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**Omega-3 fatty acids and depression: epidemiological and
experimental evidence**

PhD Thesis

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	4
LIST OF ABBREVIATIONS.....	5
ABSTRACT.....	6
GENERAL INTRODUCTION.....	8
Omega-3 polyunsaturated fatty acids.....	8
Epidemiological aspects regarding depressive disorders and diet.....	10
<i>Burden of the disease.....</i>	10
<i>Depression and diet, the association with fish consumption.....</i>	11
Hypothesized mechanisms of action.....	15
<i>Neuro-endocrine modulation of omega-3 PUFA in depression.....</i>	15
<i>Anti-inflammatory effects of omega-3 PUFA.....</i>	19
<i>Relation between omega-3 PUFA intake and structural changing of brain.....</i>	23
<i>Genetic aspects of association between omega-3 PUFA and depression.....</i>	25
CHAPTER 1.....	29
CHAPTER 2.....	81
DISCUSSION AND CONCLUSIONS.....	117
REFERENCES.....	117
LIST OF PUBLICATIONS.....	137

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LIST OF ABBREVIATIONS

5-HIAA 5-hydroxyindolacetic acid
AA arachidonic acid
AD Alzheimer's disease
BBB blood–brain barrier
BDNF brain derived neurotrophic factor
COX cyclooxygenase
CREB c-AMP response element binding protein
CRH corticotropin releasing hormone
CSF cerebrospinal fluid
CVD cardiovascular disease
DALY age-standardized disability-adjusted life year
DGLA dihomogammalinolenic acids
DHA docosahexaenoic acid
EPA eicosapentaenoic acid
GLA gamma linolenic acid
HDRS Hamilton Depression Rating Scale
HO-1 heme oxygenase-1
HPA hypothalamic-pituitary-adrenal
IL interleukin
LA linoleic acid
LT leucotrienes
MADRS Montgomery Asberg Depression Rating Scale
MAPK mitogen-activated protein kinase
MDD major depressive disorder
NARI noradrenergic reuptake inhibitors
NF-kB nuclear factor-kB
NHMRC National Health and Medical Research Council
NMDA N-methyl-D-aspartate
PUFA polyunsaturated fatty acids
RCT randomized controlled trial
SNRI serotonin noradrenaline reuptake inhibitors
SSRI selective serotonin reuptake inhibitors
TGF transforming-growth-factor β 1
TNF tumor necrosis factor
TrkB tyrosine kinase receptor B
WHO World Health Organization

ABSTRACT

The changes of omega-6/omega-3 polyunsaturated fatty acids (PUFA) in the food supply of Western societies occurred over the last 150 years is thought to promote the pathogenesis of many inflammatory-related diseases, including depressive disorders. Among the biological properties of omega-3 PUFA, their anti-inflammatory effects and their role on the structural changing of the brain should be taken into account to better understand the possible pathway through which they can be effective both in preventing or treating the depressive status.

The use of omega-3 supplement in depressed patients reached notable improvements during last years. Meta-analysis of 11 and 8 trials conducted respectively on patients with a DSM-defined diagnosis of major depressive disorder and patients with depressive symptomatology but no diagnosis of major depressive disorder demonstrated significant clinical benefit of omega-3 PUFA treatment compared to placebo (standardized mean difference in clinical measure of depression severity was 0.56 [95% CI: 0.20, 0.92] and 0.22 [95% CI: 0.01, 0.43], respectively; pooled analysis resulted in 0.38 [95% CI: 0.18, 0.59] standardized mean difference). Use of mainly eicosapentaenoic acid (EPA) within the preparation, rather than docosahexaenoic acid (DHA), influenced final clinical efficacy. Significant clinical efficacy had the use of omega-3 PUFA as adjuvant rather than monotherapy. No relation between efficacy and study size, baseline depression severity, trial duration, age of patients, and study quality was found. Omega-3 PUFA resulted effective in RCTs on patients with bipolar disorder, whereas no evidence was found for those exploring their efficacy on depressive symptoms in young populations, perinatal depression, primary disease other than depression (i.e., patients with schizophrenia, Alzheimer disease, cardiovascular disease) and healthy individuals.

The systematic review of epidemiological studies exploring the possible relation of fish or omega-3 PUFA consumption resulted in findings much harder to be interpreted. A total of 28 studies, including 251,464 individuals and over 20,000 cases of depression, were examined. Among the 18 studies exploring the possible association between fish consumption and depression, 5 out of 7 cross-sectional and 6 out of 11 prospective studies reported a significant relation. A protective effect of omega-3 PUFA intake on depression was reported in 7 out of 11 cross-sectional and 4 of 9 prospective studies. The high degree of heterogeneity did not allow to support or confute the hypothesis that dietary omega-3 PUFA decrease the risk to develop

depression. Future researches should take into account the methodological limitations retrieved, such as i) better assessment of depressive cases, ii) detailed consumption of all PUFA and their possible interactions, and iii) consider the possibility of a non-linear relationship between fish or omega-3 PUFA intake and the risk to develop depressive disorders. Whether future findings will confirm that omega-3 PUFA consumption would result effective in preventing depressive disorders, to correct the inadequate supply of omega-3 PUFA in Westernized countries' diet will be a priority in order to set food and health policies and dietary recommendations for individuals and population groups.

GENERAL INTRODUCTION

Omega-3 polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFA) are fatty acids containing two or more carbon-carbon double bonds not saturated with hydrogen atoms at multiple (poly) locations within the molecule. PUFA can be classified in various groups by their chemical structure in omega-3 and omega-6 PUFA: the omega-3 PUFA (also called ω -3 or n-3 fatty acids) refers to a group of PUFA in which the first double bond is 3 carbons from the end (omega) carbon atom of the molecule; the omega-6 (also called ω -6 or n-6 fatty acids) are a family of PUFA that have in common a final carbon-carbon double bond in the n-6 position, which is the sixth bond counting from the methyl end [1]. Omega-3 PUFA are synthesized by dietary shorter-chained omega-3 fatty acid alpha-linolenic acid (ALA), to form the more important long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Figure 1). Omega-6 PUFA derive from linoleic acid (LA), which can be converted also in the 18 carbon gamma linolenic acid (GLA), and the 20 carbon arachidonic (AA) and dihomogammalinolenic acids (DGLA) (Figure 1). Both LA and ALA are essential fatty acids because mammalian cells are unable to synthesize these fatty acids from simpler precursors. Omega-3 PUFA have been long investigated for their anti-inflammatory effects in inflammatory-related diseases [2]. Omega-6 PUFA can be converted into AA and then metabolized into the omega-6 eicosanoids, which has pro-inflammatory action (Figure 1). On the other hand, omega-3 PUFA increases EPA in the cell membrane. This competes with AA for enzymatic conversion into its own metabolites, the omega-3 derived eicosanoids. These are less active and can partly oppose or antagonize the pro-inflammatory actions of the omega-6 eicosanoids. Non-inflammatory eicosanoid balance is maintained throughout the body by way

of a homeostatic balance between omega-3 and omega-6 fatty acids in cell membranes. Eicosanoid balance then exerts a “downstream” balancing influence on cytokines.

In the context of the modern human lifestyle and diet, an absolute change of omega-6/omega-3 ratio in the food supply of Western societies has occurred over the last 150 years [3]. Despite the eicosanoid metabolites of EPA are crucial to provide anti-inflammatory effects by balancing the potentially pro-inflammatory eicosanoid metabolites of the omega-6 AA, a ratio of omega-6/omega-3 of 15:1 to 16.7:1 (instead of 1:1 characterizing prehistoric human being) has been reported [4]. Thus, the existing balance between omega-6 and omega-3 PUFA for the years during the long evolutionary history of the human being has rapidly changed over a short period of time, do not accompanied by corresponding genetic changes. In other words, humans living in modern societies are exposed to a nutritional environment that differs from their genetic constitution. Not by chance, omega-3 PUFA have been considered of particular interest for the treatment of certain forms of chronic diseases [5]. In particular, many epidemiological and experimental studies emphasized their possible role in the prevention or treatment of depressive disorders. Due to evidence from animal and human studies reporting that omega-3 deficiency lead to impaired neuronal function (especially of serotonergic and dopaminergic neurotransmitters) and altered inflammatory status, the biological plausibility of the effects of the omega-3 PUFA raised several hypothesis, although merely speculative [6]. The aim of this project was to review the current knowledge and quantitatively analyze the results on the association between the omega-3 PUFA and depression taking into account both epidemiological and experimental studies.

Epidemiological aspects regarding depressive disorders and diet

Burden of the disease

Depression is mental disorder characterized by sadness, loss of interest in activities, and decreased energy. Other symptoms include loss of confidence and self-esteem, inappropriate guilt, thoughts of death and suicide, diminished concentration, and disturbance of sleep and appetite. There are multiple variations of depression that a person can suffer from: (i) depressive episode involves symptoms such as depressed mood, loss of interest and enjoyment, and increased fatigability, categorized as mild, moderate, or severe; (ii) bipolar affective disorders typically consist of both manic and depressive episodes separated by periods of normal mood. Diagnostic criteria for a major depressive episode (DSM-IV) include a depressed mood, a marked reduction of interest or pleasure in virtually all activities, or both, lasting for at least 2 weeks. In addition, 3 or more of the following must be present: gain or loss of weight, increased or decreased sleep, increased or decreased level of psychomotor activity, fatigue, feelings of guilt or worthlessness, diminished ability to concentrate, and recurring thoughts of death or suicide. Especially when long-lasting and with moderate or severe intensity, depression may become a serious health condition. In about 20% of cases, however, depression follows a chronic course with low rates of remission, especially when adequate treatment is not available. The recurrence rate for those who recover from the first episode is around 35% within 2 years and about 60% at 12 years. The recurrence rate is higher in those who are more than 45 years of age. Depression is associated with significant disability [7] and with excess mortality, particularly increasing the risk of cardiovascular diseases [8]. By 2020, depression is projected to be the second leading cause of disease burden worldwide after heart disease. Depression is associated with dysregulation of circadian rhythms, high incidence of sleep disorders and anxiety.

Depression is estimated to affect 350 million people. The World Mental Health Survey conducted in 17 countries found that on average about 1 in 20 people reported having an episode of depression in the previous year. Depression is a leading cause of disability worldwide (in terms of total years lost due to disability), especially in high income countries, ranging from 3% in Japan to 17% in the US [9]. In most countries the number of people who would suffer from depression during their lives falls within an 8–12% range [10, 11], suggesting significant increased rates of depression in high-prevalence populations (i.e., the US population) than large-sample estimates from the 1980s and 1990s [12]. Furthermore, more recent studies reported that prospectively observed cumulative prevalence of depression estimated nearly twice as high as the lifetime prevalence of major depressive episodes reported by cross-sectional studies during same time interval [13]. Nevertheless, yet the mental health budgets of the majority of countries constitute less than 1% of their total health expenditures. More than 40% of countries have no mental health policy and over 30% have no mental health programs [14]. Moreover, both direct economic costs of depression in terms of cost of treatment and indirect costs through lost days of work and reduced productivity, represent a major issue for public health operators [15].

Depression and diet, the association with fish consumption

Mental, physical, and social health represent fundamental components for the general well-being of a person. These factors are closely interwoven and deeply interdependent. For instance, the increased prevalence of depression over last decades in Western countries has been accompanied by parallel increased prevalence of cardiovascular diseases and fundamental changing in dietary habits [15, 16]. Several studies suggest that depression may share common pathophysiologic mechanisms with the cardiovascular diseases and their risk factors [17], such as the increased production of pro-inflammatory

cytokines [18], endothelial dysfunction [19], and elevations in plasma homocysteine levels [20]. Depressive and cardiovascular disorders share blood flow abnormalities (i.e., in depression, hypoperfusion in the limbic system and prefrontal cortex) [21] and decreased glucose metabolism (i.e., low glucose utilization in a number of brain regions correlating negatively with severity of depression) [22]. Given the increases in prevalence of both depression and cardiovascular diseases, it has been hypothesized that a common underlying environmental influence may account for these changes. A comprehensive causal pathway of the relationship between depression and cardiovascular diseases included behavioral and genetic mechanisms [23]. One factor that could explain the relationship between such diseases and explain this parallel increase is the significant shift over the last century in the dietary intake of long-chain PUFA toward an increase in saturated fat and an increase in the ratio of omega-6 to omega-3 fatty acids [24]. Omega-3 PUFA have been reported to both inhibit endothelial cell proliferation [25] and influence glucose uptake [26, 27] and utilization [28] in the brain cells by reducing the expression of both isoforms of the brain glucose transporter GLUT1 in rats [29].

The fatty acid composition of the modern Western diet has changed dramatically during the last century, being characterized by an excessive amount of omega-6 PUFA and a very high omega-6/omega-3 ratio. These pattern of fatty acid intake are thought to promote the pathogenesis of many inflammatory-related diseases, including cardiovascular disease, cancer, and autoimmune diseases, whereas increased levels of omega-3 PUFA and a low omega-6/omega-3 ratio may exert suppressive effects [30]. The increased intake of saturated fatty acids and n-6 essential fatty acids and the reduced consumption of foods containing omega-3 fatty acid, which may exert anti-inflammatory properties, have been hypothesized to correlate depressive and cardiovascular diseases, increasing the incidence of both disorders [31].

The main sources of fatty acids may vary greatly among countries, mostly depending on food availability and cultural influences. Per capita consumption of EPA and DHA in the US has been reported to be about 50 mg and 80 mg/day, respectively [32]. Evidence from prospective secondary prevention studies suggests that EPA/DHA supplementation ranging from 50 to 180 mg/day (either as fatty fish or supplements) significantly reduces subsequent cardiac and all-cause mortality. For ALA, total intakes of 150 to 300 mg/day seem to be beneficial. Dietary Guidelines suggest to include at least two servings of fish per week (particularly fatty fish). In addition, the data support inclusion of vegetable oils (i.e., soybean, canola, walnut, flaxseed) and food sources (i.e., walnuts, flaxseeds) high in ALA in a healthy diet for the general population [33]. A joint expert consultation of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommend an intake of 1–2 servings of fish, where each serving is defined as providing 200 to 500 mg/week DHA and EPA [34]. Further recommendations by the National Health and Medical Research Council (NHMRC) issued Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes, which recommended an intake of combined DHA, EPA and DPA of 610 mg/day for men and 430 mg/day for women to prevent chronic disease [35].

The first observation of an inverse association between per capita fish consumption and national annual prevalence of major depression across nine countries was reported about 15 years ago [36]. Since then, several epidemiological studies on oily fish consumption and depression reported a significant inverse correlation between intake of oily fish and prevalence [37-42] and incidence [43-45] of depression and bipolar disorder [46] setting a threshold of vulnerability of about 650 mg/day. We performed an analysis using the data by the Food and Agriculture Organization of the United Nations (FAOSTAT) [47] regarding the total and marine fish consumption by

country and the last report of the WHO regarding the global burden of disease, including unipolar and bipolar depressive disorders [9] (Figure 2). Matching together these datasets, we found an inverse correlation between the fish consumption and the age-standardized disability-adjusted life year (DALY) rates for both unipolar and bipolar depressive disorders (Figure 3). A specific analysis of the same variables from 2000 to 2007 in United Kingdom [48] resulted in a significant inverse correlation between fish consumption and mixed anxiety and depressive disorders and in a significant trend of increased prevalence over time of such disorders (Figure 4). As observed in Figure 2, despite the high consumption of fish, increased rates of depression and/or depressive symptoms have been reported in western countries. Besides in industrialized countries, such as US and Japan, where stressful lifestyles and the condition of the society may counteract the potential beneficial effects of high fish consumption and increase the overall morbidity burden of depression, in the most of other countries fish consumption seems to correlate with the DALY. It has been hypothesized that this finding might be related with the low quality of diet consumed especially in such countries [49-53]. Regarding the Mediterranean countries, several studies reported a decreased prevalence [54] and incidence [55-57] of depression and/or depressive symptoms in subjects more adherent to the whole Mediterranean dietary pattern, which include a higher consumption of fish. The favorable effects of the Mediterranean diet on mental health may depend on the synergic positive actions of a variety of foods with a high content of PUFA, such as oily fish, but also olive oil and varieties of nuts with respect to the inflammation processes [58]. Such positive effects on mental health of long chain fatty acids contained in the Mediterranean diet also translate the numerous evidences of the protection of such dietary pattern against cardiovascular diseases [59]. However, conclusions are far away distant, since other components of the Mediterranean dietary pattern,

such as B vitamins, or other fish nutrients, such as iodine and selenium, may exert considerable positive effects on the brain and have a protective role on depression undermining the upcoming evidences regarding omega-3 fatty acids [60].

Hypothesized mechanisms of action

Although epidemiological data and clinical trials suggest that omega-3 PUFA may have preventive and therapeutic effects on depression, the underlying mechanisms are still unclear. The protective role of omega-3 fatty acids against depression it has been hypothesized to depend on the physiological mechanisms in which fatty acids take part.

Neuro-endocrine modulation of omega-3 PUFA in depression

The pathophysiology of depression has been dominated by the monoamine hypothesis, suggesting that an imbalance, mainly in serotonergic and noradrenergic neurotransmission, is at the core of the pathophysiology of depression. The current therapeutic strategies against depression include drugs which enhance either serotonergic neurotransmission (i.e., selective serotonin reuptake inhibitors, SSRI), noradrenergic neurotransmission (i.e., noradrenergic reuptake inhibitors, NARI), or both (i.e., tricyclic antidepressants and more recently serotonin noradrenaline reuptake inhibitors, SNRI) [87]. However, in 30% of the cases, there is little or no response to the medication, and almost half of patients treated with current antidepressant drugs do not show significant clinical improvements [87].

An effect of omega-3 intake suggested to positively influence the depressive status, is the potential interaction with the serotonergic and dopaminergic transmission, including metabolism, release, uptake, and receptor function. The highly unsaturated nature of EPA and DHA provides them the quality of highly influence membrane order (namely the fluidity) of several types of

cells [88]. Omega-3 PUFA also regulate the signal transduction by enhancing G protein-mediated signal transduction [89, 90], membrane-bound enzymes (Na/K-dependent ATPase) [91], and protein kinase C [92]. The membrane changing induced by omega-3 PUFA intake may affect different neurotransmitter system altering the regulation of dopaminergic and serotonergic neurotransmission, which are dysfunctional in depressed patients. Changes in serotonin (5-HT) and dopamine receptor (DR-2) number and function caused by changes in PUFA provide the theoretical rationale connecting fatty acids with the current receptor and neurotransmitter theories of depression. Cerebrospinal fluid (CSF) 5-hydroxyindolacetic acid (5-HIAA), a metabolite that reflects serotonin turnover, has been reported to be decreased in several psychiatric conditions, including violent suicide attempts during depression [93]. It has been reported that higher concentrations of plasma DHA predict an increase in serotonergic neurotransmission (higher CSF 5-HIAA) in healthy adults [94] and in an experimental animal model of depression [95]. Conversely, that omega-3 deficiency results in an increase of serotonin receptor (5HT2) density in the frontal cortex, probably due to an adaptation to reduced serotonergic function [96, 97]. A preclinical animal experiment reported that erythrocyte DHA was inversely correlated, and AA and the AA/DHA and AA/EPA ratios were positively correlated, with plasma IL-6, TNF α , and CRP levels, whereas plasma IL-6 levels were positively correlated with 5-HIAA/5-HT ratios in all brain regions, providing evidence for a functional link between n-3 fatty acid deficiency, elevated peripheral inflammatory signaling, and increased central 5-HT turnover [98].

Regarding the dopamine neurotransmission, in animal experimental models of depression, decreased levels of dopamine turnover in the prefrontal cortex and dopamine levels up to 6-fold higher in the nucleus accumbens have been reported [99, 100]. Similar observation was reported in omega-3 PUFA deficient rats, in which the expression of the dopamine receptor (D2R) was

decreased in the frontal cortex and increased in the nucleus accumbens [96, 101, 102]. Conversely, supplementing the diet of rats with omega 3 PUFA led to a 40% increase in dopamine levels in the frontal cortex as well as an increase in the binding to the dopamine D2 receptor [103].

