperactivity Disorder (ADHD). *Clinical Psychological Science,* 4**,** 1094-1103.
CHANG, J. P.-C., et al. 2018. Omega-3 Polyunsaturated Fatty Acids in Youths with Attention Deficit Hyperactivity Disorder: a Systematic Review and Meta-Analysis of Clinical Trials and Biological Studies. *Neuropsychopharmacology,* 43**,** 534-545.
CHANG, J. P., et al. 2019. High-dose eicosapentaenoic acid (EPA) improves attention and vigilance in children and adolescents with attention deficit hyperactivity disorder (ADHD) and low endogenous EPA levels. *Transl Psychiatry,* 9**,** 303.
CHEN, A. T., et al. 2015. A meta-analysis of placebo-controlled trials of omega-3 fatty acid augmentation in schizophrenia: Possible stage-specific effects. *Annals of clinical psychiatry: official journal of the American Academy of Clinical Psychiatrists,* 27**,** 289-296.
CHEN, X., et al. 2018a. Omega-3 polyunsaturated fatty acid attenuates the inflammatory response by modulating microglia polarization through SIRT1-mediated deacetylation of the HMGB1/NF-κB pathway following experimental traumatic brain injury. *J. Neuroinflammation,* 15**,** 1-15.

CHEN, X., et al. 2018b. Omega-3 polyunsaturated fatty acid attenuates traumatic brain injury-induced neuronal apoptosis by inducing autophagy through the upregulation of SIRT1-mediated deacetylation of Beclin-1. *J. Neuroinflammation,* 15**,** 1-15.

CHHETRY, B. T., et al. 2016. Omega-3 polyunsaturated fatty acid supplementation and white matter changes in major depression. *J. Psychiatr. Res.,* 75**,** 65-74.

CORSI, L., et al. 2015. Supplementation of omega 3 fatty acids improves oxidative stress in activated BV2 microglial cell line. *International journal of food sciences and nutrition,* 66**,** 293-299.

DE OLIVEIRA, C. L., et al. 2020. Do antidepressants promote neurogenesis in adult hippocampus? A systematic review and meta-analysis on naive rodents. *Pharmacol. Ther.,* 210**,** 107515.

DE SMEDT-PEYRUSSE, V., et al. 2008. Docosahexaenoic acid prevents lipopolysaccharide-induced cytokine production in microglial cells by inhibiting lipopolysaccharide receptor presentation but not its membrane subdomain localization. *J. Neurochem.,* 105**,** 296-307.

DEACON, G., et al. 2017. Omega 3 polyunsaturated fatty acids and the treatment of depression. *Critical reviews in food science and nutrition,* 57**,** 212-223.

DEMAR, J. C., et al. 2006. One generation of n-3 polyunsaturated fatty acid deprivation increases depression and aggression test scores in rats. *J. Lipid Res.,* 47**,** 172-180.

DENIS, I., et al. 2015. Omega-3 polyunsaturated fatty acids and brain aging. *Curr. Opin. Clin. Nutr. Metab. Care,* 18**,** 139-146.

DENIS, I., et al. 2013. Omega-3 fatty acids and brain resistance to ageing and stress: body of evidence and possible mechanisms. *Ageing research reviews,* 12**,** 579-594.

DEYAMA, S., et al. 2018. Resolvin E3 attenuates lipopolysaccharide-induced depression-like behavior in mice. *J. Pharmacol. Sci.,* 138**,** 86-88.

DYALL, S. C. & MICHAEL-TITUS, A. T. 2008. Neurological Benefits of Omega-3 Fatty Acids. *Neuromolecular Med.,* 10**,** 219-235.

EMSLEY, R., et al. 2002. Randomized, placebo-controlled study of ethyl-eicosapentaenoic acid as supplemental treatment in schizophrenia. *Am. J. Psychiatry,* 159**,** 1596-1598.

EVBUOMWAN, S. A., et al. 2022. Roles and Mechanisms of Docosahexaenoic Acid (DHA) in Neurodevelopment, Neuronal Functions, Learning and Memory. *World News of Natural Sciences,* 40**,** 104-119.