Beside the well-known deficiency in serotonergic neurotransmission as pathophysiological correlate of major depression, recent evidence points out to an important role of increased glutamate receptor activation as well [104]. Indeed, an increased activity of the glutamatergic system and N-methyl-D-aspartate (NMDA) receptor agonism has been associated with depressed mood, whereas a reduction of the glutamatergic activity may exert antidepressant action. These effects of the glutamatergic system on mood may depend on its direct or indirect influence on the serotonergic and noradrenergic neurotransmission, since NMDA receptor antagonists increase the serotonin levels in the brain [105, 106]. Omega-3 deficiency has been demonstrated to promote age-induced degradation of glutamatergic transmission and its associated astroglial regulation in the hippocampus [107] by slowing astroglial glutamate transport via a specific signal-like effect [108]. Further experimental models confirmed that dietary omega-3 content is relevant for glutamatergic system development and for behavioral performance in adulthood [109]. At molecular level, it has been demonstrated that NMDA receptor can be stimulated by the protein kinase C, which conformational changes and optimal activation depend on membrane content of omega-PUFA [110, 111]. The putative correlation among the neurochemical and behavioral alterations caused by dietary omega-3 PUFA and glutamatergic transmission must be further investigated in future research.

Glucocorticoids play a fundamental role in attenuating the inflammation processes following exposure to a variety of stress-related conditions [112]. Glucocorticoids suppress critical inflammatory signaling pathways including

nuclear factor-kB (NF-kB) and inhibit stress-related outflow pathways including corticotropin releasing hormone (CRH), the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system [113]. Failure of glucocorticoids to inhibit inflammatory and neuroendocrine responses to challenge may contribute to disease development, although the etiology of glucocorticoid resistance in both inflammatory and neuropsychiatric disorders is unknown. Depression has been associated with a high level of cortisol in blood due to the hyper activity of HPA axis, largely due to a hyper secretion of CRH [114, 115]. EPA may regulate the HPA axis dysfunction associated with depression by reducing corticotrophin releasing factor expression and corticosterone secretion [116]. Some animal studies reported that the response to chronic stress can be modulated by the omega-3 fatty acid supply, in that a dietary deficiency has been found to be deleterious while enrichment has protected against stress [117]. These effects were associated with the reduction of corticosterone levels promoted by the PUFA supplementation in the stress-induced animals [118, 119]. From a mechanistic point of view, it has been demonstrated that omega-3 PUFA inhibit the P-glycoprotein (P-gp) activity [119], which are transport proteins responsible of the increase in cortisol transport through the blood–brain barrier (BBB) in depressive subjects [120-124]. The normalization in brain penetration of cortisol would normalize the feedback control of the HPA axis. Another study demonstrated a modulatory effect of omega-3 PUFA by increasing the cortisol transport in the BBB models not through the inhibition of P-gp efflux but thanks to membrane fluidification and some effect on tight junction integrity [125].

Anti-inflammatory effects of omega-3 PUFA

Recent studies indicate that factors other than monoamine deficiency or hyperactivation of HPA axis, must be considered when examining the

pathogenesis of major depression such as an altered activation of immune system and chronic inflammation with a specific impairment in the signaling of neurotrophins such as transforming-growth-factor β 1 (TGF- β 1) [126, 127]. According to recent evidence, chronic stress can elicits a neuroinflammatory response through the activation of microglia in CNS, with ensuing release of inflammatory mediators such as interleukin-1 β (IL-1 β), and tumor necrosis factor- α (TNF α) [128]. The neuroinflammatory response leads to inhibition of neurotrophin signaling and can also elicit both sickness behavior and psychological pain. In addition, chronic stress alters activation of immune system in the periphery, which might account for the state of chronic inflammation observed in depressed patients [129]. Different studies have demonstrated a positive correlation between the severity of the symptoms of depression and the increase in the inflammatory status [129]. Pro-inflammatory cytokines interfere with many of the pathophysiological mechanisms that characterize the pathogenesis of depression, altering serotonin metabolism, and reducing both synaptic plasticity and hippocampal neurogenesis [129]. On the other hand, reduced levels of anti-inflammatory cytokines, such as interleukin-4 (IL-4), interleukin-10 (IL-10) and TGF- β 1, have been found in the plasma of depressed patients [129, 130].

Chronic systemic inflammation also contributes to the progression of neurodegeneration [131]. The key anti-inflammatory effect of omega-3 fatty acids has been long recognized to depend on their action on eicosanoids. Eicosanoids are biologically active lipid mediators produced from PUFA which play a role in inflammation and regulation of immune function [132]. To produce these eicosanoids, AA is released from membrane phospholipids through the action of phospholipase A2 enzymes, and then acts as a substrate for cyclooxygenase (COX), lipoxygenase or cytochrome P450 enzymes. COX enzymes lead to PG and thromboxanes, lipoxygenase enzymes lead to leucotrienes (LT) and cytochrome P450 enzymes lead to

hydroxyeicosatetraenoic and epoxyeicosatrienoic acids. Omega-3 EPA and DHA incorporation in cell membrane decreases their AA content and reduces the amount of substrate available to produce inflammatory and immunoregulatory eicosanoids [133]. LTB₅, a product of EPA, is a competitive antagonist to LTB₄, a highly pro-inflammatory eicosanoid derived from AA [134]. A series of studies gave important information regarding the omega-3 fatty acids as mediators of inflammatory response in depressive status. Indeed, it has been demonstrated that severity of depression varies with the degree of omega-3 fatty acids in erythrocyte membranes, which are decreased in more severe status, as an indicator of oxidative damage [135-138]. It has been also reported that plasma fatty acids composition and depression are associated with a significant higher ratio of omega-6 to omega-3 PUFA in depressed subjects [139-142]. Many studies also focused on analysis of fasting bloods for detection of plasma fatty acid analysis in risk population. Results from a case-control study conducted on 16 depressed and 22 non-depressed women recruited during the third trimester of pregnancy demonstrated that high DHA, high total n-3 and a low n-6:n-3 ratio were associated with significantly lower odds of depression [143]. Similar finding were reported in some studies conducted on depressed post-myocardial infarction [144] and acute coronary syndromes patients [145, 146] in which, compared with control group, lower levels of long-chain omega-3 PUFA as measured by mean AA/EPA ratio were found. Moreover, a low DEA percentage and low omega-3 proportions of lipid profile predicted risk of suicidal behavior among depressed patients over the 2-year period [147]. Another evidence come from a case-control study conducted on 150 subjects reporting an association between fatty acids with serotonergic and immunological markers in depressive patients but not in patients with somatization syndrome suggesting a different biological mechanisms of depression and somatoform disorders [148]. This may lead to the speculation

of a potential bias in previous studies on depression assessment concerning the indiscriminate merging together of both disorders that could affect the studies outcome. Similarly, an association between omega-3 fatty acids in adipose tissue and major depression has been showed [149-151], although not univocally reported [152, 153].

Dysregulation of the functional activity of the immune system in depression is a phenomenon that has been widely reviewed [154, 155]. As discussed above, the peripheral immune activation observed in major depression, through the release of pro-inflammatory cytokines, is responsible for the variety of behavioral, neuroendocrine and neurochemical alterations that are associated with this psychiatric condition [154]. Depression has been associated with excessive production (during an acute phase response) of pro-inflammatory cytokines, such as IL-1beta, IL-12, and interferon-gamma [155]. A recent meta-analysis of experimental studies reported a significantly higher concentration of the pro-inflammatory cytokines tumor necrosis factor -alpha and IL-6 in depressed subjects compared with control subjects [156]. The actions of omega-3 on cells include the changing of the expression of key cell surface proteins and the modulation of the production of pro-inflammatory cytokines. Indeed, omega-3 PUFA have been reported to decreased the production of TNF, IL-1b and IL-6 in *in vitro* studies, and decrease production of TNF, IL-1b, IL-6 and various growth factors in healthy human subjects, although not all studies confirm this effect [157]. At the cellular level, they have been demonstrated to interact with to decrease activation of NF-kB, a key transcription factor involved in upregulation of inflammatory cytokine[157]. The question arises as to whether the decreased prevalence of depressive symptoms accompanying the higher plasma content of omega-3 PUFA is also associated with improved central inflammation, i.e. cytokine activation, in the brain. Recent studies have pointed out the possible role of the omega-3 inducing a central antidepressant-like effect by

modulating oxidative reactions and inflammatory cytokine production in microglial and neuronal cells, determining a reduction of expressions of tumor necrosis factor- α , interleukin-6, nitric oxide synthase, and cyclooxygenase-2, and an induction by interferon- γ , and induction of upregulation of heme oxygenase-1 (HO-1) in BV-2 microglia [158]. However, results of experimental studies on cytokines response after administration of omega-3 fatty acids are not univocal. In example, long term intake of omega-3 increased plasma serotonin concentration and hippocampus c-AMP response element binding protein (CREB) and reducing interleukin (IL)-6 expression in rats but a clear dose-dependent effects and significant differences in expressions of IL-1 β , tumor necrosis factor- α , brain-derived neurotrophic factor or phosphorylated CREB were not found [159]. Moreover, another experimental study on mice demonstrated that high level of brain DHA was associated with a decrease in depressive-like symptoms throughout aging independently on the cytokines response (in fact increased interleukin-6 and decreased IL-10 expression were found in the cortex of aged mice independently of the diets) [160].

Among the anti-inflammatory actions of omega-3, it is noteworthy that they have been recently discovered as source of docosanoids, metabolites with a novel stereospecificity unlike that of the known eicosanoids [161]. The three known classes, namely docosatrienes, resolvins, and protectins, are produced mainly from controlled oxidative breakdown of DHA within the membrane and demonstrated anti-inflammatory properties [162]. Novel research on depression focused on the role of resolvins, which are thought to terminate ongoing inflammatory cascades and may be responsible of the potential anti-inflammatory effects of omega-3 PUFA in preventing or ameliorating the depressive status [163]. Resolvins are grouped in E-series and D-series, depending if derived by EPA or DHA, respectively. Resolvin E1 has been reported to reduces inflammation by suppressing the activation of the

transcription factor nuclear factor κ B and subsequent synthesis of inflammatory cytokines and chemokines [163].

As discussed above, major depression is characterized by increased levels of pro-inflammatory cytokines and reduced levels of anti-inflammatory cytokines such as IL-10 and TGF- β 1 [126, 129]. Plasma TGF- β 1 levels are reduced in major depressed patients and show a significant negative correlation with the Hamilton Depression Rating Scale [130, 164, 165]. Interestingly, TGF- β 1 levels significantly increase after antidepressant treatment [130], and SSRI drugs such as sertraline might exert immunomodulatory effects *in vivo* through a decrease in the pro-inflammatory cytokine IL-12 and an increase in the anti-inflammatory cytokines such as IL-4 and TGF- β 1 [165]. Similarly, therapeutic concentrations of venlafaxine prevent microglial activation, reduce pro-inflammatory cytokine secretion, and finally increase the release of TGF- β 1 in an astroglia–microglia co-culture model [166]. Recent studies suggest that omega-3 fatty acids can increase both *in vitro* and *in vivo* the synthesis of TGF- β 1 [167, 168] and in particular in pregnant women [169], although no studies have been yet conducted in depressed patients. On the basis of this evidence it might be worth to assess whether TGF- β 1 signaling is a common target both for omega-3 fatty acids and antidepressant drugs, and whether omega-3 fatty acids can exert their antidepressant effects *in vivo* effects via the rescue of TGF- β 1 signaling.

Relation between omega-3 PUFA intake and structural changing of brain

Lipids, especially phospholipids, are indispensable for the construction of biological membranes. About 60% of phospholipids in the brain are polyunsaturated fatty acids and, among them, about 1/3 are omega-3 PUFA, depending on the brain area [170]. The DHA content of the adult cerebral cortex is approximately 3% of the dry weight and 0.4% of the white matter

[171]. Lack of dietary DHA has been demonstrated to affect the normal of brain development by altering the composition of the membranes of brain cells, neurons, oligodendrocytes, astrocytes and organelles like myelin and nerve terminals [172]. Indeed, a large corpus of reports from animal [173] and human [174] studies agree that regular dietary intakes of omega-3 fatty acids during at least the first 6 months of life and suggest that PUFA should be added in formulas at the level generally found in human milk [175].

Since omega-3 PUFA play a fundamental role in the structural development and maintenance of a normal brain, it has been hypothesized that chronic supplementation with PUFA may induce antidepressant-like effects in parallel to brain structural changes. Morphological changes, such as reduction in the gray matter volume within the prefrontal cortex, the hippocampus, and the striatum, have been repeatedly reported in the brain organization of depressed patients [176-179]. The Cardiovascular Health Study and the Oregon Brain Aging Study did not report any significant relation of fish consumption or marine omega-3 nutrient biomarker pattern, respectively, to whole brain volumes [180, 181]. Conversely, the Framingham Study recently reported an association between lower red blood cell DHA and smaller whole brain volume [182]. It has been suggested that specific areas of the brain may be involved in depression and, thus, associated with omega-3 consumption. For instance, the amygdala is the core structure of the limbic emotion-processing circuit which plays a key role in the regulation of mood and in the memory of emotional stimuli [183]. Several studies reported an association between medial temporal lobe atrophy and recurrent major depression [184-187]. The neurotrophic hypothesis of antidepressant action is supported by the evidence that amygdala volume is significantly decreased in untreated depressed patients whereas increases after medication [185]. An experimental study conducted on mice demonstrated that besides the beneficial effects of PUFA on depressive

symptoms, PUFA supplementation was associated with an increase in the hippocampal volume [188]. In line with the aforementioned finding, another study demonstrated that higher plasma EPA, but not DHA, was associated with lower gray matter atrophy of the right hippocampal/parahippocampal area and of the right amygdala [189]. These findings confirmed the results of previous studies in which higher EPA and DHA intake was associated with greater gray matter volume in the subgenual anterior cingulate cortex, the right hippocampus, and the right amygdala in healthy adults [190] whereas DHA significantly affected hippocampal neuronal development and synaptic function in embryonic neuronal cultures [191].

It has been hypothesized that omega-3 PUFA may have a modulating action on brain derived neurotrophic factor (BDNF), a brain trophic factor involved in the neuronal growth and plasticity [192]. Serum BDNF has been found to be negatively correlated with the severity of depressive symptoms [193]. DHA has been reported to have the ability to regulate BDNF via a p38 mitogen-activated protein kinase (MAPK)-dependent mechanism [194] and through modulating the neurotrophin tyrosine kinase receptor B (TrkB) and p75(NTR) expression [195]. Conversely, the omega-3 deficiency has been demonstrated to reduce the levels of BDNF and signaling through the TrkB, in proportion to brain DHA levels, and reduced the activation of the BDNF-related signaling molecule cAMP response element-binding protein (CREB) in selected brain regions [196].

Genetic aspects of association between omega-3 PUFA and depression

The modern scientific approach indicate that mental disorders do not suffer from the artificial separation of biological from psychological factors, changing the old paradigm “genetics *versus* environment” in “genetics *plus* environment”, as the result of the interaction of biology with psychological and environmental factors. The improvements in genetic discovery may yield

important new insights into the understanding of genetic predisposition, alteration, or condition leading to increased risk of mental disorders, especially with interaction with environmental factors, such as diet.

In major depression, distinct anatomical abnormalities may not occur, rather, risk of illness may be due to abnormality in phospholipid metabolism, namely alteration of omega-3 PUFA uptake mechanism with specific impairment of the enzyme Type IV cytosolic phospholipase A2 and the fatty acid CoA ligase 4 [197-200], although this genetic alteration was not found in bipolar mood disorder [201]. These recent observations at molecular level could explain the low omega-3 PUFA levels in patients with depression, accompanying (or enhancing) the decreased dietary intake. Other experimental studies have demonstrated a partial correlation between Apolipoprotein (APO) E polymorphism and response to an omega-3 fatty acid supplement. Indeed, EPA and DHA plasma content seems to be somehow related with the APO E4 [202, 203] and EPA, but not DHA, resulted effective in slow cognitive decline in depressed subjects [204]. However, future investigators examining the effects of EPA on lipoprotein characteristics may consider including APO E genotype in their analyses.

Omega-3 PUFA-enriched diets lead to significant changes in expression of several genes in the central nervous tissue, and these effects appear to be mainly independent of their effects on membrane composition and as second messengers [205]. However, if these genetic modulation may exert some specific effect on prevention or treatment of depression is still unknown and further research on this field is needed.

Figure 1. Biosynthesis of the principal polyunsaturated fatty acids and their metabolism.

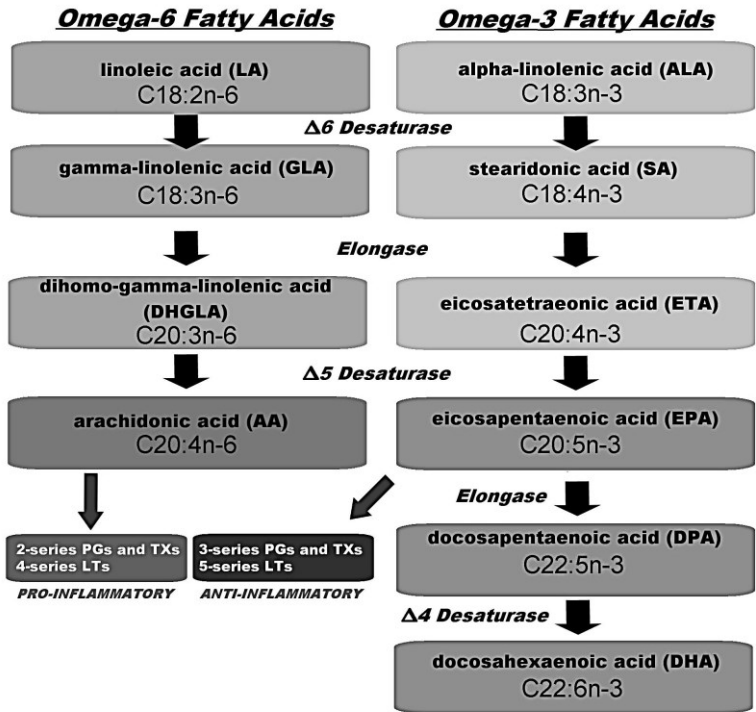
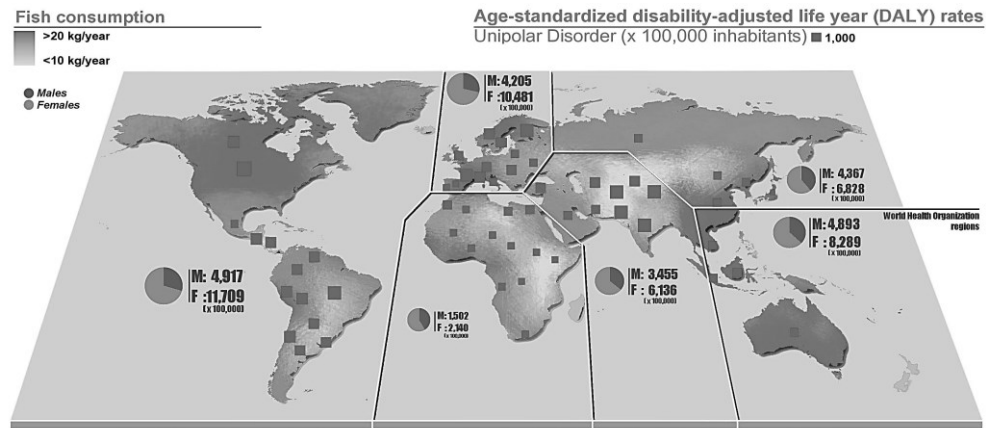


Figure 2. Per capita annual fish consumption and age-standardized disability-adjusted life year for unipolar disorder distribution across countries. DALY rates by gender are also reported per all World Health Organization regions (year 2004). High-income regions reported higher rates of DALY despite their increased consumption of fish, suggesting the role of social environment in the establishment of unipolar depressive disorder.



Source: Consumption of Fish and Fishery Products, Fishery and Aquaculture Department 2011, Food and Agriculture Organization of the United Nations (FAOSTAT); The Global Burden of Disease: 2004 update, World Health Organization, Geneva, 2008.