FAN, C., et al. 2016. Maternal n-3 polyunsaturated fatty acid deprivation during pregnancy and lactation affects neurogenesis and apoptosis in adult offspring: associated with DNA methylation of brain-derived neurotrophic factor transcripts. *Nutr. Res.,* 36**,** 1013-1021.

FEDOROVA, I., et al. 2009. An n-3 fatty acid deficiency impairs rat spatial learning in the Barnes maze. *Behav. Neurosci.,* 123**,** 196.

FOURRIER, C., et al. 2017. Docosahexaenoic acid-containing choline phospholipid modulates LPS-induced neuroinflammation in vivo and in microglia in vitro. *J. Neuroinflammation,* 14**,** 1-13.

FRANGOU, S., et al. 2007. Preliminary in vivo evidence of increased N-acetyl-aspartate following eicosapentanoic acid treatment in patients with bipolar disorder. *J. Psychopharm.,* 21**,** 435-439.

FREEMAN, M. P., et al. 2006. Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. *Journal of Clinical psychiatry,* 67**,** 1954.

FURUKAWA, T. A., et al. 2015. Initial severity of schizophrenia and efficacy of antipsychotics: participant-level meta-analysis of 6 placebo-controlled studies. *JAMA psychiatry,* 72**,** 14-21.

FUSAR-POLI, P. & BERGER, G. 2012. Eicosapentaenoic acid interventions in schizophrenia: meta-analysis of randomized, placebo-controlled studies. *J. Clin. Psychopharmacol.,* 32**,** 179-185.

GABBS, M., et al. 2015. Advances in our understanding of oxylipins derived from dietary PUFAs. *Adv. Nutr.,* 6**,** 513-540.

GIACOBBE, J., et al. 2020. The Anti-Inflammatory Role of Omega-3 Polyunsaturated Fatty Acids Metabolites in Pre-Clinical Models of Psychiatric, Neurodegenerative, and Neurological Disorders. *Front Psychiatry,* 11**,** 122.

GOH, K. K., et al. 2021. Effects of omega-3 polyunsaturated fatty acids supplements on psychopathology and metabolic parameters in schizophrenia: A meta-analysis of randomized controlled trials. *J. Psychopharm.,* 35**,** 221-235.

GROSSO, G. 2021. Nutritional Psychiatry: How Diet Affects Brain through Gut Microbiota. Multidisciplinary Digital Publishing Institute.

GROSSO, G., et al. 2016. Dietary n-3 PUFA, fish consumption and depression: A systematic review and meta-analysis of observational studies. *J. Affect. Disord.,* 205**,** 269-281.

GROSSO, G., et al. 2014. Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. *PLoS One,* 9**,** e96905.

GUU, T.-W., et al. 2020. A multi-national, multi-disciplinary Delphi consensus study on using omega-3 polyunsaturated fatty acids (n-3 PUFAs) for the treatment of major depressive disorder. *J. Affect. Disord.,* 265**,** 233-238.

HAAG, M. 2003. Essential fatty acids and the brain. *The Canadian Journal of Psychiatry,* 48**,** 195-203.

HALLAHAN, B., et al. 2016. Efficacy of omega-3 highly unsaturated fatty acids in the treatment of depression. *The British Journal of Psychiatry,* 209**,** 192-201.

HAMAZAKI, K., et al. 2013. Abnormalities in the fatty acid composition of the postmortem entorhinal cortex of patients with schizophrenia, bipolar disorder, and major depressive disorder. *Psychiatry Res.,* 210**,** 346-350.

HASHIMOTO, K. 2019. Role of soluble epoxide hydrolase in metabolism of PUFAs in psychiatric and neurological disorders. *Front. Pharmacol.,* 10**,** 36.

HASHIMOTO, M. & HOSSAIN, S. 2018. Fatty acids: from membrane ingredients to signaling molecules. *Biochemistry and Health Benefits of Fatty Acids*.

HEALY-STOFFEL, M. & LEVANT, B. 2018. N-3 (Omega-3) fatty acids: effects on brain dopamine systems and potential role in the etiology and treatment of neuropsychiatric disorders. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders),* 17**,** 216-232.