Figure 3. A. Association between per capita annual fish consumption and age-standardized disability-adjusted life year for unipolar and bipolar disorders by country (year 2004). B. Countries ordered by increasing fish consumption and relative depressive disorders trends. Both type of graphs demonstrate that DALY rates for unipolar and bipolar disorders are decreased in countries with increased fish consumption.

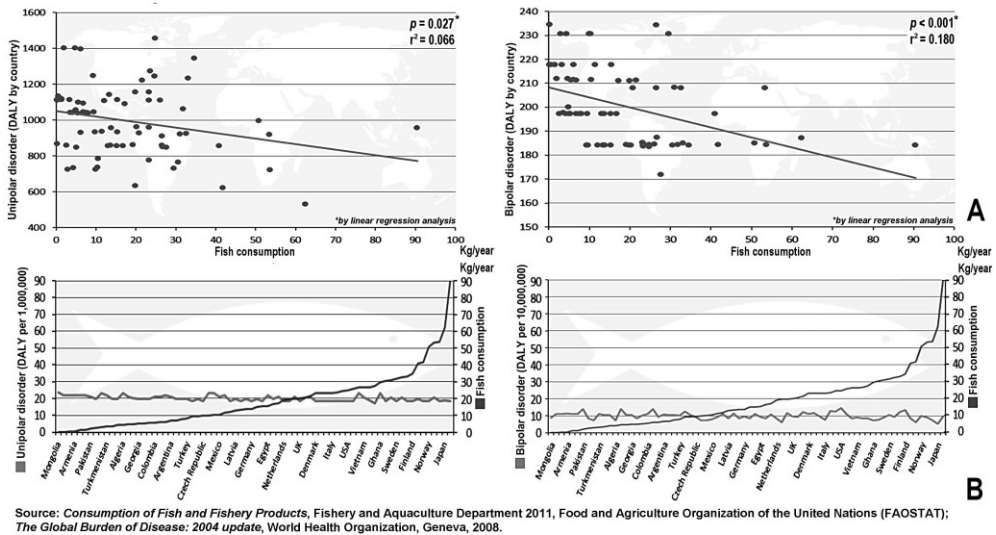
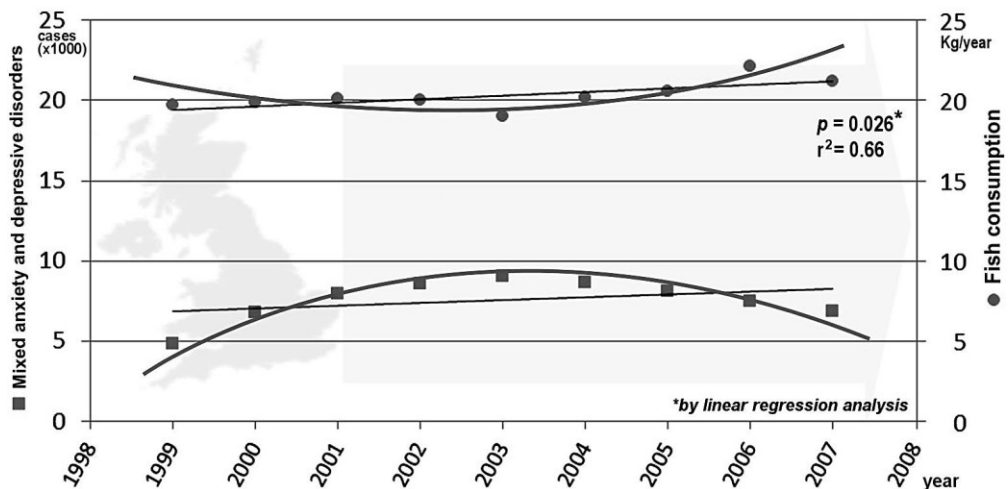


Figure 4. Trend over time (1999-2007) of per capita annual fish consumption and mixed anxiety and depressive disorders in United Kingdom. Despite a diametric-shaped distribution of cases and relative fish consumption, a significant increasing trend has been found.



Source: Consumption of Fish and Fishery Products, Fishery and Aquaculture Department 2011, Food and Agriculture Organization of the United Nations (FAOSTAT); Recent trends in the incidence of anxiety diagnoses and symptoms in primary care, Walters et al. PLoS One, 2012.

CHAPTER 1

Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials

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Abstract

Background. Despite omega-3 polyunsaturated fatty acids (PUFA) supplementation in depressed patients have been suggested to improve depressive symptomatology, previous findings are not univocal.

Objectives. To conduct an updated meta-analysis of randomized controlled trials (RCTs) of omega-3 PUFA treatment of depressive disorders, taking into account the clinical differences among patients included in the studies.

Methods. A search on MEDLINE, EMBASE, PsycInfo, and the Cochrane Database of RCTs using omega-3 PUFA on patients with depressive symptoms published up to August 2013 was performed. Standardized mean difference in clinical measure of depression severity was primary outcome. Type of omega-3 used (particularly eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) and omega-3 as mono- or adjuvant therapy was also examined. Meta-regression analyses assessed the effects of study

size, baseline depression severity, trial duration, dose of omega-3, and age of patients.

Results. Meta-analysis of 11 and 8 trials conducted respectively on patients with a DSM-defined diagnosis of major depressive disorder (MDD) and patients with depressive symptomatology but no diagnosis of MDD demonstrated significant clinical benefit of omega-3 PUFA treatment compared to placebo (standardized difference in random-effects model 0.56 SD [95% CI: 0.20, 0.92] and 0.22 SD [95% CI: 0.01, 0.43], respectively; pooled analysis was 0.38 SD [95% CI: 0.18, 0.59]). Use of mainly EPA within the preparation, rather than DHA, influenced final clinical efficacy. Significant clinical efficacy had the use of omega-3 PUFA as adjuvant rather than mono-therapy. No relation between efficacy and study size, baseline depression severity, trial duration, age of patients, and study quality was found. Omega-3 PUFA resulted effective in RCTs on patients with bipolar disorder, whereas no evidence was found for those exploring their efficacy on depressive symptoms in young populations, perinatal depression, primary disease other than depression and healthy subjects.

Conclusions. The use of omega-3 PUFA is effective in patients with diagnosis of MDD and on depressive patients without diagnosis of MDD.

Introduction

Omega-3 polyunsaturated fatty acids (PUFA) eicosapentaeoic acid (EPA) and docosahexaenoic acid (DHA) have been demonstrated to be effective in cardiovascular disease (CVD) prevention due to their anti-inflammatory and cardio-protective effects [1]. Recently, new therapeutic indications for omega-3 PUFA have been proposed, such as treatment for certain forms of mental illness, including depressive disorders [2]. Indeed, some psychiatric diseases as depression may share certain pathophysiological mechanisms with CVD, namely increased production of pro-inflammatory cytokines, endothelial dysfunction, and elevations in plasma homocysteine levels [3-5]. The positive effects of omega-3 PUFA on depression may depend on their physiological abundant content in the human nervous system and their involvement in neurogenesis and neuroplasticity [6]. Moreover, their anti-inflammatory capacity may counteract inflammatory processes occurring in depression [7,8]. Several ecological, cross-sectional, and prospective studies supported such hypotheses by reporting an inverse association between omega-3 intake and prevalence of depression [2]. Further clinical studies demonstrated lower concentration of omega-3 PUFA in plasma or red blood cell membranes of depressed subjects [9-13]. All together, these observations suggest a correlation between omega-3 PUFA and depressive disorders, justifying the rationale of a number of randomized controlled trials (RCTs) of omega-3 PUFA supplementation for the treatment of depressive disorders. The overall analysis of these studies from previous meta-analyses suggested a general benefit of omega-3 PUFA on depressive symptoms, despite certain variability in results weakened the possible validity of the findings. Indeed, results of such studies are not univocal, jeopardizing the evidence of therapeutic implications of omega-3 PUFA in depressed patients. It has been suggested that the heterogeneity between studies may depend on clinical and methodological issues, such as severity of baseline depression and methods

of assessment and diagnosis of depression. Some important issues regarding therapeutic regimen have been explored in more recent meta-analysis, reporting that the positive effects of omega-3 PUFA on depressive symptoms appeared to depend more on EPA administration rather than DHA, severity of depression, and study quality [14]. However, some concerns regarding these findings still persist [15,16]. The analyses previously conducted focused on the effects of omega-3 PUFA supplementation on depressive symptoms, but features associated with the pathophysiological nature of the depression occurring in the patients and their comorbidity status were often lacking. It is reasonable to believe that the biological effects of omega-3 PUFA may result effective in certain subtypes of depressive disorders rather than in others due to the different type of depression or clinical phenotype of the patient. Despite a full understanding of the processes leading to the depressive status is lacking, primary psychiatric disorders, such as major depression disorder (MDD) and bipolar disorders, are specific psychiatric conditions as recognized in the American Psychiatric Association's revised fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [17], marking out specific depressive symptoms that should be present as inclusion criteria to determine MDD diagnosis. The mental health examination may include the use of rating scales, such as the Hamilton Rating Scale for Depression [18], the Beck Depression Inventory [19], or the MARDS [20] for MDD, and the bipolar spectrum diagnostic scale [21] for bipolar disorders. These psychiatric diseases have indeed specific biological causes and are often known to be treated with and respond to different pharmacological interventions [22]. Another specific pathological condition is perinatal depression, which indicates the occurrence of depressive and other mood-associated symptoms during pregnancy and lactation, with a range of 5-25% of women developing post-partum depression [23]. Pregnancy and lactation are challenging periods due to a higher demand of

omega-3 PUFA from the fetus and the newborn, respectively, and a low DHA status may induce depressive symptoms [24]. Despite the fact that it is not clear if the depressive status is caused by or simply precipitated by pregnancy and lactation conditions, it is however likely associated with these conditions rather than with the aforementioned causes of MDD. Similarly, psychiatric disorders occurring in young populations need special attentions because major differences between adult and juvenile depression have been well-documented, despite the reasons for such dissimilarities are not clear [25]. Actually, there is very limited evidence upon which to base conclusions about the relative effectiveness of psychological interventions or antidepressant medication, but effectiveness of these interventions cannot be fully established [26]. Finally, the occurrence of depression secondary to a different primary disease, for instance schizophrenia, Alzheimer's disease (AD), Parkinson's disease, and CVD, may raise doubts on the pathophysiological mechanisms that cause the depressive symptomatology. In addition, despite it is of interested to examine the role of omega-3 PUFA on potential mood depression in healthy subjects, it is important to underline that preventive and therapeutic pathways may differ each other. Thus, altogether, the choice in previous meta-analyses to pool together studies with such different baseline conditions, in which depression occurred, may have affected the quality of the studies as well as utilizability of the results [27,28]. Moreover, the last meta-analysis included studies up to 2010 [29]. Thus, the aim of this study was to update the current knowledge about the overall clinical efficacy of omega-3 fatty acids (particularly EPA and DHA) in previous and more recent RCTs published in the last years, minimizing, from a clinical point of view, the differences among the populations of patients included in the studies, finally focusing on patients with a DSM-defined diagnosis of MDD.

Methods

A comprehensive search on MEDLINE, EMBASE, PsycInfo, and the Cochrane Database systematic Reviews of all RCTs using omega-3 PUFA on patients with depressive symptoms published up to August 2013 was performed. Articles of potential interest were identified by using the following search terms: “omega-3”, “polyunsaturated fatty acids”, “PUFA”, “trial”, “EPA”, “DHA”, combined with the following terms: “depression”, “depressive disorder”, “depressed mood”, “bipolar”, combined with “perinatal”, “post-partum”, “CVD”, “schizophrenia”, “Parkinson”, “Alzheimer”, “diabetes”, “angina”. Among the 192 articles retrieved, RCTs were identified and screened by reading the abstract and, when necessary, the full text, in order to select those articles relevant for the analysis. The reference list of the relevant reports was also inspected to identify any additional trials not previously identified. The process of identification and inclusion of trials is summarized in Figure 1. Inclusion criteria were the following: (i) studies conducted on humans; (ii) randomized design; (iii) placebo controlled; (iv) use of omega-3 PUFA supplement which relative amount could be quantified; (v) exploring changes in depressive symptoms as primary or secondary outcome. Exclusion criteria were the following: (i) studies reporting insufficient statistics or results; (ii) adopted a dietary intervention design. Study quality was measured in a 13-point scale including the Jadad criteria [30] and specific information regarding (i) registration of RCT before conducting the study, (ii) adequate blinding of the researchers, (iii) the use of an intention-to-treat analysis, (iv) control for patients’ diet (i.e., number of servings of fish), (v) assessment of compliance through measurement of plasma fatty acids, (vi) significant differences at baseline, (vii) adequate sample calculation, whether (viii) depression was the primary outcome, and (ix) number and reasons of withdrawal were mentioned. Data were abstracted independently from each identified trial by GG and SM using

a standard data abstraction form. This process was independently performed by two researchers and discordances were discussed and resolved.

Out of 59 originally selected studies, one [31] was excluded because of having a non-randomize non-placebo controlled design; two [32,33], because there was used a dietary intervention design; five [34-38], because the depressive status was reported as a categorical variable rather than a rating scale; two [39,40], because an inadequate or poorly comparable rating score of depression was used; two [41,42], because poorly comparable omega-3 PUFA or placebo preparations. This selection strategy resulted in a final selection of 47 studies eligible to be included in the present systematic review.

The clinical outcome of interest was the standardized mean difference in the change from baseline to endpoint scores on a depression rating scale, in patients taking omega-3 PUFA supplements *vs.* patients taking placebo. Preferred rating scales for measuring depression severity were the Hamilton Depression Rating Scale (HDRS), either the 9-item short form, 17-item, 21-item or 25-items scales, and the Montgomery Asberg Depression Rating Scale (MADRS) [20,43,44]. When available, HDRS scores from each study were used. If the HDRS was not available we used the MADRS. If neither HDRS nor MADRS data were available, we used the clinician rated measure of depression that the investigators identified as their primary outcome.

Among selected RCTs lacking in data, such as means and/or standard deviations (SDs), the data of one study [45] were provided by authors; SDs and 95% confidence intervals (CIs) of five studies [46-50] were retrieved from graphs; data of one study [51] were medians; and data of three studies [52-54] were imputed from data from all other trials using the same measure for depression as described elsewhere [55]. Eight studies [56-63] were finally excluded from the meta-analysis due to lacking data, resulting in a total number of 39 studies to be included in the analysis.

Effects due to participant diagnosis were investigated by grouping studies according to the most relevant clinical characteristics of the population on which they were conducted, as follows: (i) Depressed patients (including DSM-defined diagnosis of MDD and general assessment of depression without clinical visit); (ii) Bipolar disorder patients (including bipolar disorder during pregnancy); (iii) Children or adolescents with depression or bipolar disorder; (iv) Women with perinatal depression (including DSM-defined diagnosis of MDD and prevention of post-partum depression); (v) Mild-cognitive impairment or AD patients; (vi) Schizophrenic patients; (vii) Parkinson's disease patients; (viii) Patients with concomitant CVDs; and (ix) Healthy subjects.

Data regarding type of diagnosis, number of subjects enrolled in the trial, ongoing therapy, (TRATT.) type of supplement used in the intervention, type of placebo, daily dose, duration of the intervention, outcome measures, and information to retrieve the study quality were collected. Those RCTs reporting more than one dose of omega-3 PUFA [54,64-68] or more than one formulation (i.e., EPA or DHA separately) [48,51,69], were considered as separate studies in the pooled analyses. One study [70] enrolled different populations (MDD and non-MDD patients), thus each population was also included in the meta-analysis as a separate study.

Statistical analysis

Continuous data were reported as mean and SDs and listed in descriptive tables. All depression scales' means and SDs at baseline and end of follow-up period of both intervention and control groups were combined [71] and the standardized mean effect for all trials was calculated by using Hedges adjusted g in order to correct for small sample bias [72]. Both random- and fixed-effects models were used to estimate the overall effect size. Heterogeneity was investigated by using Higgins' I^2 statistic [73,74]. When

heterogeneity between results of the studies exists, the random-effect models were preferred.

Possible publication bias for the analysis regarding RCTs conducted on MDD patients (MDD group, $n = 11$) and those not diagnosed with DSM-IV criteria (non-MDD group, $n = 9$) was investigated by drawing a funnel plot to look for funnel plot asymmetry [71] and meta-regression based on study size. Meta-regression was performed using linear regression, with the effect size (SMD) of trials as the dependent variable and the variables of interest as the independent variable. The generic inverse variance method was used to weight trials. Effects due to severity of depressive symptoms, age of patients, and study quality were also investigated by using meta-regression based on standardized baseline depression scores, mean age of the study participants, and our modified Jadad scores of the studies, respectively. The effects of trial duration, EPA and DHA dose in omega-3 preparations, and the use as mono or adjuvant therapy were also examined. Particularly, the qualitative analysis of the type of supplementation used was investigated grouping the studies in those using mainly EPA (EPA >50% of the dose) and mainly DHA (DHA >50% of the dose). A further analysis was computed by splitting the grouping in mainly EPA, pure EPA, mainly DHA, and pure DHA supplementation. As well, the therapeutic approach was investigated by grouping studies using omega-3 in monotherapy or as adjuvant therapy together with antidepressant drugs. The quantitative analysis of the dose was computed by a meta-regression analysis of the EPA and DHA doses used.

Random- and fixed-effects models, forest and funnel plots, and Higgins' I^2 statistics were performed in Review Manager (RevMan) version 5.2 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration), meta-regression analyses were performed in SPSS version 17 (SPSS Inc., Chicago, IL, USA).

Results

Overall studies

The most relevant features of the 47 studies included in this systematic review and meta-analysis are displayed in Table 1. Considerable differences among studies were found for all characteristics examined. The average quality of the studies was about 9 over a maximum score of 13 (range 5-13). The mean length of the trials was about 16 weeks (range 4-160), 36 studies used a mixed intervention with EPA+DHA, 14 pure EPA and 4 pure DHA. The average dose of EPA+DHA was 1.39 g (range 0.63-6.2 of EPA and 0.27-3.4 of DHA), whereas 1.93 g (range 1-6) and 0.86 g (range 0.22-2) were the average doses of pure EPA and DHA, respectively (Table 1). The most of RCTs used the Hamilton Depression Rating Scale [46,48,49,54,57,58,63,65,69,75-84], 10 studies [46,49,56,68,79,85-89] used the Beck Depression Inventory, and 13 studies [47,50,54,62,64,67,77,84,90-94] the Montgomery-Asberg Depression Scale as the main outcome measure. Among the studies not included in the quantitative analysis, due to lack of data, one was conducted on patients with obsessive-compulsive disorder [57] and one on patients with chronic fatigue syndrome [56], both reporting no relevant effects of omega-3 fatty acids compared with placebo; four studies conducted in bipolar depressed patients [58,59,61,63] reporting that there were no significant differences on any outcome measure between the EPA and placebo groups; one study on diabetes mellitus patients with MDD [62] reporting no effect of omega-3 fatty acids on depression severity; and one on older adults with mild cognitive impairment suggesting that increased intakes of DHA and EPA can reduce depressive symptoms and the risk of progressing to dementia [60].

Depression (MDD and non-MDD groups)

A total of 19 studies were included in the first pooled analysis conducted in patients with depressive symptoms (Figure 2). Among them, 11 trials were conducted in patients with a DSM-defined diagnosis of MDD, including 8 studies conducted in adults [46,48,70,76-78,83,84] and 3 studies in elderly patients [53,95,96]. The pooled standardized difference in means using a fixed-effects model for the MDD group was 0.47 SD (95% CI: 0.29, 0.66), which suggests a beneficial effect of omega-3 fatty acids on depressed mood compared with placebo in patients with diagnosis of MDD. The pooled standardized difference in means in a random-effects model was 0.56 SD (95% CI: 0.20, 0.92). The remaining 8 were those conducted on patients with an assessment of depression but not rigorously diagnosed according to the DSM criteria, and included patients with depressive symptoms despite ongoing treatment [54,69,79,97], women with borderline personality disorder [91], patients with recurrent self-harm [49], people with mild to severe depressed mood not taking medications [86], post-menopausal women with psychological distress and depressive symptoms [70], and subjects with a history of at least one major depressive episode [89], whereas two studies were excluded due to lack of data [56,57]. Despite patients pooled in this analysis were not homogeneous in terms of health status, all studies clearly reported to have included subjects with no other psychiatric or neurological illnesses such as AD, Parkinson's disease, as well as no history of any end-stage diseases, CVDs, or any unstable medical conditions, thus to make them comparable each other for our purposes. Similar results were found for this group of patients (standardized mean difference – fixed effects-model: 0.15 SD, 95% CI: 0.01, 0.30; random-effects model: 0.22 SD, 95% CI: 0.01, 0.43). For both MDD and non-MDD groups, there was evidence of heterogeneity (MDD group, $I^2 = 71\%$, $P < 0.001$; non-MDD group, $I^2 = 46\%$, $P = 0.04$).

The overall analysis including both groups was conducted to assess whether results were different considering a mood-improving effect on depressive symptoms in patients with non-organic, metabolic, nor genetic-related neurodegenerative disease. The pooled standardized difference in means using a fixed-effects model was 0.27 SD (95% CI: 0.16, 0.39), and the pooled standardized difference in means using a random-effects model was 0.38 SD (95% CI: 0.18, 0.59). However, there was evidence of heterogeneity ($I^2 = 65\%$, $P < 0.001$). To test this heterogeneity, a funnel plot was drawn and is shown in Figure 3. The funnel plot did not show considerable evidence of asymmetry. Meta-regression of study effect size, based on study size, did not present significant association (regression coefficient = -0.108, 95% CI: -0.224, 0.012; $P = 0.066$) indicating no role of the sample size in determining the results of the analysis.

A meta-regression analysis was performed of standardized mean depression scores on baseline depression scores to test whether the gravity of depression at baseline may play a role in the efficacy of omega-3 fatty supplementation. The analysis showed no relation between baseline depression scores and efficacy for all studies (regression coefficient = 0.019, 95% CI: -0.009, 0.047; $P = 0.167$) as well as for MDD patients (regression coefficient = 0.008, 95% CI: -0.053, 0.068; $P = 0.787$) and non-MDD (regression coefficient = 0.019, 95% CI: -0.017, 0.054; $P = 0.270$) separately. Even taking into account the comparison of studies using the same depression scale (HDRS), no significant relation between baseline depression scores and efficacy was found (data not shown).

Analysis conducted to explore the role of type (namely, the administration of mainly EPA or DHA supplementation) and dose (separately for EPA and DHA) of omega-3 supplement used showed that the use of mainly EPA within the preparation, rather than DHA, appeared to influence final clinical

efficacy (standardized mean difference – fixed effects-model: 0.46 SD, 95% CI: 0.31, 0.61; random-effects model: 0.50 SD, 95% CI: 0.27, 0.72) (Figure 4). Despite heterogeneity fallen by 55%, it remained significantly high ($P = 0.002$). When the analysis was split in mainly EPA, pure EPA, mainly DHA, and pure DHA supplementation, both the EPA preparations were significant (for pure EPA, standardized mean difference – fixed effects-model: 0.40 SD, 95% CI: 0.19, 0.61; random-effects model: 0.43 SD, 95% CI: 0.18, 0.68) and the heterogeneity fallen to 28% ($P = 0.19$). This result indicates that despite the overall heterogeneity represented an underlying true difference in effect sizes across studies, it may be strongly affected by type of formulation of omega-3 fatty acids used.

The meta-regression analyses exploring the role of the dose of omega-3 fatty acids revealed that the total dose of DHA were unrelated to efficacy (regression coefficient = -0.066, 95% CI: -0.471, 0.603; $P = 0.801$), whereas the dose of EPA formulation resulted related to efficacy both for all MDD plus non-MDD patients (regression coefficient = 0.477, 95% CI: 0.084, 0.869; $P = 0.02$). However, when the analyses was repeated separately for each group, the association remained significant only for MDD patients (regression coefficient = 0.746, 95% CI: 0.100, 1.392; $P = 0.028$) whereas lost significance for non-MDD patients (regression coefficient = 0.215, 95% CI: -0.288, 0.718; $P = 0.359$).

No relation between study size (regression coefficient = -0.109, 95% CI: -0.231, 0.012; $P = 0.075$, baseline depression severity (regression coefficient = 0.026, 95% CI: -0.007, 0.060; $P = 0.116$), trial duration (regression coefficient = -0.058, 95% CI: -0.153, 0.038; $P = 0.223$), age of patients (regression coefficient = 0.013, 95% CI: -0.10, 0.036; $P = 0.879$), and study quality (regression coefficient = -0.142, 95% CI: -0.357, 0.072; $P = 0.183$)

and omega-3 PUFA efficacy was found, despite study quality almost reached significance when considered only for RCTs conducted on patients with MDD (regression coefficient = -0.403, 95% CI: -0.857, 0.052; $P = 0.077$). On the contrary, fixed- and random-effect models of RCTs grouped by use of omega-3 PUFA as mono- or adjuvant therapy revealed a significant effect when they were used in combination with standard antidepressant therapy (standardized mean difference – fixed effects-model: 0.26 SD, 95% CI: 0.09, 0.44; random-effects model: 0.39 SD, 95% CI: 0.06, 0.71).

Bipolar disorder

In our systematic review we collected 7 trials conducted on patients with bipolar disorder (both type I and II) [58,59,61,63,65,75,80] (Table 1). The only three studies pooled for the analysis included one study [65] that accounted for more than 70% of the weight of the analysis, that together with others [75,80] resulted in a significant effect of omega-3 fatty acids in ameliorating depressive symptoms in adults with bipolar disorder (standardized mean difference – fixed effects-model: 0.73 SD, 95% CI: 0.39, 1.07; random-effects model: 0.74 SD, 95% CI: 0.38, 1.10; $I^2 = 9\%$, $P = 0.35$) (Figure 5).

Depression or bipolar disorder in children and adolescents

Among the studies conducted on depression occurring in youth, one study [98] documented a positive effect of omega-3 fatty acids in improving the mood of children diagnosed of MDD and one study conducted on adolescents at high risk of psychosis [92] reported that omega-3 fatty acids significantly reduced positive symptoms, negative symptoms, and improved functioning compared with placebo, but no significant effect was observed on depressive symptoms.

Perinatal depression

There were six trials aiming to explore the effects of omega-3 PUFA on perinatal depression. We distinguished between those studies conducted on pregnant women with MDD [82,93,99] (Figure 6) and those on apparently healthy women (primary prevention) [51,68,85] (Figure 7). However, both analyses led to inconclusive results (MDD in pregnancy, standardized mean difference – fixed effects-model: 0.08 SD, 95% CI: -0.29, 0.45; random-effects model: 0.24 SD, 95% CI: -0.73, 1.21; prevention of post-partum depression, standardized mean difference – fixed effects-model: 0.05 SD, 95% CI: -0.24, 0.15; random-effects model: -0.05 SD, 95% CI: -0.24, 0.15). Only one study [82] concluded that omega-3 fatty acids might have therapeutic benefits in depression during pregnancy. Besides the clinical efficacy of omega-3, in regard to the safety issue, it is important to underline that omega-3 fatty acids supplementation was well tolerated and no adverse effects were reported on the subjects treated and newborns in all studies.

Depression as secondary outcome

Among the trials conducted in patients with primary disease other than depression, those conducted on AD or mild cognitive impairment [50,81] (Figure 8), schizophrenia [54,90] (Figure 9), and CVDs [87,94,100] (Figure 10) reported inconclusive results, whereas the only study conducted on Parkinson's disease patients in comorbidity with MDD [47], including those treated with antidepressants and those without, reported improvement in depressive symptoms and indicate that the intake of omega-3 PUFA can be used as adjuvant therapy in Parkinson's disease patients. However, in one study conducted on schizophrenic patients with persistent ongoing symptoms [54], the authors reported a large placebo effect in patients on typical and new atypical antipsychotics and no difference was observed between active treatment and placebo, but in patients on clozapine, there was a clinically

important and statistically significant effect of 2 g/day omega-3 PUFA treatment on the PANSS and its sub-scales.

Depressive symptoms in healthy subjects

The trials conducted on healthy subjects aimed to explore potential beneficial effects of omega-3 fatty acids as mood improving medicaments in the general population (Figure 11). Among the tot studies included [52,66,67,88,101,102], the overall analysis showed a nearly null effect of this supplement on depressive symptoms in healthy subjects (standardized mean difference – fixed and random effects-model: 0.00 SD, 95% CI: -0.13, 0.13).

Discussion

We demonstrated that the use of omega-3 PUFA as therapeutic agents was effective in patients with diagnosis of MDD and on depressive patients without a diagnosis of MDD, whereas inconclusive results were found for patients with other pathological conditions (namely schizophrenia and AD) as well as in healthy subjects and perinatal depression. The analysis of the studies on bipolar disorder showed a positive effect of the omega-3 PUFA, but the evidence is weakened due to the exclusion from the quantitative analysis of three studies that may affect the overall effect of the supplement. When the studies conducted on patients with MDD or those on patients with depressive symptoms but not rigorous evaluation by health professionals were pooled together, a general positive effect of omega-3 PUFA was found.

As previously reported [15], the studies that mostly negatively influenced the pooled results of the non-MDD patients included non-homogenous individuals, since their enrolment was in settings such as general practice surgeries, shopping malls, and university freshman fairs [86], newspaper, radio and television advertising, and flyers posted [70], and through a Community Mental Health Service, general practices, and advertisements in

community newspapers [79]. Despite the idea of a widely available low cost supplement that could assist those being treated for a current depressive episode in a community setting is highly desirable, a lack of rigor in patients' selection may lead to the inclusion of subjects with normal emotional states, eventually affecting the results and, thus, challenging the model's credibility. It is noteworthy that negative results came out mostly from studies sharing this methodology [70,79,86]. Moreover, as reported by the authors [79,86], both experimental and control groups improved significantly, usually indicative of a major placebo response which is expected to exert a meaningful clinical effect in the treatment of such "subthreshold" depressed subjects [103]. A recent meta-analysis demonstrated that the relative efficacy of the active drug compared to placebo in clinical trials for MDD is highly heterogeneous across studies, with a worse performance in showing a superiority of the drug *versus* placebo for studies with placebo response rates $\geq 30\%$ [104]. Thus, the studies quality decreased when placebo response rates were not maintained below this critical threshold that may depend on the non-homogenous depressive "phenotypes" of the subjects enrolled. The non-MDD group also included four studies conducted on patients with depressive disorders despite ongoing antidepressant therapy [54,69,79,97]. These results should be considered with caution, because these studies may include those "non-responder" subjects that generally fail to reach remission with the first anti-depressant therapy and have higher relapse rates and poorer outcomes than those who remit [105]. Studies conducted in this subgroup of patients can explain not clearly favorable effects of omega-3 PUFA on depressive symptoms in these studies and puzzling results.

Previous meta-analyses included all RCTs with little distinction among population groups, leading to controversial results, such as overall benefit [106,107] and negligible effects [27,28] of omega-3 PUFA against depressive

symptoms, especially due to the high heterogeneity of studies. The following studies improved some methodological issues (i.e., better definition of inclusion criteria, especially in the distinction between the definition of MDD and other depressive disorders) and focused attention on specific aspects of omega-3 administration (i.e., dosage, EPA:DHA ratio) leading to the conclusion that administration of EPA, rather than DHA, is responsible for the beneficial effects of omega-3 PUFA intake as therapeutic agents in patients with depressive disorders [108,109] and supplements containing EPA \geq 60%, in dose range from 200 to 2200 mg EPA in excess of DHA, were effective against primary depression . On the contrary, the last meta-analytic study [14] reported small, non-significant benefit of omega-3 PUFA for the treatment of MDD, generally in contrast with the aforementioned previous meta-analyses, but some methodological issues in study selection have arisen [15,16]. Taking into account that pathophysiological processes of depressive symptoms involved in MDD patients are likely to be very different from those in patients with depression occurring in other clinical conditions (i.e., bipolar disorder, pregnancy, primary diseases others than depression) and in non-homogenous patients (i.e., community sample of individuals), we used a different approach to analyze the RCTs using omega-3 PUFA supplementation against depressive symptoms, grouping the studies by type of diagnosis of depression and taking into account any possible health condition that may influence the onset of the depression as well as the response to therapy. Other meta-analyses reported that the more severe was the depression, the more likely omega-3 PUFA supplementation would reduce depressive symptoms. We failed to demonstrate such a result, and we consider this finding as a surrogate of our observation that, overall, the efficacy of omega-3 PUFA was mostly related to a specific DSM-based diagnosis of MDD. Hence, this latter has been translated in a correlation of efficacy to more severe symptoms whereas, according to our results, we

hypothesized that this efficacy may be more related to the specific pathophysiological processes of the MDD rather than to its severity. Compared with previous meta-analyses, the differences of findings may depend on the additional number of RCTs published since the publication of the last study [37,40,53,60,61,66,68,69,84,89,92,94-97,100-102], the increasing number of participants which vary the overall weight of previous studies, the requirement for public registration of trials resulting in an increase of general studies' quality and may be responsible for the decreased evidence of publication bias.

Since the pathophysiological mechanisms and the therapeutic approach for bipolar disorder differ from those of MDD [22,23], when previous analyses included and pooled findings of studies conducted on these groups of different patients, they led to inconclusive results. It has been hypothesized that the efficacy of omega-3 PUFA may be different in the depressive phase rather than the manic episode [110], and recent systematic analysis of trials focused on this topic showed positive effects of omega-3 PUFA as an adjunctive treatment for depressive but not mania in bipolar disorder patients [111,112]. Thus, we separately grouped the studies conducted on patients with bipolar disorder and explored efficacy of omega-3 PUFA in ameliorating the depressive symptoms, finding a significant efficacy of the supplement in two [65,75] out of the three trials. Despite the positive results, it is noteworthy to underline that we had to exclude, due to missing of data, four studies [58,59,61,63] conducted on bipolar patients reporting poor effect of the omega-3 PUFA intervention, thus weakening our findings. There is a need of well-designed, high quality studies, which may clarify the potential effects of omega-3 PUFA supplement in patients with rigorously diagnosed bipolar disorder.

Regarding the substantial inefficacy of the omega-3 PUFA in patients with

primary diseases other than depression, it may be possible that these studies are more likely to suffer from publication bias, since depression was often a secondary outcome. Despite this methodological issue, the effects of the omega-3 PUFA may have been also affected by factors particularly related to the primary disease. Regarding the studies conducted on patients with CVDs, the analysis included very heterogeneous populations, namely patients with coronary heart disease [87], with diabetes mellitus [94], and post myocardial infarction [100], that may have been responsible for the inconclusive results. Moreover, it has been recently reported that supplementation of EPA in diabetes mellitus patients with comorbid MDD poorly affect biological risk factors for adverse outcome observed in this category of patients [113]. The RCTs conducted on patients with mild cognitive impairment or AD revealed poor efficacy of omega-3 PUFA in ameliorating the depressive symptoms. It has been reported that molecular mechanisms and pathways that underlie the pathogenesis of depression (i.e., impairment in the signaling of some neurotrophins such as Transforming-Growth-Factor- β 1 and Brain-derived-neurotrophic-factor) are also involved in the pathogenesis of AD [114,115], thus the omega-3 PUFA supplementation may not be the optimal pharmacological approach for this specific group of patients [116-118]. The two trials (including different dosages) conducted on schizophrenic patients with persistent ongoing symptoms resulted in limited effects of the omega-3 PUFA on patients' affective states. These results may be attributable to some psychotic symptoms (i.e., negative symptoms) that may directly influence (i.e., improve) depression-rating scores. Moreover, these patients were receiving different types of antipsychotics such as first- and second-generation antipsychotics that may differently affect (positively or negatively) the final effects of omega-3 PUFA on depressive symptoms. Finally, depression examined as secondary outcome could suffer by changing of the measurement depending on the improvement (or worsening) of the

underlying primary disease.

Regarding the different efficacy of EPA compared with DHA and EPA-DHA combinations, the analysis of RCTs grouped according to type of omega-3 PUFA administered confirmed the findings of previous meta-analysis and substantial stronger pooled results of studies using EPA rather than DHA. However, as previously reported [109], the aforementioned methodological issues may have biased the results in favor of efficacy for EPA-containing preparations suggesting that the reported benefits on depressive symptoms in this group of studies may not therefore be definitively attributed only to the EPA content of the supplementation regimen and also that further studies are needed in this field. Whether EPA, rather than DHA, is effective in ameliorating depression in specific groups of patients, the different effects of these classes of omega-3 PUFA is a challenge to be explained convincingly, since DHA is a major structural component of neuronal membranes, and we can hypothesize that increasing its nutritional availability would have beneficial effects on brain function, rather than EPA, which is present at levels several hundred-fold lower [120]. Possible explanations of the beneficial role of EPA are the following: (i) the anti-inflammatory effects of EPA-derived eicosanoids [121] and its oxidized derivatives [122] (ii) its efficacy at reducing the inflammatory cytokines tumor necrosis factor- α (TNF- α), IL-6, and IL-1b [123] through inhibition of the activity of nuclear factor kappa-B (NF-kB) [124]; (iii) *in vivo* evidence of a more effective anti-inflammatory action of dietary EPA compared with DHA [125]. Moreover, DHA has been reported to be poorly incorporated in the human brain [126], and EPA may facilitate an increase in brain DHA levels after its conversion [127]. Finally, EPA supplementation has been associated with N-acetyl-aspartate increase in brain, a marker for neuronal homeostasis, suggesting its role as a neuroprotective agent [80]. Together with the inflammation theory of depression [8], chronic intake of omega-3 fatty acids has been reported to

play an important role in neuronal structure and function [128]. However, such hypotheses are not completely exhaustive and further research is needed to better identify the specific molecular mechanisms underlying clinical efficacy of omega-3 PUFA (both EPA and DHA) in preventing or ameliorating depression.

The studies excluded from this systematic review were not comparable in terms of methodology used, and their exclusion was needed in order to reduce differences among RCTs and improve data quality (i.e., reduce selection bias). On the other hand, these trials may still be directly relevant to the topic of the present study, and a specific discussion (e-discussion) may strengthen conclusion retrieved from this meta-analysis. Moreover, we discussed in a specific section of the e-discussion about the studies quality and potential sources of heterogeneity.

The main limitation of this study was the inability to control all the many potential sources of heterogeneity. Despite the fact that a logical grouping of trials was performed, a non-modifiable degree of heterogeneity, due to specific characteristics of all trials included, still weakened the pooled analysis of these studies. However, compared with older studies, the inclusion of the updated RCTs strengthened the conclusions of the effects of omega-3 PUFA intake on depressive disorders.

To sum up, trials conducted in individuals with a diagnosis of MDD provided evidence that omega-3 PUFA supplementation has beneficial clinical effects on depressive status. Evidence of their efficacy was provided also for patients with bipolar disorder, whereas no evidence was found for individuals included in the other diagnostic groups. According to our findings, in RCTs with omega-3 PUFA supplementation in healthy subjects and patients with schizophrenia, AD and CVD seems to result ineffective.

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Figure 1. Process of inclusion of trials for systematic review and meta-analysis of studies on omega-3 fatty acids and depressive symptoms.