HENNEBELLE, M., et al. 2014. Omega-3 polyunsaturated fatty acids and chronic stress-induced modulations of glutamatergic neurotransmission in the hippocampus. *Nutr. Rev.,* 72**,** 99-112.

HENNEBELLE, M., et al. 2017. Altered soluble epoxide hydrolase-derived oxylipins in patients with seasonal major depression: an exploratory study. *Psychiatry Res.,* 252**,** 94-101.

HESS, A. S. & ABD-ELSAYED, A. 2019. Observational studies: uses and limitations. *Pain.* Springer.

HU, Y., et al. 2019. Marine omega-3 supplementation and cardiovascular disease: an updated meta-analysis of 13 randomized controlled trials involving 127 477 participants. *Journal of the American Heart Association,* 8**,** e013543.

HUANG, S.-Y., et al. 2008. Omega-3 fatty acids on the forced-swimming test. *J. Psychiatr. Res.,* 42**,** 58-63.

IKEMOTO, A., et al. 2000. Dietary n-3 fatty acid deficiency decreases nerve growth factor content in rat hippocampus. *Neurosci. Lett.,* 285**,** 99-102.

INOUE, T., et al. 2017. Omega-3 polyunsaturated fatty acids suppress the inflammatory responses of lipopolysaccharide-stimulated mouse microglia by activating SIRT1 pathways. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids,* 1862**,** 552-560.

IRVING, C. B., et al. 2006. Polyunsaturated fatty acid supplementation for schizophrenia. *Cochrane Database Syst. Rev.*

ISHIKAWA, Y., et al. 2017. Rapid and sustained antidepressant effects of resolvin D1 and D2 in a chronic unpredictable stress model. *Behav. Brain Res.,* 332**,** 233-236.

JANSSEN, C. I., et al. 2015. Impact of dietary n-3 polyunsaturated fatty acids on cognition, motor skills and hippocampal neurogenesis in developing C57BL/6J mice. *J. Nutr. Biochem.,* 26**,** 24-35.

JOFFRE, C., et al. 2014. n-3 LCPUFA improves cognition: the young, the old and the sick. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 91**,** 1-20.

KAWAKITA, E., et al. 2006. Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. *Neuroscience,* 139**,** 991-997.

KIM, S.-W., et al. 2016. Relationship between erythrocyte fatty acid composition and psychopathology in the vienna omega-3 study. *PLoS One,* 11**,** e0151417.

KISHI, T., et al. 2021. Omega-3 fatty acids for treating residual depressive symptoms in adult patients with bipolar disorder: A systematic review and meta-analysis of double-blind randomized, placebo-controlled trials. *Bipolar Disorders*.

KÖNIGS, A. & KILIAAN, A. J. 2016. Critical appraisal of omega-3 fatty acids in attention-deficit/hyperactivity disorder treatment. *Neuropsychiatr. Dis. Treat.,* 12**,** 1869.

LAYÉ, S., et al. 2018. Anti-inflammatory effects of omega-3 fatty acids in the brain: physiological mechanisms and relevance to pharmacology. *Pharmacol. Rev.,* 70**,** 12-38.

LIAO, Y., et al. 2019. Efficacy of omega-3 PUFAs in depression: A meta-analysis. *Translational Psychiatry,* 9.

LIN, P.-Y., et al. 2010. A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. *Biol. Psychiatry,* 68**,** 140-147.

LIN, P.-Y. & SU, K.-P. 2007. A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. *Journal of Clinical Psychiatry,* 68**,** 1056-1061.

LIN, P. Y., et al. 2012. Are omega-3 fatty acids antidepressants or just mood-improving agents? The effect depends upon diagnosis, supplement preparation, and severity of depression. *Mol. Psychiatry,* 17**,** 1161-1163.

LOGAN, A. C. & JACKA, F. N. 2014. Nutritional psychiatry research: an emerging discipline and its intersection with global urbanization, environmental challenges and the evolutionary mismatch. *J. Physiol. Anthropol.,* 33**,** 1-16.