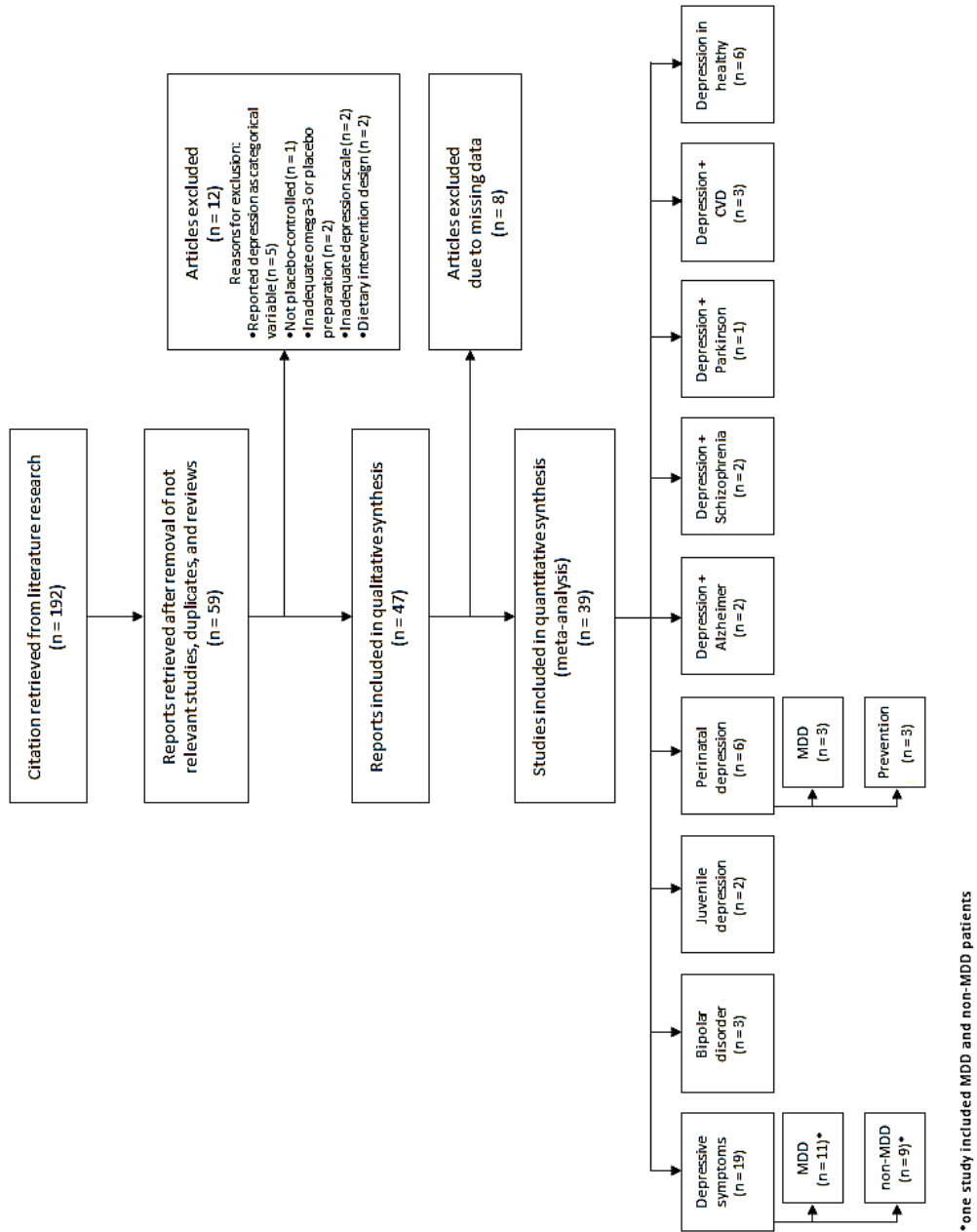


Figure 2. Forest plot showing individual and combined effect size estimates and 95% CIs for 19 trials grouped in those conducted on patients with a DSM-defined diagnosis of major depressive disorder (MDD group, n = 11) and those on patients with an assessment of depression but not rigorously diagnosed according to the DSM criteria (non-MDD group, n = 8). Black squares: indicate the weighting given to the trial in the overall pooled estimate; lines: indicate the 95% CIs; rhombus: indicate the combined effect size.

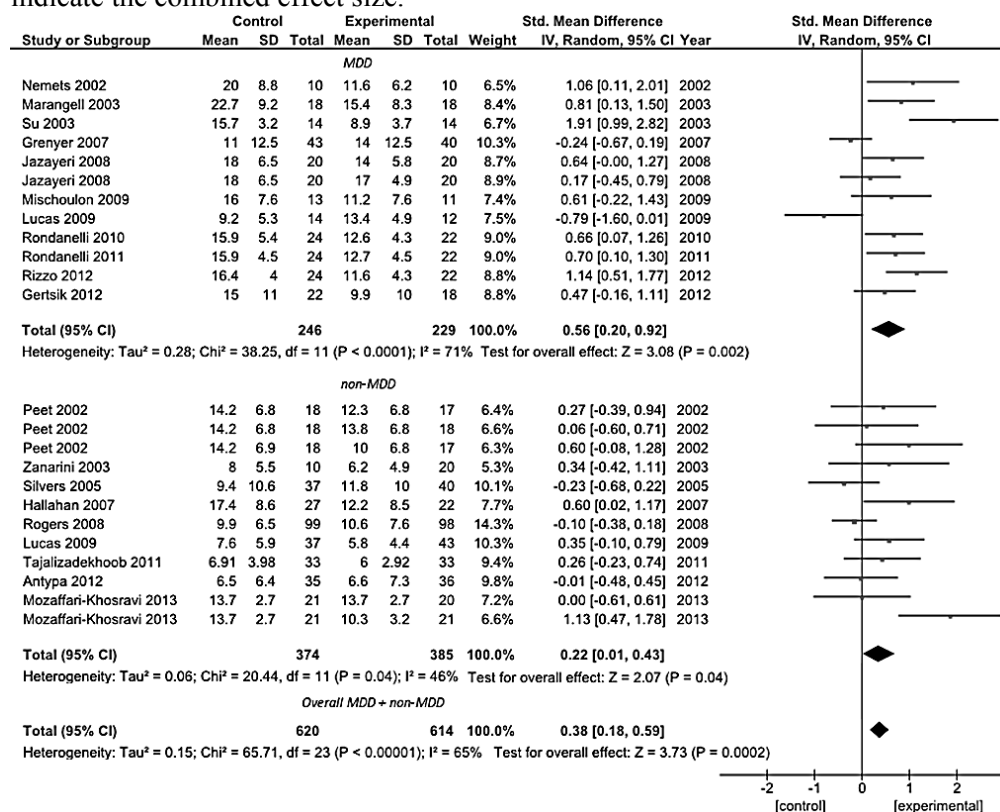


Figure 3. Funnel plot of effect size estimates for individual trials conducted on patients with depressive disorder without secondary comorbidities (MDD group and non-MDD group, $n = 19$).

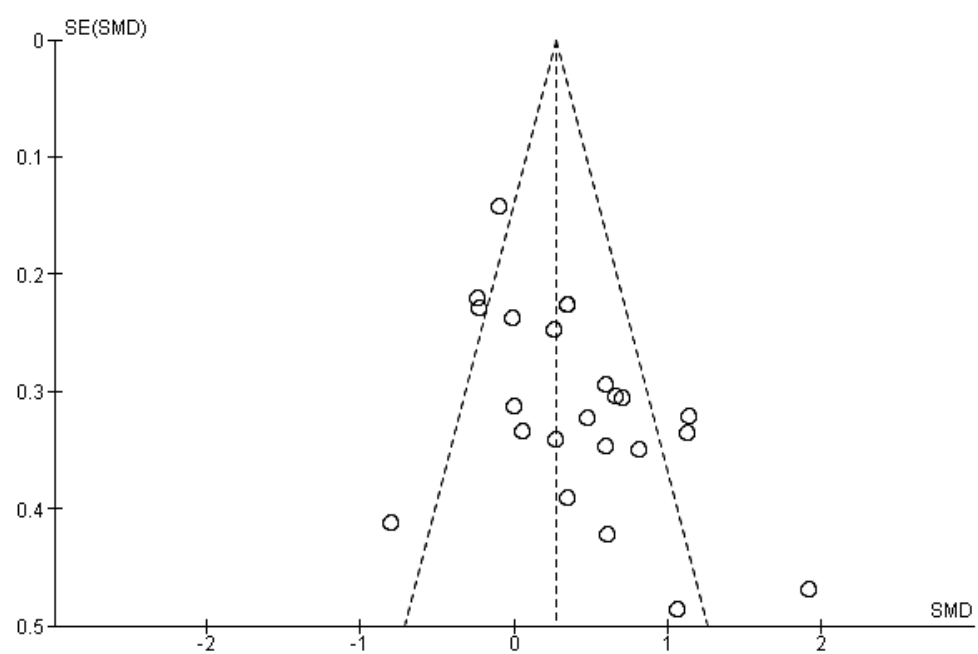


Figure 4. Forest plot examining the effect of the type of omega-3 PUFA supplementation employed on the reduction in depressive symptoms (MDD group and non-MDD group, n = 19).

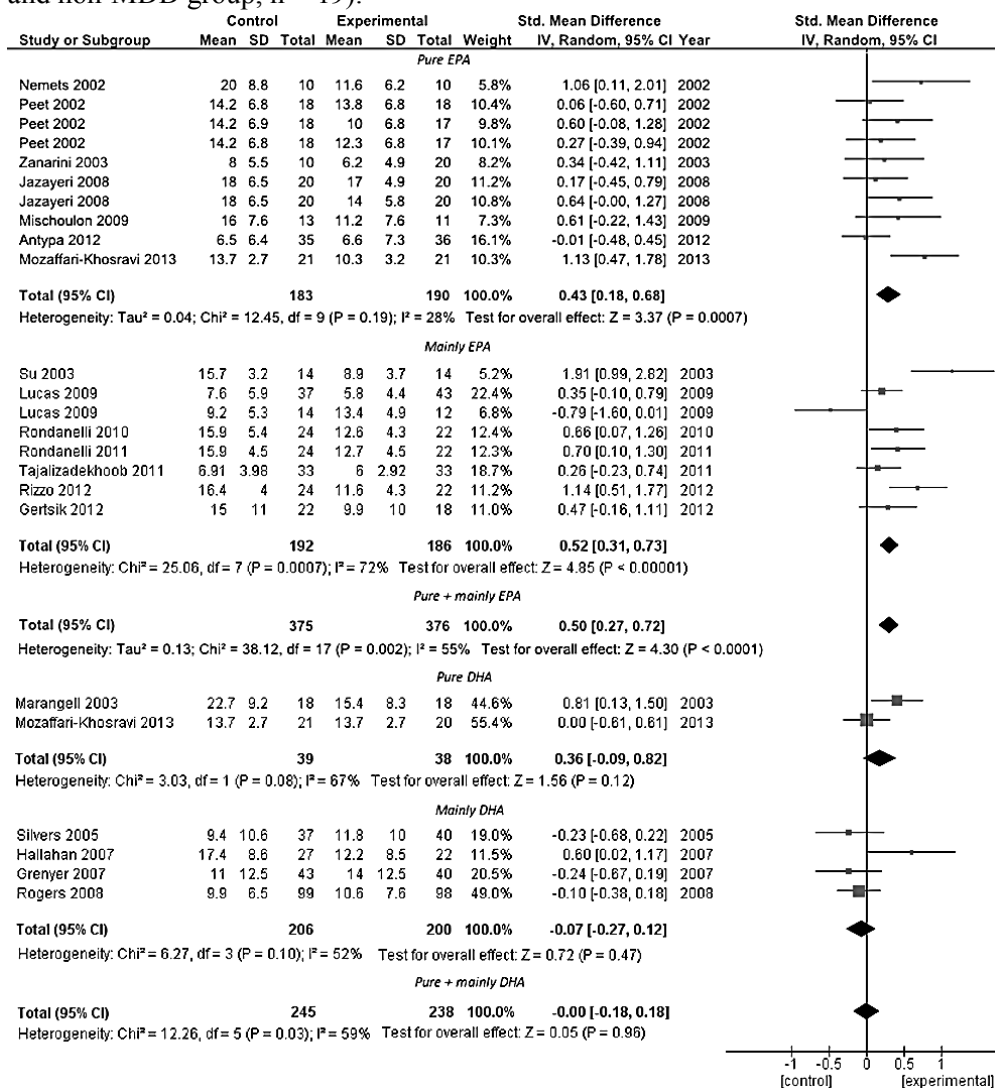


Figure 5. Forest plot showing individual and combined effect size estimates and 95% CIs for 3 trials conducted on patients with bipolar depression.

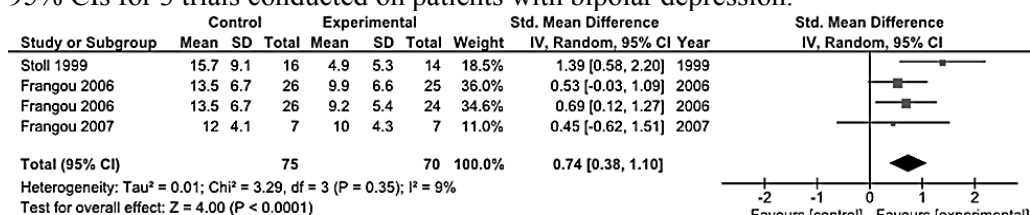


Figure 6. Forest plot showing individual and combined effect size estimates and 95% CIs for 3 trials conducted on pregnant women with major depressive disorder.

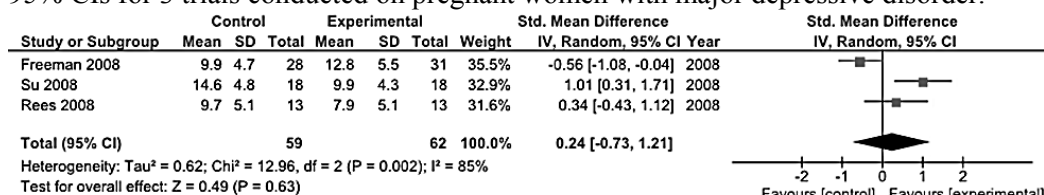


Figure 7. Forest plot showing individual and combined effect size estimates and 95% CIs for 3 trials conducted on healthy pregnant women for prevention of postpartum depression.

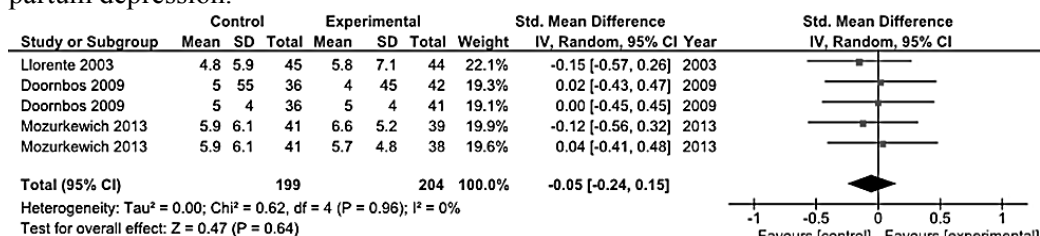


Figure 8. Forest plot showing individual and combined effect size estimates and 95% CIs for 2 trials conducted on patients with Alzheimer or mild cognitive impairment.

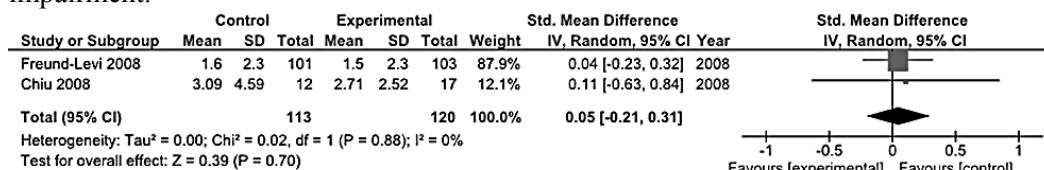


Figure 9. Forest plot showing individual and combined effect size estimates and 95% CIs for 2 trials conducted on patients with schizophrenia.

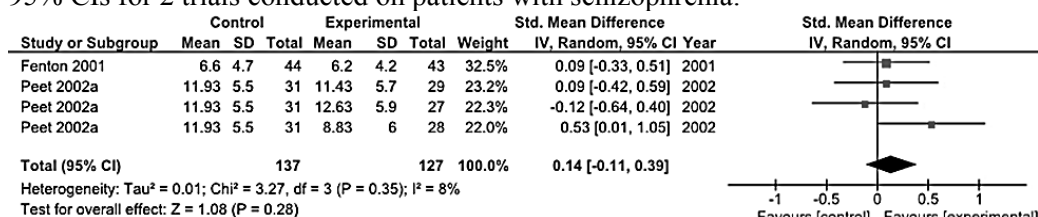


Figure 10. Forest plot showing individual and combined effect size estimates and 95% CIs for 3 trials conducted on patients with cardiovascular disease.

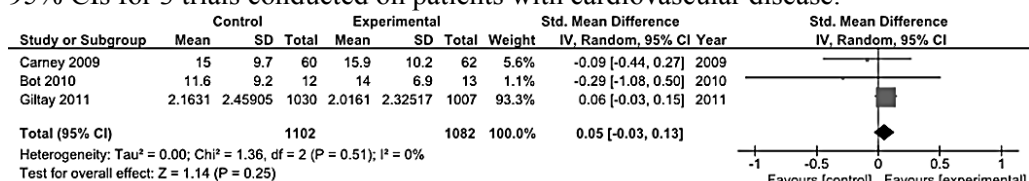


Figure 11. Forest plot showing individual and combined effect size estimates and 95% CIs for 6 trials conducted on healthy individuals for prevention of depressive symptoms.

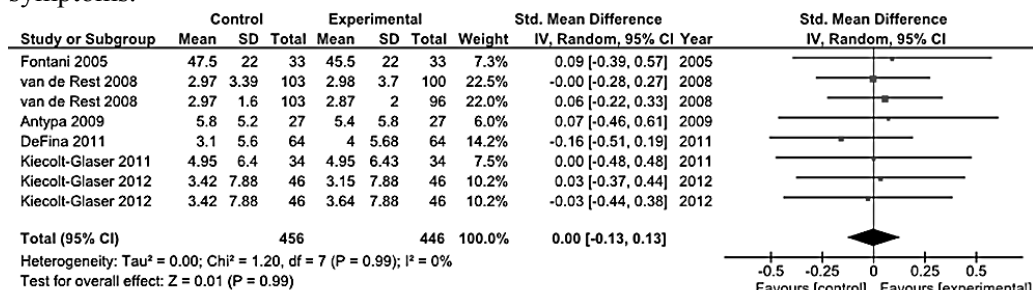


Table 1. Randomized controlled trials investigating effects of omega-3 polyunsaturated fatty acids (PUFAs) on depressed mood listed in chronological order by type of depressive disorder.

Author	Year	Participating Group	Subjects, n (I/C)	Type of treatment	Intervention	Placebo	Daily dose	Duration (weeks)	Outcome measure	Study quality
<i>MDD</i>										
Nemets [76]	2002	Pz with MDD	20 (10/10)	All but 1 used antidepressant	E-EPA	NR	2 g	4	HDRS	8
Marangell [77]	2003	Pz with MDD	36 (18/18)	None	DHA	NR	2 g	6	MADRS HDRS	7
Su [78]	2003	Pz with MDD	28 (14/14)	Mixed antidepressants	EPA + DHA	Olive oil ethyl esters	4.4 g EPA + 2.2 g DHA	8	HDRS	8
Grenyer [46]	2007	Pz with MDD	83 (40/43)	Mixed antidepressants	EPA + DHA	Olive oil	0.6 g EPA + 2.2 g DHA	16	BDI, HDRS	9
Jazayeri [48]	2008	Pz with MDD	60 (20/20/20)	Fluoxetine	E-EPA, E-EPA + fluoxetine	Rapeseed oil	1.0 g E-EPA	8	HDRS	10
Mischoulon [83]	2009	Pz with MDD	57 (28/29)	Psychotherapy	EPA (+0.2% dl-alpha-tocopherol)	Paraffin oil and 0.2% dl-alpha-tocopherol	1 g EPA	8	HDRS -17	11
Rondanelli [95]	2010	Pz with MDD (only women >66)	46 (22/24)	None	EPA + DHA	Paraffin oil	1.67 g EPA + 0.83DHA	8	GDS, SF-36	10
Rondanelli [53]	2011	Pz with MDD (only women >66)	46 (22/24)	None	EPA + DHA	Paraffin oil	1.67 g EPA + 0.83DHA	8	GDS, SF-36	10
Gertsik [84]	2012	Pz with MDD	42 (21/21)	Citalopram	EPA + DHA	Olive oil	0.9 g EPA + 0.2 g DHA	8	HDRS, BDI, MADRS, CGI	7

Rizzo [96]	2012	Pz with MDD (only women >66)	46 (22/24)	NR	EPA + DHA	Paraffin oil, 2.5 g of n-3 PUFA with EPA/DHA 2:1	8	GDS	8
<i>Non-MDD</i>									
Behan [39]	1990	Pz with post viral fatigue	63 (39/24)	NR	EPA + DHA	Liquid paraffin + 0.4 g LA	13	4-point Linkert scale	8
Warren [56]	1999	Pz with chronic fatigue syndrome	50 (24/26)	None	EPA + DHA	Sunflower oil	13	BDI	8
Peet [54]	2002	Pz treated for depression	70 (17/18/17/18)	Mixed antidepressants	E-EPA	Liquid paraffin	12	HDRS, MADRS, BDI	7
Zanarini [91]	2003	Pz with borderline personality disorder	30 (20/10)	Heterogeneous	E-EPA	Mineral oil	8	MADRS	6
Fux [57]	2004	Pz with obsessive compulsive disorder	11 (11/11)	Heterogeneous (within-subjects crossover design)	E-EPA	Liquid paraffin	6	HDRS	6
Silvers [79]	2005	Pz treated for depression	77 (40/37)	Mixed antidepressants	EPA + DHA	Olive oil	12	HDRS-SF, BDI	10
Hallahan [49]	2007	Pz with recurrent self-harm	49 (22/27)	Mixed antidepressants	EPA + DHA	Corn oil + 1% n23 PUFAs	12	BDI, HDRS	9
Rogers [86]	2008	Untreated pz with mild-to-moderate depression	218 (109/109)	None	EPA + DHA	Olive oil	12	DASS, BDI, GHQ, Mood Diary	12

Lucas [70]	2009	Pz with psychological distress	120 (59/61)	None	EPA +DHA (ethyl esters)	Sunflower oil	1.05 g EPA. 0.15 g DHA	8	PGWB, HDRS, CGI, HSCL-D-20	12
Tajalizadekhsob [97]	2011	Pz with mild-to-moderate depression (>66 yrs)	66 (33/33)	55 mixed antidepressants, 11 none	EPA + DHA	Coconut oil	0.180 g EPA + 0.120 g DHA	24	GDS-15	10
Antypa [89]	2012	Pz with a history of at least one major depressive episode	71 (36/35)	7 mixed antidepressants, 6 heterogeneous, 58 none	EPA + DHA	Olive oil	1.74 g EPA + 0.25g DHA	4	BDI-II	9
Mozaffari-Khosravi [69]	2013	Pz with mild-to-moderate depression	81 (27/27/27)	Mixed antidepressants	EPA or DHA	Coconut oil	1 g EPA or 1 g DHA	12	HDRS-17	12
Sohrabi [40]	2013	Pz with pre-menstrual syndrome	139 (70/69)	113 sedative, 26 none	EPA + DHA	NR	0.24 EPA +0.36 DHA	12	VAS	8
<i>Bipolar disorder</i>										
Stoll [75]	1999	Pz with bipolar disorder	30 (14/16)	Heterogeneous	EPA + DHA	Olive oil	6.2 g EPA + 3.4 g DHA	16	HDRS	8
Hirashima [58]	2004	Pz with bipolar disorder	21 (12/9)	Heterogeneous	EPA + DHA	NR	5-5.2 g EPA + 3-3.4 g DHA or 1.3 g EPA + 0.7 g DHA	4	HDRS	4
Chiu [63]	2005	Pz with bipolar disorder	15 (NR)	Lorazepam, valproate	EPA + DHA	Olive oil	0.44 g EPA + 0.24 g DHA	4	HDRS	5
Frangou [65]	2006	Pz with bipolar disorder	75 (24/25/26)	Heterogeneous	E-EPA	Paraffin oil	1 g; 2 g	12	HDRS	9
Keek [59]	2006	Pz with bipolar disorder	116 (59/57)	Mood stabilizing	E-EPA	Liquid paraffin	6 g	17	IDS-C	9

Frangou [80]	2007	Pz with bipolar disorder	14 (7/7)	Lithium	E-EPA	Liquid paraffin	2 g E-EPA	12	HDRS	8
<i>Depression or bipolar disorder in children and adolescents</i>										
Nemets [98]	2006	Children with MDD	28 (13/15)	5 Methylphenidate	EPA+DHA	Olive oil or safflower oil	0.38–0.40 g EPA, 0.18–0.20 g DHA	16	CDRS, CDI, CGI	7
Gracious [61]	2010	Children and adolescents with bipolar disorder	51 (NR)	lithium, atypical antipsychotic	α -LNA	Olive oil	0.55–6.6 α -LNA	16	CDRS-R, CPRS, CGI-BP	11
Amminger [92]	2010	Adolescents at risk of psychosis	81 (41/40)	Heterogeneous	EPA+DHA	Coconut oil	0.70 g EPA + 0.48 g DHA	48	MADRS, SCID	11
<i>Perinatal MDD</i>										
Freeman [99]	2008	Pz with MDD during pregnancy	59 (31/28)	Psychotherapy	EPA + DHA	Corn oil + 1% fish oil	1.1 g EPA + 0.8 g DHA	8	EPDS, HDRS, CGI	8
Su [82]	2008	Pz with MDD during pregnancy	36 (18/18)	None	EPA+DHA	Olive oil ethyl esters	2.2 g EPA 1.2 g DHA	8	HDRS, EPDS, BDI-21	10
Rees [93]	2008	Pz with MDD during pregnancy	26 (13/13)	None	EPA+DHA	Sunola oil	0.42 g EPA, 1.64 g DHA	6	EPDS, HDRS, MADRS	11
<i>Prevention of post-partum depression</i>										
Llorente [85]	2003	Healthy pregnant women	99 (44/45)	None	DHA	NR	0.2 g	16	BDI	10
Doombos [51]	2009	Healthy pregnant women	119 (42/41/36)	Unclear	DHA DHA+AA	Soybean oil	0.22 g DHA, 0.22 g DHA, 0.22 g AA	28	EPDS (Dutch), PPBQ	5
Mozurkewich [68]	2013	Healthy pregnant women	126	Unclear	EPA + DHA	soy oil	1.06 g EPA + 0.27 DHA or		BDI	12

Depressive symptoms in pz with Alzheimer disease or mild cognitive impairment

Chiu [81]	2008	Pz with Alzheimer disease or mild cognitive impairment	46 (24/22)	Unclear	EPA + DHA	Olive oil ethyl esters	1.08 g EPA + 0.72 g DHA	24	MMSE, HDRS	9
Freund-Levi [50]	2008	Pz with Alzheimer disease	204 (103/101)	Various	EPA+DHA	Corn oil + 0.6 g LA	0.6 g EPA, 1.72 g DHA	26	MADRS, NPI	8
Sinn [60]	2012	Pz with mild cognitive impairment (> 65)	50 (17/18/15)	Unclear	EPA+DHA	LA 2,2 g	1.67 g EPA + 0.16 g DHA or 1.55 g DHA + 0.40 g EPA	24	GDS	9
<i>Depressive symptoms in pz with schizophrenia</i>										
Fenton [90]	2001	Pz with schizophrenia	87 (43/44)	All but 1 used neuroleptic	E-EPA	Mineral oil	3 g	16	MADRS	10
Peet [64]	2002	Pz with schizophrenia	115 (29/28/27/31)	31 clozapine, 48 atypical antipsychotics, 36 typical psychotic	E-EPA	Liquid paraffin	1 g; 2 g; 4 g	12	MADRS	9
<i>MDD in pz with Parkinson's disease</i>										
Da Silva [47]	2008	Pz with Parkinson's disease and MDD	29 [NAD: 13 (6/7) AD: 16 (8/8)]	26 levodopa, 19 pramipexol, 5 amantadine, 4 COMT inhibitors, 6 SSRI, 4 tricyclics, 2 trazodone	EPA + DHA	Mineral oil	0.72 g EPA, 0.48 g DHA	12	MADRS, BDI, CGI	10

Depressive symptoms in pz with CVD

Carney [87]	2009	Pz with coronary heart disease and MDD	122 (62/60)	sertraline 50 mg/day	EPA + DHA	Corn oil	0.93 g EPA; 0.75 g DHA	10	BDI-II, HDRS-17	10
Bot [94]	2010	Pz with diabetes mellitus and MDD	25 (13/12)	antidepressant medication	EPA	Rapeseed oil and medium chain triglycerides	1 g	12	MADRS	12
Giltay [100]	2011	Pz post myocardial infarction	4116	antidepressant medication	EPA + DHA		0.4 EPA-DHA/d. 2 ALA/d. 0.4 EPA-DHA + 2 ALA	160	GDS, LOT-R	10
Bot [62]	2011	Pz with diabetes mellitus and MDD	25 (13/12)	antidepressant medication	EPA	Rapeseed oil and medium chain triglycerides	1 g	12	MADRS	10
Andreeva [37]	2012	Pz CVD survivors	2501 (620/633/622/626)	Antidepressant used by 130 (63/67)	B vitamins and n3 fatty acids (EPA + DHA), n3 fatty acids, B vitamins	B vitamins	600 mg EPA and DHA in a 2:1 ratio	52	GDS	9
<i>Depressive symptoms in healthy subjects</i>										
Fontani [52]	2005	Healthy subjects	33 (cross-over design)	None	EPA + DHA	Olive oil	1.60 g EPA + 0.80 g DHA + 0.40 g other omega-3 fatty acids	5	POMS	7

Van de Rest [68] 2008 Healthy subjects 302 (96/100/106) Unclear EPA + DHA Sunflower oil High: 1,093 g EPA, 0.847 g DHA; Low: 0.226 g EPA, 0.176 g DHA CES-D, MADRS, GDS-15 26 12

Antypa [88]	2009	Healthy subjects	(56;>27/ >27)	None	EPA + DHA	Olive oil	1.74 g EPA, 0.25 g DHA	4	MINI, BDI-II, POMS, LEIDS-R	9
Kiecolt-Glaser [102]	2011	Healthy subjects	68 (34/34)	None	EPA + DHA	Palm, olive, soy, canola, and coco butter oils	2.085 g EPA 0.348 g DHA	12	CES-D	11
DeFina [101]	2011	Healthy subjects (overweight)	128 (64/64)	None	EPA + DHA	Soybean and corn oils	3.0 g EPA and DHA in a 5:1 ratio (5 g EPA 1 g DHA)	24	POMS	8
Kiecolt-Glaser [66]	2012	Healthy subjects (overweight)	138 (46/46/46)	None	EPA + DHA	Palm, olive, soy, canola, and coco butter oils	2.09 g EPA + 0.35 g DHA; n3 1.25 g middle group	16	CES-D	13

AD: anti-depression; BDI: Beck Depression Inventory; CES-D: Center for Epidemiological Studies Depression Scale; CDRS: Children Depression Rating Scale; CPRS: Comprehensive Psychopathological Rating Scale; GDS: Geriatric Depression Scale; CGI: Clinical Global Impression; CGI-BP: Clinical Global Impression Bipolar; DHA: docosahexaenoic acid; E-EPA: e-icosapentaenoic acid; EPDS: Edinburgh Postnatal Depression Scale; HDRS: Hamilton Depression Rating Scale; I/C: intervention/control; IDS-C: Inventory of Depressive Symptomatology Clinician; LEIDS-R: Leiden Index of Depression Severity Revised; LOT-R: Revised Life Orientation Test; MADRS: Montgomery Åsberg Depression Rating Scale; MINI: Mini International Neuropsychiatric Interview; MMSE: Mini-Mental State Evaluation; NAD: non anti-depression; NPI: Neuropsychiatric Inventory; POMS: Profile of Mood States; PPBQ: Papolos Pediatric Bipolar Questionnaire; SCID: Structural Clinical Interview for Depression; VAS: Visual Analog Scale.

CHAPTER 2

Dietary omega-3 PUFA consumption and depression: a systematic review of observational studies.

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Abstract

Aims. To systematically review existing observational epidemiological studies exploring the potential association between fish and/or omega-3 polyunsaturated fatty acids (PUFA) dietary consumption and prevalence or incidence of depression.

Methods. A comprehensive search on MEDLINE, EMBASE, PsycInfo, and the Cochrane Database of Systematic Reviews of all observational studies evaluating the effects of omega-3 PUFA on depression in cohort of individuals published up to August 2014 was performed.

Results. A total of 28 studies, including 251,464 individuals and over 20,000 cases of depression, were examined. Among the 18 studies exploring the possible association between fish consumption and depression, 5 out of 7 cross-sectional and 6 out of 11 prospective studies reported a significant relation. A protective effect of omega-3 PUFA intake on depression was reported in 7 out of 11 cross-sectional and 4 of 9 prospective studies. Possible explanation for heterogeneity in results and limitations of the studies included were discussed.

Conclusions. The present analysis did not support or confute the hypothesis that dietary omega-3 PUFA decrease the risk to develop depression due to significant contrasting findings among studies. Future researches should into account the methodological limitations retrieved, such as i) better assessment of depressive cases, ii) detailed consumption of all PUFA and their possible interactions, and iii) consider the possibility of a non-linear relationship

between fish or omega-3 PUFA intake and the risk to develop depressive disorders.

Introduction

Over the last four decades, polyunsaturated fatty acids (PUFA) have been studied in relation to prevention and treatment of cardiovascular diseases (CVD) (Sanchez-Villegas and Martinez-Gonzalez, 2013). The highest representative compounds consumed by humans belong to the omega-3 and omega-6 family of PUFA and are represented by alpha-linolenic acid (ALA) and linoleic acid (LA), respectively. Once consumed in the diet, LA undergoes to transformation in arachidonic acid (AA), which is precursor of pro-inflammatory cytokines, whereas ALA is converted in eicosapentaenoic acid (EPA), with a subsequent elongation to docosahexaenoic acid (DHA), which is endowed with anti-inflammatory and neuroprotective effects (Grosso et al., 2014a). EPA and DHA may also be consumed directly through the diet as highly contained in fishes and seafoods. Since their beneficial effect is supposed to depend on their capacity to positively modulate the immune and inflammatory response, omega-3 PUFA have been recently studied as a new treatment for disorders with an inflammatory component (Parletta et al., 2013). An emerging body of data indicates efficacy of omega-3 PUFA intake also in psychiatric illnesses that may involve inflammation, such as depressive disorder (Grosso et al., 2014a). Ecological studies suggest that dietary intakes of fish, high in omega-3 PUFA content, correlate with major depression (Hibbeln, 1998), bipolar disorder (Noaghiul and Hibbeln, 2003), and postpartum depression (Hibbeln, 2002). There is evidence that depressed patients had decreased content of omega-3 PUFA in plasma and erythrocyte membrane, supporting the mechanistic links between omega-3 PUFA deficiency and increased risk to develop depressive disorders (Edwards et al., 1998, Peet et al., 1998, De Vriese et al., 2003). Altogether, these data suggested that cross-national variations in omega-3 PUFA intake were inversely correlated with the prevalence of depression, despite a causal relation could not be demonstrated.

The potential benefits of omega-3 PUFA intake in preventing or treating psychiatric illnesses, such as depressive disorders, are biologically plausible. Major depression has been demonstrated to share certain pathophysiological mechanisms with CVD, such as increased production of pro-inflammatory cytokines, endothelial dysfunction, and elevations in plasma homocysteine levels (Hepgul et al., 2010). Several studies have shown a positive correlation

between the severity of the symptoms of depression and the increase in the inflammatory status (Maes et al. 2009). Major depression has been recently considered not only as a central nervous system disease, rather as a systemic inflammatory disorder in which pro-inflammatory cytokines alter serotonin metabolism, reduce synaptic plasticity, and increase the risk to develop CVD (Maes et al., 2009, Caraci et al., 2010, Huffman et al., 2013).

According to this scenario, the anti-inflammatory capacity of omega-3 PUFA has been hypothesized to play a central role in counteracting inflammatory processes occurring in depression (Parletta et al., 2013). Another possible mechanism may be connected with their neuro-endocrine effects on metabolism, release, uptake, and receptor function (Hibbeln et al., 1998). Their positive effects on depression may also depend on their physiological abundant content in the human nervous system and their involvement in neurogenesis and neuroplasticity (Bourre, 2004).

A number of cross-sectional and prospective investigations have been performed in order to better identify the extent of the potential protective role of omega-3 PUFA consumption in preventing depression. However, up to now, results presented in literature are contrasting (Giles et al., 2013). The use of omega-3 PUFA supplements as add-on therapy for major depression (in addition to first and second generation antidepressant drugs) appears promising (Grosso et al., 2014b) but observational studies analyzing the association of exposure to disease in free-living populations did not yield to strong conclusions on the potential preventive role of omega-3 PUFA. More important, it is still unclear whether a biological response of omega-3 PUFA can be obtained in a dose-dependent manner with clinically relevant effects or, alternatively, if overcoming a certain cut-off dietary intake might be considered the minimum for dietary recommendations planned to prevent the occurrence of depressive disorders.

The aim of this study was to systematically review existing observational epidemiological studies exploring the potential association between fish and/or omega-3 PUFA dietary consumption and prevalence or incidence of depression.

Methods

Search strategy and selection of the studies

A comprehensive search on MEDLINE, EMBASE, PsycInfo, and the

Cochrane Database of Systematic Reviews of all observational studies evaluating the effects of omega-3 PUFA on depression in cohort of individuals published up to August 2014 was performed. Articles of potential interest were identified by using the following search terms: “omega-3”, “polyunsaturated fatty acids”, “PUFA”, “EPA”, “DHA”, combined with the following terms: “depression”, “depressive disorder”, “depressed mood”, combined with “cohort”, “prospective”, and “cross-sectional”. Studies were considered only when reported the analyses on fish or total omega-3 PUFA, or EPA, DHA, or ALA separately. Exclusion criteria were the following: i) studies with different design, such as ecological approach, case-control studies or randomized controlled trials; ii) studies conducted on individuals with depression as secondary disease. No other restrictions were placed on the duration of follow-up or dietary assessment method. Studies that reported patterns of food consumption (such as a Mediterranean-style diet) were included only if analysis regarding fish consumption was reported separately. Only article in English were included.

The process of identification and inclusion of studies is summarized in Figure 1. Among the 231 articles retrieved, observational studies were identified and screened by reading the abstract and, when necessary, the full text, in order to select those articles relevant for the review. The reference list of the relevant reports was also inspected to identify any additional study not previously identified. Of the 54 articles considered potentially relevant, 26 were excluded and not assessed further for the following reasons: 2 reported insufficient statistics or crucial data not presented, 7 studies were conducted on omega-3 PUFA in tissues, 7 studies had different study design (i.e., case-control studies), 10 were conducted on individuals with primary disease other than depression. This process led to a final number of 28 studies included in this systematic review.

Quality assessment of the studies

Assessment of study quality was determined according to the reporting of the study design, method and statistical analysis. Quality assessments considered the duration of follow-up, the validity and content of the dietary assessment method (whether FFQ measured frequency and amount, and specifically asked questions on intake and type of two or more core fish foods), primary conducted to investigate the relation between omega-3 PUFA and/or fish intake and depression, the application of standardized criteria to measure

clinical end points and the severity of depression, the appropriateness of adjustment for confounding factors. We also considered indicative of the quality of the study other features providing important information for the interpretation of data, such as (i) the possibility to identify the mean or median amount of fish or omega-3 PUFA in the categories of exposure, (ii) the evaluation of the ratio omega-6:omega-3 PUFA and its association with the outcome.

Data extraction and synthesis

Data were abstracted from each identified trial by GG and SM using a standard data abstraction form. This process was independently performed by two researchers and discordances were discussed and solved. Qualitative data from each study including author, year of publication, study design, participant characteristics, methodology, results and statistical analysis were extracted and tabulated for comparative analysis.