LU, D.-Y., et al. 2010. Docosahexaenoic acid suppresses neuroinflammatory responses and induces heme oxygenase-1 expression in BV-2 microglia: implications of antidepressant effects for omega-3 fatty acids. *Neuropsychopharmacology,* 35**,** 2238-2248.

LUO, X.-D., et al. 2020. High-dose omega-3 polyunsaturated fatty acid supplementation might be more superior than low-dose for major depressive disorder in early therapy period: a network meta-analysis. *BMC Psychiatry,* 20.

MADORE, C., et al. 2014. Nutritional n-3 PUFAs deficiency during perinatal periods alters brain innate immune system and neuronal plasticity-associated genes. *Brain, behavior, and immunity,* 41**,** 22-31.

MANOR, I., et al. 2012. The effect of phosphatidylserine containing Omega3 fatty-acids on attention-deficit hyperactivity disorder symptoms in children: A double-blind placebo-controlled trial, followed by an open-label extension. *Eur. Psychiatry,* 27**,** 335-342.
MARTINS, J., et al. 2012. Eicosapentaenoic acid appears to be the key omega-3 fatty acid component associated with efficacy in major depressive disorder: a critique of Bloch and Hannestad and updated meta-analysis. *Mol. Psychiatry,* 17**,** 1144.
MCNAMARA, R. K. 2010. DHA deficiency and prefrontal cortex neuropathology in recurrent affective disorders. *The Journal of nutrition,* 140**,** 864-868.
MCNAMARA, R. K., et al. 2018. Deficits in docosahexaenoic acid accrual during adolescence reduce rat forebrain white matter microstructural integrity: an in vivo diffusion tensor imaging study. *Dev. Neurosci.,* 40**,** 84-92.
MCNAMARA, R. K., et al. 2008. Omega-3 fatty acid deficiency augments amphetamine-induced behavioral sensitization in adult DBA/2J mice: Relationship with ventral striatum dopamine concentrations. *Synapse,* 62**,** 725-735.
MCPHILEMY, G., et al. 2021. A 52-week prophylactic randomised control trial of omega-3 polyunsaturated fatty acids in bipolar disorder. *Bipolar Disorders,* 23**,** 697-706.
MECHELLI, A., et al. 2017. Using clinical information to make individualized prognostic predictions in people at ultra high risk for psychosis. *Schizophr. Res.,* 184**,** 32-38.
ORR, S. K., et al. 2013. Unesterified docosahexaenoic acid is protective in neuroinflammation. *J. Neurochem.,* 127**,** 378-393.
ORTEGA, R., et al. 2012. Effects of omega 3 fatty acids supplementation in behavior and non-neurodegenerative neuropsychiatric disorders. *Br. J. Nutr.,* 107**,** S261-S270.
PALACIOS-PELAEZ, R., et al. 2010. Omega-3 essential fatty acids modulate initiation and progression of neurodegenerative disease. *Mol. Neurobiol.,* 41**,** 367-374.
PARK, S.-C. 2019. Neurogenesis and antidepressant action. *Cell and tissue research,* 377**,** 95-106.
PARLETTA, N., et al. 2013. Nutritional modulation of cognitive function and mental health. *J. Nutr. Biochem.,* 24**,** 725-743.
PAWEŁCZYK, T., et al. 2018. Omega-3 fatty acid supplementation may prevent loss of gray matter thickness in the left parieto-occipital cortex in first episode schizophrenia: a secondary outcome analysis of the OFFER randomized controlled study. *Schizophr. Res.,* 195**,** 168-175.
PERERA, H., et al. 2012. Combined ω3 and ω6 Supplementation in Children With Attention-Deficit Hyperactivity Disorder (ADHD) Refractory to Methylphenidate Treatment. *J. Child Neurol.,* 27**,** 747-753.
PERINI, G., et al. 2019. Cognitive impairment in depression: recent advances and novel treatments. *Neuropsychiatr. Dis. Treat.,* 15**,** 1249.
PLANCHEZ, B., et al. 2020. Adult hippocampal neurogenesis and antidepressants effects. *Current opinion in pharmacology,* 50**,** 88-95.
PUSCEDDU, M. M., et al. 2016. The Omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) reverses corticosterone-induced changes in cortical neurons. *Int. J. Neuropsychopharmacol.,* 19**,** pyv130.
QIAO, Y., et al. 2018. Effects of Omega-3 in the treatment of violent schizophrenia patients. *Schizophr. Res.,* 195**,** 283-285.
RAO, J., et al. 2007. n-3 polyunsaturated fatty acid deprivation in rats decreases frontal cortex BDNF via a p38 MAPK-dependent mechanism. *Mol. Psychiatry,* 12**,** 36-46.
REEVES, J. L., et al. 2017. DHA mediates the protective effect of fish consumption on new episodes of depression among women. *Br. J. Nutr.,* 118**,** 743-749.
RICHARDSON, A. J. 2006. Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. *Int. Rev. Psychiatry,* 18**,** 155-172.