Results

Summary of studies reviewed

The most relevant characteristics of the studies included in this systematic review are reported in Table 1. Overall, this systematic review comprised 28 studies (Tanskanen et al., 2001, Hakkarainen et al., 2004, Timonen et al., 2004, Barberger-Gateau et al., 2005, Kamphuis et al., 2006, Appleton et al., 2007a, Appleton et al., 2007b, Sanchez-Villegas et al., 2007, Sontrop et al., 2008, Astorg et al., 2008, Murakami et al., 2008, Bountziouka et al., 2009, Colangelo et al., 2009, Kyrozis et al., 2009, Golding et al., 2009, Strom et al., 2009, Murakami et al., 2010, Suominen-Taipale et al., 2010, Li et al., 2011, Lucas et al., 2011, Oddy et al., 2011, Kesse-Guyot et al., 2012, Albanese et al., 2012, Beydoun et al., 2013, Daley et al., 2014, Miyake et al., 2006, da Rocha and Kac, 2012, Jacka et al., 2013) including 251,464 individuals and over 20,000 cases of depression. Eight studies explored the effects of both fish and omega-3 PUFA dietary consumption on depression (Hakkarainen et al., 2004, Astorg et al., 2008, Colangelo et al., 2009, Murakami et al., 2010, Lucas et al., 2011, Sontrop et al., 2008, Strom et al., 2009, Miyake et al., 2006), 10 were focused only on fish consumption (Tanskanen et al., 2001, Timonen et al., 2004, Barberger-Gateau et al., 2005, Sanchez-Villegas et al., 2007, Bountziouka et al., 2009, Kyrozis et al., 2009, Suominen-Taipale et al.,

2010, Li et al., 2011, Albanese et al., 2012, Appleton et al., 2007b), and 7 considered the potential association only with omega-3 PUFA intakes (Kamphuis et al., 2006, Murakami et al., 2008, Oddy et al., 2011, Kesse-Guyot et al., 2012, Beydoun et al., 2013, Golding et al., 2009, Daley et al., 2014, Appleton et al., 2007a, Jacka et al., 2013). Among them, 5 studies explored also the role of the ratio omega-6:omega-3 on depression (Lucas et al., 2011, Beydoun et al., 2013, Kesse-Guyot et al., 2012, Miyake et al., 2006, da Rocha and Kac, 2012). Thirteen studies (Tanskanen et al., 2001, Barberger-Gateau et al., 2005, Kamphuis et al., 2006, Murakami et al., 2008, Bountziouka et al., 2009, Murakami et al., 2010, Suominen-Taipale et al., 2010, Albanese et al., 2012, Beydoun et al., 2013, Sontrop et al., 2008, Golding et al., 2009, Daley et al., 2014, Appleton et al., 2007a) adopted a cross-sectional design, 13 were prospective studies (Hakkarainen et al., 2004, Timonen et al., 2004, Sanchez-Villegas et al., 2007, Astorg et al., 2008, Colangelo et al., 2009, Kyroziis et al., 2009, Li et al., 2011, Lucas et al., 2011, Strom et al., 2009, Appleton et al., 2007b, da Rocha and Kac, 2012, Miyake et al., 2006, Jacka et al., 2013), and 2 presented data with both designs (Oddy et al., 2011, Kesse-Guyot et al., 2012). The duration of follow-up for the prospective studies ranged from 2 to 30 years. Overall, on an 8-point scale, 7 studies (Hakkarainen et al., 2004, Astorg et al., 2008, Lucas et al., 2011, Beydoun et al., 2013, Kesse-Guyot et al., 2012, Strom et al., 2009, Miyake et al., 2006) scored better (≥ 5) than the others.

Participant characteristics and the methods for assessing consumption and classification of omega-3 consumption and foods varied between studies. Most of the studies were conducted on general population (Tanskanen et al., 2001, Hakkarainen et al., 2004, Timonen et al., 2004, Appleton et al., 2007a, Appleton et al., 2007b, Sanchez-Villegas et al., 2007, Astorg et al., 2008, Colangelo et al., 2009, Suominen-Taipale et al., 2010, Li et al., 2011, Kesse-Guyot et al., 2012, Beydoun et al., 2013), 2 on community dwellers (Barberger-Gateau et al., 2005, Albanese et al., 2012), 3 on health professionals or employee (Murakami et al., 2008, Suominen-Taipale et al., 2010, Lucas et al., 2011), 5 on women during or after pregnancy (Sontrop et al., 2008, Golding et al., 2009, Strom et al., 2009, Miyake et al., 2006, da Rocha and Kac, 2012), 2 on adolescents (Murakami et al., 2010, Oddy et al., 2011), and 3 on elderly (60+ years) subjects (Kamphuis et al., 2006, Bountziouka et al., 2009, Kyroziis et al., 2009). Diet assessment was measured with a validated food frequency questionnaire (FFQ) in almost all

studies, with the exception of 3 studies (Astorg et al., 2008, Kesse-Guyot et al., 2012, Beydoun et al., 2013) that used 24-h recalls, and one study (Albanese et al., 2012) using specific assessment questions. The majority of studies separated individuals into categories of exposure (quantiles) based on daily amount of fish consumed or omega-3 intake whereas 7 studies (Appleton et al., 2007a, Appleton et al., 2007b, Bountziouka et al., 2009, Kyrozis et al., 2009, Lucas et al., 2011, Oddy et al., 2011, Daley et al., 2014) considered fish and omega-3 PUFA intakes as continuous variables. Most of the studies identified cases of depression according the cut-off points of the scores respectively used, 5 accounting clinical diagnosis, hospitalization, or medicament prescription (Hakkarainen et al., 2004, Timonen et al., 2004, Colangelo et al., 2009, Lucas et al., 2011, Jacka et al., 2013), and 6 used values of the score as a continuous variable for the analyses (Appleton et al., 2007a, Appleton et al., 2007b, Bountziouka et al., 2009, Kyrozis et al., 2009, Lucas et al., 2011, Oddy et al., 2011).

Association between fish and omega-3 consumption and depression

The characteristics associated with fish and omega-3 PUFA consumption and depression are listed in Table 2. The amount of dietary omega-3 PUFA and fish intake varied with a great extent across studies, with a general higher consumption in all studies conducted in Japan (Murakami et al., 2010, Murakami et al., 2008, Miyake et al., 2006), reporting almost 2-3 g/d or 2% of energy for omega-3 PUFA and 70 g/d of fish intakes in the highest quantiles of consumption. In contrast, the lowest intakes among the highest quantiles of consumption were reported in studies conducted in Germany (Kamphuis et al., 2006) and England (Golding et al., 2009).

All studies adjusted analysis for confounders, but number and type of potential confounders used for statistical adjustment varied between studies, with most of them including age, gender (when both sexes were included), smoking, and education. Among other confounders, 10 studies adjusted for occupational, social or poverty-income status (Tanskanen et al., 2001, Lucas et al., 2011, Li et al., 2011, Beydoun et al., 2013, Daley et al., 2014, Sontrop et al., 2008, Golding et al., 2009, Strom et al., 2009, Oddy et al., 2011, Miyake et al., 2006) and 5 studies adjusted for overall dietary fats (Tanskanen et al., 2001, Hakkarainen et al., 2004, Li et al., 2011, Lucas et al., 2011, Kesse-Guyot et al., 2012).

Among the 18 studies exploring the possible association between fish consumption and depression, 5 (Tanskanen et al., 2001, Barberger-Gateau et al., 2005, Bountziouka et al., 2009, Murakami et al., 2010, Suominen-Taipale et al., 2010) out of 7 cross-sectional and 6 (Timonen et al., 2004, Astorg et al., 2008, Kyroziis et al., 2009, Li et al., 2011, Strom et al., 2009, Miyake et al., 2006) out of 11 prospective studies reported a significant relation. Together, these studies involved 107,098 men and women of various ages, followed in prospective studies up to 13 years. Studies demonstrating association between fish consumption and depression accounted to different categories of exposure. Overall, the amount of fish associated with decreased odds or risk of depression was about 80 g/d and over, despite similar amount was not significantly associated with decreased risk of post-partum depression (Miyake et al., 2006). Two studies conducted in Greece (Bountziouka et al., 2009, Kyroziis et al., 2009) and one in Northern Ireland (Appleton et al., 2007b) reported a linear association between fish intake and scores of depression. There was no specific association between the results and methodology used or any other study characteristic.

A protective effect of omega-3 PUFA intake on depression was reported in 7 (Kamphuis et al., 2006, Murakami et al., 2008, Golding et al., 2009, Murakami et al., 2010, Kesse-Guyot et al., 2012, Beydoun et al., 2013, Daley et al., 2014) out of 11 cross-sectional and 4 (Sanchez-Villegas et al., 2007, Colangelo et al., 2009, Lucas et al., 2011, Jacka et al., 2013) of 9 prospective studies. The studies by Murakami et al. (Murakami et al., 2008) and Lucas et al (Lucas et al., 2011) reported a significant association between depression score and ALA, but not all omega-3 PUFA, whereas the study of Jacka et al (Jacka et al., 2013) reported an association with EPA intake, but not DHA. Besides the study of Lucas et al (Lucas et al., 2011), all other articles exploring a linear association between intake of omega-3 PUFA and scores of depression showed no significant results. Overall, the amount of omega-3 PUFA estimated across studies varied to a great extent and results of studies seem to partially reflect the categories of exposure chosen. Specifically, studies comparing the effect of extreme omega-3 PUFA intakes, for instance, 10 mg/d vs. 100 mg/d (Sontrop et al., 2008, Strom et al., 2009) or 350 mg/d vs. up to 4000 mg/d (Murakami et al., 2008, Miyake et al., 2006) reported inconclusive results, whereas other studies comparing amount of consumption of omega-3 PUFA between which it would be reasonable to

appreciate a difference, for instance, up to 350 mg/d vs. more than 600 mg/d (Kamphuis et al., 2006, Murakami et al., 2010, Kesse-Guyot et al., 2012, Beydoun et al., 2013, Jacka et al., 2013) showed significant results. Out the 5 investigations evaluating the possible relation between the ratio omega-6:omega-3 and depression, 3 studies (Lucas et al., 2011, Beydoun et al., 2013, da Rocha and Kac, 2012) reported a significant relationship.

Among the studies reporting specific results by gender, a greater association between fish or omega-3 PUFA intake and depressive symptoms in women or men cannot be retrieved, since 3 studies showed a significant effect on men but not in women (Murakami et al., 2010, Murakami et al., 2008, Li et al., 2011) whereas 3 reported significant results only for women (Colangelo et al., 2009, Timonen et al., 2004, Beydoun et al., 2013).

Discussion

This systematic review showed no consistent findings to support or discourage the hypothesis that dietary fish or omega-3 PUFA intake is associated with decreased risk of depression. To date, this is the first systematic review pooling together all relevant epidemiological studies assessing the relation between fish, omega-3 PUFA intake and depression. The evidence extracted from cross-sectional studies consistently suggested a possible relationship between fish, omega-3 PUFA consumption and depression, but findings retrieved by prospective cohort studies are contrasting and often inconclusive. Considering that studies exploring the relation of fish consumption and depression were more likely to have significant results than those analyzing omega-3 PUFA intakes, it has been suggested that fish consumption could be a proxy variable for a healthier lifestyle, which in turn may lead to lower risk of depression (Kesse-Guyot et al., 2012, Appleton et al., 2007a, Appleton et al., 2007b, Strom et al., 2009). Despite we cannot retrieve any definitive conclusions by the review of these studies, several reasons that may account for such inconsistency should be discussed, suggesting new strategies to be considered and taken into account for future research.

First, the definition of the outcome of interest and the methodology used to ascertain it may bias the results. Omega-3 PUFA have been suggested to protect by major depression through several possible pathways, such as anti-inflammatory effects (Hibbeln and Salem, 1995), neuro-endocrine

modulation (Hibbeln et al., 1998), and neuroprotective/neurotrophic mechanisms (Chang et al., 2009). Whichever is the mechanism of action, it has been suggested that their effects may be clinically relevant specifically acting on the pathophysiological mechanisms involved in major depression, whereas omega-3 PUFA cannot be viewed as mood modulating agents useful in all those individuals with depressive symptoms but without an established diagnosis of major depression. Psychometric tools used in most of the observational studies cannot diagnose cases of major depression, rather may identify cases of depressive status that can be biased by cases of depressed mood, emotional or anxious distress, for which omega-3 PUFA are not supposed to be equally effective. Indeed, it has been recently reported that omega-3 PUFA supplement demonstrates antidepressant effects especially in patients with DSM-defined major depressive disorder rather than in individuals with only non-clinically relevant depressive symptoms (Lin et al., 2012). We recently demonstrated in a meta-analysis of clinical randomized trials that omega-3 PUFA supplement exert a beneficial effect in patients affected by depression, but results were more convincing in those studies conducted on patients with an established diagnosis of major depression based on clinical evaluation, weaker in patients without a clinical diagnosis, and inconsistent in randomly recruited individuals among the general population (Grosso et al., 2014b). Among epidemiological studies included in this systematic review, only few (Hakkarainen et al., 2004, Timonen et al., 2004, Colangelo et al., 2009, Lucas et al., 2011, Jacka et al., 2013) reported diagnosis of depression as hospitalization or medication prescription, still with contrasting results. Nevertheless, this issue may explain, at least in part, the complex variability in the results obtained in cohort studies.

A second limitation raised by this systematic review was the lack of proper adjustment for potential confounders. While randomized controlled trials have the benefit to accurately select individuals, virtually countering potential confounding factors, observational studies conducted on general population must take into account the possibility that background characteristics have a role in the final associations between the variables of interest. In light of this observation, lack of adjustment for certain variables such as socio-economic status, which is a powerful predictor of morbidity and mortality, has been suggested to be a significant limitation of most of the studies included in this systematic review. Particular attention on variables that may show interaction or influence associations between variables of interest should be taken into

account, as incomplete control may result in biased effect measures due to residual confounding.

Another criticism might be related to the psychometric tools used to assess the outcome (namely the depressive status) when they have been used as continuous variables to analyze the correlation with the dietary intakes of fish or omega-3 PUFA. All the tools used in the observational studies included in this systematic review have been extensively validated, but these scales can only suggest the presence of depression when a cut-off point has been reached, whereas a deep clinical evaluation is needed for an established diagnosis of major depression. Furthermore, a cut-off point of the scale may indicate the presence of depression, but it does not necessarily mean that different scores accounted in the scale correspond to different clinical conditions. This issue rises up in those studies attempting to demonstrate a linear dose-response association between fish or omega-3 PUFA consumption and the scale used to measure the depression, which reported mostly entirely negative results (Lucas et al., 2011, Kyrozis et al., 2009, Appleton et al., 2007a). Moreover, this analytic approach may elicit doubts also regarding the dietary intake of omega-3 PUFA, since by testing the linear association between omega-3 PUFA or fish consumption and the scale used to measure the depression, authors assume a linear dose-response effect of omega-3 PUFA, which is actually not demonstrated. This may also explain why even the quantile analysis in certain studies failed to demonstrate the association between omega-3 PUFA and depression, since individuals being in the highest category of exposure did not necessarily indicated that they reached an adequate amount of omega-3 PUFA to be effective in preventing the onset of depressive disorders. On the other hand, the non-linearity could indicate that the “low” amount of omega-3 PUFA would become active as a possible etiological factor favoring the onset of depression at a certain low level, as suggested in some studies included in this systematic review (Sanchez-Villegas et al., 2007, Lucas et al., 2011, Jacka et al., 2013, Appleton et al., 2007a). Another recent study conducted to evaluate the possible relation between omega-3 PUFA and post-partum depression revealed that a non-linear curve best described the inverse relationship between the omega-3 index (defined as the content of EPA+DHA in red blood cells membranes expressed as a percent of total fatty acids) in pregnancy and maternal level of depressive symptoms three months postpartum (Markhus et al., 2013). The curve that best described the

association was a rectangular, hyperbolic relationship, with the kink in the curve at an omega-3 index of about 5%, which was also the 25-percentile level in the study population (Markhus et al., 2013). This non-linear inverse relationship has been previously reported at ecological level by other studies (Hibbeln, 2002, Hibbeln and Salem, 1995). A cross-national ecological examination of both omega-6 and omega-3 PUFA reported that a threshold of omega-3 PUFA consumption of 750 mg/d (0.35% of energy, based on a 2000 kcal/d diet) could be sufficient to protect 98% of the population from the risk to develop depressive disorders (Hibbeln et al., 2006). Considering such threshold, none of the epidemiological studies included in this systematic review reported similar amounts, even among individuals in higher quantiles, with the exception of those conducted in Japan (Murakami et al., 2010, Murakami et al., 2008, Miyake et al., 2006). Moreover, the ecological study by Hibbeln et al (Hibbeln et al., 2006) suggested that healthy omega-3 PUFA intakes must be made dependent on concurrent intakes of omega-6 PUFA, which based on the current per capita background available intake of LA in the United States, reaches a healthy dietary allowance of 3.5 g EPA + DHA/d (Hibbeln et al., 2006). Indeed, omega-6 PUFA have been associated themselves to unhealthy outcomes, such as increased suicide rates in pregnant women (Vaz et al., 2014). Furthermore, dietary omega-6 PUFA lowering has been reported to significantly reduce LA and increase omega-3 PUFA concentrations in plasma, without altering plasma AA concentration (Taha et al., 2014). Considering the extent of influence of omega-6 on omega-3 PUFA intake to detect a protective effect on the most of population, this may explain the substantial inconsistency among the studies included in this review. This hypothesis is supported by the fact that three studies (Lucas et al., 2011, da Rocha and Kac, 2012, Beydoun et al., 2013) examining both omega-6 and omega-3 PUFAs reported a significant protective effect of decreased omega-6:omega-3 ratio, even when depression was not associated with omega-3 PUFA consumption alone (Lucas et al., 2011). Moreover, the study of Lucas et al. (Lucas et al., 2011) pointed out the attention also on the ratio ALA:LA, reporting an inverse association between depression, higher intakes of ALA and low of LA. By contrast, other two studies (Kesse-Guyot et al., 2012, Miyake et al., 2006) evaluating the omega-3:omega-6 PUFA ratio did not lead to similar results, despite possible explanations can be hypothesized. In the study of Kesse-Guyot et al (Kesse-Guyot et al., 2012) the estimated ratio seemed to be much lower than the one reported in the previous studies, as the highest quantile of exposure corresponded to the

lowest reported by Beydoun et al. (Beydoun et al., 2013) and even lower than the one reported by Lucas et al. (Lucas et al., 2011). In contrast, in the study of Miyake et al. (Miyake et al., 2006), the estimated omega-3 PUFA consumption and omega-3:omega-6 ratio in lower quantile was much higher than that reported in the other studies, thus more likely to provide an equal protection across quantiles resulting in no significant differences. Despite the omega-3:omega-6 ratio itself may not be independently predictive of outcome (Harris, 2006), its evaluation may result of major importance to further evaluate, perhaps adjust findings according a more complete estimation of dietary source of PUFA.

In addition to the aforementioned observations, a limitation of this review depends on the methodology of the studies included. Cross-sectional studies do not allow demonstrating a causal relationship between the factors studied because the temporal variable is lacking. As well, prospective studies may suffer by misclassification of exposure, since omega-3 PUFA dietary intake was assumed to be constant over the entire follow-up periods. Finally, omega-3 PUFA estimation methods by FFQs may lead to recall biases in both types of study.

In conclusion, present analysis of available observational studies do not support or confute the hypothesis that dietary omega-3 PUFA decrease the risk to develop depression. Findings are generally split between studies finding beneficial *versus* null effects of omega-3 PUFA consumption on depression, potentially owing to methodological limitations as discussed throughout the article. Despite merely speculative, we suggested several explanations for such inconsistency and addressed some limitations inherent to the methodological approaches that may explain such contrasting results. Further studies are needed to better identify whether this relation is consistent taking into account the methodological limitations retrieved in this systematic reviews, such as: i) a better assessment of depressive cases; ii) the detailed consumption of all PUFA, including ALA, LA, and the omega-6:omega-3 ratio, and their possible interaction; and iii) consider the possibility of a non-linear relationship between fish or omega-3 PUFA intake and the risk to develop depressive disorders.

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Figure 1. Process of studies identification and inclusion.