SAINI, R. K. & KEUM, Y.-S. 2018. Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance—A review. *Life Sci.,* 203**,** 255-267.
SAINI, R. K., et al. 2021. Omega− 3 Polyunsaturated Fatty Acids (PUFAs): Emerging Plant and Microbial Sources, Oxidative Stability, Bioavailability, and Health Benefits—A Review. *Antioxidants,* 10**,** 1627.
SALEM, N., et al. 2001. Alterations in brain function after loss of docosahexaenoate due to dietary restriction of n-3 fatty acids. *J. Mol. Neurosci.,* 16**,** 299-307.
SARRIS, J., et al. 2011. Omega-3 for bipolar disorder: meta-analyses of use in mania and bipolar depression. *The Journal of clinical psychiatry,* 72**,** 0-0.
SAUNDERS, E. F., et al. 2016. Omega-3 and omega-6 polyunsaturated fatty acids in bipolar disorder: a review of biomarker and treatment studies. *The Journal of clinical psychiatry,* 77**,** 0-0.
SCHLÖGELHOFER, M., et al. 2014. Polyunsaturated fatty acids in emerging psychosis: a safer alternative? *Early intervention in psychiatry,* 8**,** 199-208.
SHAHIDI, F. & AMBIGAIPALAN, P. 2018. Omega-3 polyunsaturated fatty acids and their health benefits. *Annu. Rev. Food Sci. Technol.,* 9**,** 345-381.
SHI, J.-P., et al. 2018. Effect of ω-3 polyunsaturated fatty acid on oxidative stress production and apoptosis in hippocampus of neonatal rats with brain injury induced by lipopolysaccharide. *Journal of Xinxiang Medical College,* 35**,** 455-458,463.
SHI, Z., et al. 2017. Fish oil prevents lipopolysaccharide-induced depressive-like behavior by inhibiting neuroinflammation. *Mol. Neurobiol.,* 54**,** 7327-7334.
SINCLAIR, A., et al. 2007. Omega 3 fatty acids and the brain: review of studies in depression. *Asia Pac. J. Clin. Nutr.,* 16**,** S391-S397.
SINN, N., et al. 2008. Cognitive effects of polyunsaturated fatty acids in children with attention deficit hyperactivity disorder symptoms: a randomised controlled trial. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 78**,** 311-326.
SOLBERG, D. K., et al. 2016. Lipid profiles in schizophrenia associated with clinical traits: a five year follow-up study. *BMC Psychiatry,* 16**,** 1-9.
SONG, C., et al. 2003. Effects of dietary n-3 or n-6 fatty acids on interleukin-1β-induced anxiety, stress, and inflammatory responses in rats. *J. Lipid Res.,* 44**,** 1984-1991.
SONG, C., et al. 2016. The role of omega-3 polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids in the treatment of major depression and Alzheimer's disease: acting separately or synergistically? *Prog. Lipid Res.,* 62**,** 41-54.
STEVENS, L. J., et al. 1995. Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. *The American Journal of Clinical Nutrition,* 62**,** 761-768.
STOLL, A. L., et al. 1999. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. *Arch. Gen. Psychiatry,* 56**,** 407-412.
SU, K.-P. 2008. Mind-body interface: the role of n-3 fatty acids in psychoneuroimmunology, somatic presentation, and medical illness comorbidity of depression. *Asia Pac. J. Clin. Nutr.,* 17.
SU, K.-P. 2009. Biological mechanism of antidepressant effect of omega–3 fatty acids: how does fish oil act as a 'mind-body interface'? *Neurosignals,* 17**,** 144-152.
SU, K.-P., et al. 2010. Phospholipase A2 and cyclooxygenase 2 genes influence the risk of interferon-α–induced depression by regulating polyunsaturated fatty acids levels. *Biol. Psychiatry,* 67**,** 550-557.
SU, K.-P., et al. 2000. Effects of polyunsaturated fatty acids on psychiatric disorders. *The American Journal of Clinical Nutrition,* 72**,** 1241-1241.
SU, K. P., et al. 2014. Omega-3 fatty acids in the prevention of interferon-alpha-induced depression: results from a randomized, controlled trial. *Biol. Psychiatry,* 76**,** 559-66.

SUBLETTE, M. E., et al. 2011. Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. *The Journal of clinical psychiatry,* 72**,** 0-0.

TALAMONTI, E., et al. 2020. Impairment of DHA synthesis alters the expression of neuronal plasticity markers and the brain inflammatory status in mice. *The FASEB Journal,* 34**,** 2024-2040.

TANG, M., et al. 2015. Antidepressant-like effect of n-3 PUFAs in CUMS rats: role of tPA/PAI-1 system. *Physiol. Behav.,* 139**,** 210-215.

TREMBLAY, M.-E., et al. 2016. Remodeling of lipid bodies by docosahexaenoic acid in activated microglial cells. *J. Neuroinflammation,* 13.

TU, C.-H., et al. 2020. Brain responses to emotional stimuli after eicosapentaenoic acid and docosahexaenoic acid treatments in major depressive disorder: toward personalized medicine with anti-inflammatory nutraceuticals. *Journal of personalized medicine,* 10**,** 283.

VAISMAN, N., et al. 2008. Correlation between changes in blood fatty acid composition and visual sustained attention performance in children with inattention: effect of dietary n−3 fatty acids containing phospholipids. *The American journal of clinical nutrition,* 87**,** 1170-1180.

VANCAMPFORT, D., et al. 2019. Sedentary behavior and anxiety-induced sleep disturbance among 181,093 adolescents from 67 countries: a global perspective. *Sleep Med.,* 58**,** 19-26.

VINES, A., et al. 2012. The role of 5-HT1A receptors in fish oil-mediated increased BDNF expression in the rat hippocampus and cortex: a possible antidepressant mechanism. *Neuropharmacology,* 62**,** 184-191.

WU, Y.-Q., et al. 2016. Long chain omega-3 polyunsaturated fatty acid supplementation alleviates doxorubicin-induced depressive-like behaviors and neurotoxicity in rats: involvement of oxidative stress and neuroinflammation. *Nutrients,* 8**,** 243.

WURTMAN, R. J. 2014. A nutrient combination that can affect synapse formation. *Nutrients,* 6**,** 1701-1710.

XU, F., et al. 2019. Effects of omega-3 fatty acids on metabolic syndrome in patients with schizophrenia: a 12-week randomized placebo-controlled trial. *Psychopharmacology,* 236**,** 1273-1279.

YOUNG, G. & CONQUER, J. 2005. Omega-3 fatty acids and neuropsychiatric disorders. *Reprod. Nutr. Dev.,* 45**,** 1-28.

YU, J.-Z., et al. 2020. N-3 polyunsaturated fatty acids promote astrocyte differentiation and neurotrophin production independent of cAMP in patient-derived neural stem cells. *Mol. Psychiatry*.

ZHOU, X., et al. 2019. Polyunsaturated fatty acids metabolism, purine metabolism and inosine as potential independent diagnostic biomarkers for major depressive disorder in children and adolescents. *Mol. Psychiatry*, 24**,** 1478-1488.