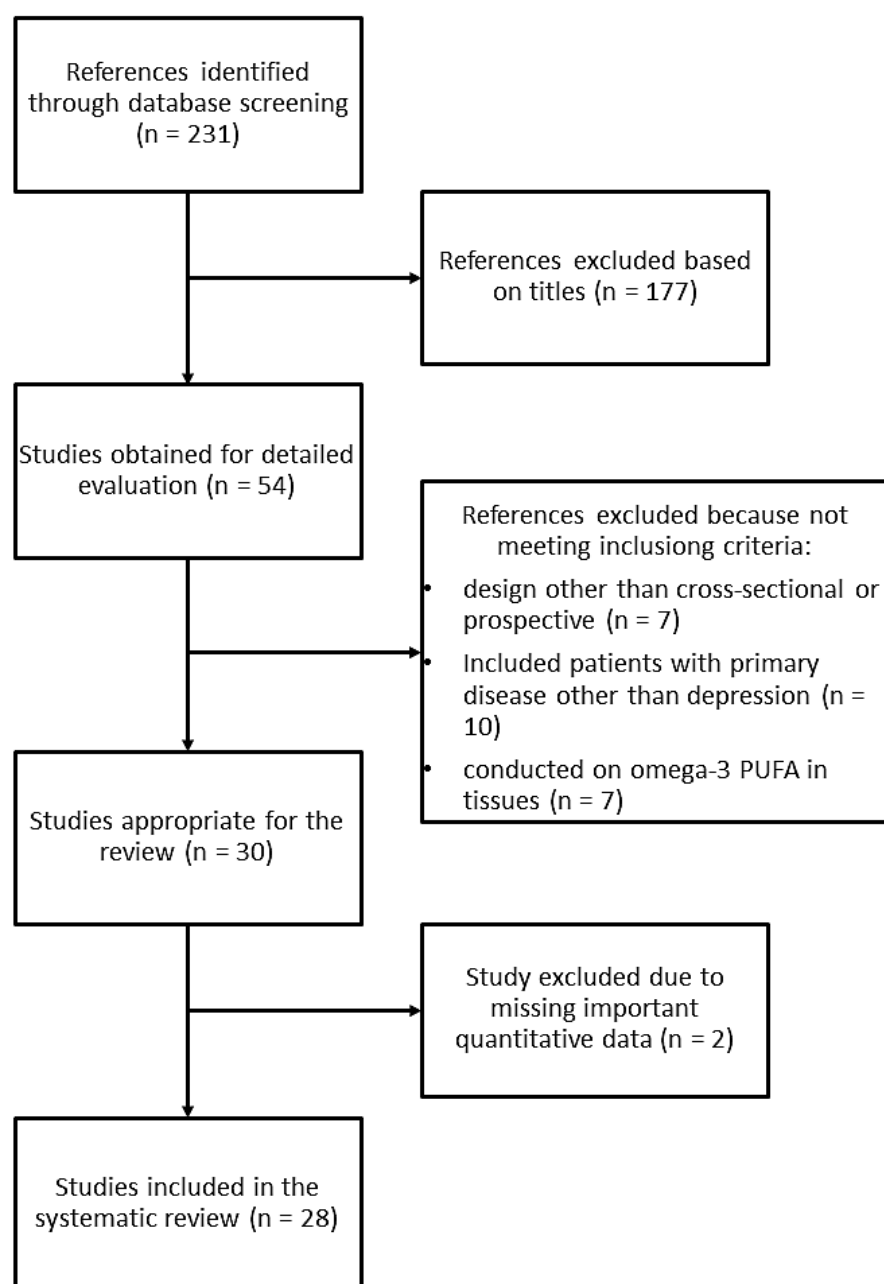


Table 1. Characteristics of the epidemiological studies conducted on fish and/or omega-3 PUFA consumption and depression.

Author, year	Country (cohort)	Study design	Dietary assessment	Depression assessment	Participants	Number of participants	Age (range)	Omega-6:omega-3 ratio	Omega-3	Fish	Follow-up
Tanskanen et al, 2001	Finland	Cross-sectional	FFQ	BDI-Y	General population	3204	25-64	no	no	yes	-
Hakkarainen et al, 2004	Finland (ATBC study)	Prospective	FFQ	Hospital treatment	General population	29,133	50-69	no	yes	yes	5-8 years
Timonen et al, 2004	Finland (Northern Finland 1966 Birth Cohort)	Prospective	FFQ	Hopkins Symptom Check List-25 subscale and diagnosis by medical doctor	General population	5689	up to 31	no	no	yes	from born up to the age of 31 years
Barberger-Gateau et al, 2005	France (Three-City Study)	Cross-sectional	FFQ	CES-D	Community dwellers	9280	64+	no	no	yes	-
Kamphuis et al, 2006	German (Zurphen Elderly Study)	Cross-sectional	FFQ	Self-rating Depression Scale	Elderly general population	332	70-90	no	yes	no	-
Miyake et al, 2006	Japan (Osaka Maternal and Child Health Study)	Prospective	FFQ	Edinburgh Postnatal Depression Scale	Pregnant women	865	<29-32+	yes	yes	yes	2-9 months post-partum
Appleton et al, 2007a	UK	Cross-sectional	FFQ	Depression, Anxiety and Stress Scales (DASS-21)	General population	2982	<25-65+	no	yes	no	-
Appleton et al, 2007b	Northern Ireland and France (PRIME cohort)	Prospective	FFQ	Welsh Pure Depression subscale of the Minnesota Multiphasic	General population (men)	10,602	50-59	no	no	yes	5 years

Sanchez-Villegas et al, 2007	Spain (SUN cohort)	Prospective	FFQ	Self-reported physician diagnosis of depression, anxiety or stress or use of antidepressant medication or tranquilizers	General population	7903	38 (mean age)	no	no	yes	2 years
Astorg et al, 2008	France (SU.VI.MAX)	Prospective	24-h recall	Antidepressant or lithium prescription	General population	3748	35-60	no	yes	yes	2 years
Murakami et al, 2008	Japan	Cross-sectional	FFQ	CES-D	Municipal employees	517	21-67	no	yes	no	-
Sontrop et al, 2008	Canada (Prenatal Health Project)	Cross-sectional	FFQ	CES-D	Pregnant women (10- and 22-week gestation)	2394	<21-35+	no	yes	yes	-
Golding et al, 2009	England (Avon Longitudinal Study of Parents and Children)	Cross-sectional	FFQ	Edinburgh Postnatal Depression Scale (depression=13)	Women (32 weeks' gestation)	14,541	Not specified	no	yes	no	-
Strom et al, 2009	Denmark (Danish National Birth Cohort)	Prospective	FFQ	Post-partum depression hospital admission or medication prescription	Women	54,202	<25-40+	no	yes	yes	1 year post-partum
Bountziouka et al, 2009	Greece and Cyprus (MEDIS study)	Cross-sectional	FFQ	GDS (depression >5)	Elderly general population	1190	65+	no	no	yes	-

Colangelo et al, 2009	US (Coronary Artery Risk Development in Young Adults study [CARDIA])	Prospective	FFQ	CES-D	General population (women)	3317	24-42	no	yes	yes	10 years
Kyrozis et al, 2009	Greece (EPIC-Greece)	Prospective	FFQ	GDS	Elderly general population	610	60+	no	no	yes	6 to 13 years
Murakami et al, 2010	Japan	Cross-sectional	FFQ	CES-D	Adolescents (school students)	6517	12-15	no	yes	yes	-
Suominen-Taipale et al, 2010	Finland (Health 2000 Survey)	Cross-sectional	FFQ	M-CIDI	General population	5492	45-74	no	no	yes	-
	Finland (Fishermen Study)			CIDI-SF	Fishermen with their families	1265					
Li et al, 2011	US (First National Health and Nutrition Examination Survey Follow-up Study)	Prospective	FFQ	CES-D (depression =22)	General population	5068	25-74	no	no	yes	10.6 years
Lucas et al, 2011	US (Nurses' Health Study)	Prospective	FFQ	Physician-diagnosed depression and regular antidepressant medication use	Nurses (women)	54,632	50-77	yes	yes	yes	10 years

Oddy et al, 2011	Australia [Western Australian Pregnancy Cohort (Raine) Study]	Cross-sectional	FFQ	BDI-Y	Adolescents	1407	14	no	yes	no	-
Prospective											
						995	17				3 years
Da Rocha	Brazil	Prospective	FFQ	Edinburgh Post-partum Depression Scale	Pregnant women (between 8th and 13th week of gestation)	106	18-41	yes	no	no	Within 30 days post-partum
Kesse-Guyot et al, 2012	France (SU.VI.MAX) (subsample)	Cross-sectional	24-h recall	CES-D	General population	2744	35-60	yes	yes	no	-
Prospective											
						1235					13 years
Albanese et al, 2012	Multicenter (10/66 research programme)	Cross-sectional	Standardized questions	ICD-10 depressive episode	Community dwellers	14,926	65+	no	no	yes	-
Jacka et al, 2012	Australia (Geelong Osteoporosis Study)	Prospective	FFQ	Structured Clinical Interview for DSM-IV-TR	General population (women)	935	20-94	no	yes	no	10 years
Beydoun et al, 2013	US (Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS))	Cross-sectional	24-h recall	CES-D (depression =16)	General population	1746	30-64	yes	yes	no	-

Daley et al, 2014	Australia (Australian Longitudinal Study on Women's Health [ALSWH] Young Cohort Survey 3)	Cross- sectional	FFQ	CES-D (depression =10)	General population (women)	7635	25-30	no	yes	no	-
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BDI-Y, Beck Depression Inventory for Youth ; CES-D, Center for Epidemiologic Studies Depression Scale; CIDI-SF, Short Form of the Composite International Diagnostic Interview; M-CIDI, Munich-Composite International Diagnostic Interview ; GDS, Geriatric Depression Scale.

Table 2. Summary results of the epidemiological studies conducted on fish and/or omega-3 PUFA consumption and depression.

Author	Number of cases	Adjustments	Results	Low category of exposure	High category of exposure	Study quality
Tanskanen et al, 2001	896	Age, marital status, occupation, smoking status, physical activity, BMI, alcohol intake, coffee intake, educational level, serum cholesterol level	Fish consumption (rare vs. regular eaters), OR 1.31 (95% CI: 1.10, 1.56)			3
Hakkarainen et al, 2004	8612	Age, body mass index, energy intake, serum total cholesterol level, high-density lipoprotein cholesterol level, consumption of alcohol, education, marriage, self-reported depression, self-reported anxiety, and smoking.	Fish consumption (highest vs. lowest tertile), OR 0.97 (95% CI: 0.70, 1.33)			5
Omega-3 (highest vs. lowest tertile), OR 0.96 (95% CI: 0.70, 1.30)						
Timonen et al, 2004	Doctor diagnosed women 236 (5.3%)	Alcohol intake, smoking, physical inactivity, and marital status.	Women - Fish consumption (rare vs. regular eaters), OR 2.4 (95% CI: 1.4, 4.2)			2
HSCL-25 diagnosed women 312 (7%)						
	Doctord diagnosed men 146 (3.6%)		Men - Fish consumption (rare vs. regular eaters), OR 0.8 (95% CI: 0.4, 1.6)			

HSCL-25 diagnosed men 171 (4.2%)				
Barberger-Gateau et al, 2005	Not specified	Age, sex, education, and city.	Fish consumption (> once a week vs. < once a week), OR 0.63 (95% CI: 0.52, 0.75)	2
Kamphuis et al, 2006	72	Age, years of education, BMI, smoking status, alcohol consumption, systolic blood pressure, physical activity, living alone	Omega-3 (high [>156 mg/d] vs. low [<59 mg/d] consumption), OR 0.46 (95% CI: 0.22, 0.95), P for trend 0.04	3
Appleton et al, 2007a	Not specified	Age, gender, Index of Multiple Deprivation score, date of questionnaire completion.	Omega-3 (linear component), B 0.08 (95% CI: -0.07, 0.23)	3
Appleton et al, 2007b	Not specified	All diet and demographic variables.	(Northern Ireland) Fish consumption (linear term), B -0.09 (95% CI: -2.25, -0.01), P = 0.05	3
(France) Fish consumption (linear term), B -0.14 (95% CI: -2.73, -1.17), P <0.01				
Sanchez-Villegas et al, 2007	173	Age, gender, incapacitating disease, energy intake, physical activity during leisure time, and change in physical activity since baseline.	Fish consumption (highest vs. lowest quintile), OR 1.08 (95% CI: 0.51, 2.31), P for trend 0.660	4
Omega-3 (highest vs. lowest quintile), OR 1.04 (95% CI: 0.78, 1.40), P for trend 0.376				
				1.89 g/d

Astorg et al, 2008	664	Age, sex, intervention group, family status, education level, and tobacco use.	Fish consumption (highest vs. lowest tertile), OR 0.71 (95% CI: 0.52, 0.97), P for trend 0.029	12.8 g/d	79.7 g/d	5
Murakami et al, 2008	112 men and 76 women	Age, BMI, work place, marital status, occupational physical activity, leisure-time physical activity, smoking status, alcohol drinking, and job stress score.	Omega-3 (highest vs. lowest tertile), OR 0.79 (95% CI: 0.58, 1.15) Men - Omega-3 (highest vs. lowest quartile), OR 0.58 (95% CI: 0.28, 1.19), P for trend 0.13	0.075% energy	0.135% energy	3
Bountziouka et al, 2009	407	Not specified	Fish consumption (linear times/week), B -0.529 (95% CI: -0.45, -0.73)			3
Colangelo et al, 2009	3317	Age, race, gender, educational level, BMI, smoking status, alcohol intake, total physical activity, and marital status.	Men - Fish consumption (highest vs. lowest quintile), OR 0.89 (95% CI: 0.62, 1.28), P for trend 0.96			4

			Women - Fish consumption (highest vs. lowest quintile), HR 0.75 (95% CI: 0.55, 1.01), P for trend 0.02	
			Men - Omega-3 (EPA+DHA) (highest vs. lowest quintile), HR 0.91 (95% 0.64, 1.30), P for trend 0.93	
			Women - Omega-3 (EPA+DHA) (highest vs. lowest quintile), OR 0.71 (95% 0.52, 0.95), P for trend 0.001	
Kyrozis et al, 2009	Not specified	Gender, age, marital status, years of education, height, BMI, physical activity, smoking, alcohol intake, coffee intake, energy daily intake, hypertension at baseline, diabetes at baseline, Mediterranean diet adherence, cancer at follow-up, and cardiac disease.	Fish consumption (linear 1 SD more), B -0.08 (95% CI: -0.30, 0.15), P = 0.513	3
Murakami et al, 2010	1766	Age, habitual exercise, paternal educational level, and maternal educational level.	Males - Fish consumption (highest vs. lowest quintile), OR 0.73 (95% CI: 0.55, 0.97), P for trend 0.002	5
			Females - Fish consumption (highest vs. lowest quintile), OR 1.01 (95% CI: 0.80, 1.28), P for trend 0.79	
			Males - Omega-3 (highest vs. lowest quintile), OR 0.72 (95% CI: 0.55, 0.98), P for trend 0.08	

			Females - Omega-3 (highest vs. lowest quintile), OR 1.05 (95% CI: 0.83, 1.33), P for trend 0.43	0.071% energy	0.295% energy	
Suominen-Taipale et al, 2010	590	Age, total energy intake for fish consumption, BMI, level of education, marital status, smoking history, physical activity, and alcohol intake.	Fish consumption (highest vs. lowest quartile), OR 0.6 (95% CI: 0.3, 0.9), P for trend 0.03	11 g/d	76 g/d	4
				23 g/d	116 g/d	
Li et al, 2011	11,7% men and 17,89% of women	Age, race/ethnicity, education attainment, family income level, marital status, types of residence area, occupation, employment, BMI, alcohol drinking, cigarette smoking, serum total cholesterol, total dietary energy intake, saturated fatty intake, fruit and vegetable intake, health status.	Males - Fish consumption (<1/week vs. >1 week), OR 2.08 (95% CI: 1.08, 4.09), P for trend 0.03			3
Lucas et al, 2011	2,823	Age, time interval of the study, hormonal status, race, obesity, smoking status, physical activity, diagnosis of diabetes, cancer, myocardial infarction, multivitamin use, average intake of energy, protein, trans fatty acids, saturated fatty acids, monounsaturated fatty acids, alcohol,	Females - Fish consumption (<1/week vs. >1 week), OR 1.15 (95% CI: 0.83, 1.59), P for trend 0.40 Fish consumption (>5 times/month vs. <1 time/month), RR 1.07 (95% CI: 0.74, 1.55)			7

			Omega-3 (0.3 g/d increment), RR 0.99 (95% CI: 0.88, 1.10)	0.07 g/d	0.41 g/d
			Omega-3:omega-6 ratio intake (increase of 0.1 U), RR 0.74 (95% CI: 0.61, 0.90)	0.11	0.17
			ALA (0.5 g/d increment), RR 0.81 (95% CI: 0.69, 0.65)		
Oddy et al, 2011	14% men and 28% women	BMI, physical activity level, and socioeconomic status.	Omega-3 (linear regression on BDI-Y scores), beta -0.03 (95% CI: -0.23, 0.17), P = 0.76		3
	14% men and 29% women		Omega-3 (linear regression on BDI-Y scores), beta -0.14 (95% CI: -0.40, 0.18), P = 0.29		
Kesse-Guyot et al, 2012	298 men and 617 women	Age, gender, physical activity, educational level, intervention group, energy intake, marital status, number of 24-h recalls, alcohol consumption, BMI, saturated fatty acids intake, vegetable and fruit consumption, omega-3 and omega-6 PUFA.	Omega-3 (highest vs. lowest quartile), OR 0.74 (95% CI: 0.58, 0.95), P for trend <0.001	0.2 g/d	0.9 g/d
			Omega-6/omega-3 ratio intake (highest vs. lowest quartile), OR 1.14 (95% CI: 0.90, 1.43)	0.04	0.07

47 men and 93 women		Omega-3 (highest vs. lowest quartile), OR 1.02 (95% CI: 0.60, 1.75), P for trend <0.76			
		Omega-6/omega-3 ratio intake (highest vs. lowest quartile), OR 0.98 (95% CI: 0.58, 1.65)			
Albanese et al, 2012	Not clear	Age, gender, educational level, number of household assets, marital status, self-reported diagnosed diabetes, coronary heart disease and stroke, number of physical illnesses, overall cognitive status, weekly meat intake, fruits and vegetables consumption, alcohol intake, physical activity.	Fish consumption, never, OR 0.93 (95% CI: 0.78, 1.10); some days, OR 1 (reference); most days, OR 1.07 (95% CI: 0.85, 1.86)		2
Jacka et al, 2012	51	Energy intake and diet quality score.	EPA (highest vs. lowest tertile), OR 1.31 (95% CI: 1.64, 2.69) DHA (highest vs. lowest tertile), OR 1.44 (95% CI: 0.73, 2.83)	210 mg/d 480 mg/d	4
Beydoun et al, 2013	18.1% men and 25.6% women	Age, race/ethnicity, marital status, education, poverty-income ratio, smoking and drug use status, measured BMI, selected nutrients and total energy intake.	Men - Omega-3 (highest vs. lowest tertile), OR 1.40 (95% CI: 0.66, 2.97) Women - Omega-3 (highest vs. lowest tertile), OR 0.51 (95% CI: 0.27, 0.95)	0.41% energy 1.14% energy	6

			Men - Omega-3/Omega-6 ratio (highest vs. lowest tertile), OR 1.24 (95% CI: 0.60, 2.56)	0.07	0.15
			Women - Omega-3/Omega-6 ratio (highest vs. lowest tertile), OR 0.47 (95% CI: 0.27, 0.83)		
Daley et al, 2014	1949	BMI, energy intake, physical activity, chronic illnesses, alcohol intake, education, drug use, smoking status, pregnancy status, abuse, area of residence, managing on income, marital status, major life events, symptoms, depression medications.	EPA (continuous), OR 1.31 (95% CI: 0.75, 2.34)		4
			DHA (continuous), OR 1.20 (95% CI: 0.89, 1.61)		
			ALA (continuous), OR 0.77 (95% CI: 0.60, 0.90)		
Miyake et al, 2006	14%	Age, gestation, parity, cigarette smoking, family structure, family income, education, changes in diet in the previous month, season when data at baseline were collected, body mass index (continuous), time of delivery before the second survey, medical problems in pregnancy, baby's sex and baby's birthweight.	Fish consumption (highest vs. lowest quartile), OR 0.89 (95% CI: 0.50, 1.59), P for trend 0.37	23.1 g/d	72.9 g/d 5

			Omega-3 (highest vs. lowest quartile), OR 0.90 (95% CI: 0.53, 1.53), P for trend 0.61	1.6 g/d	3 g/d
			Omega-3/omega-6 ratio, OR 0.97 (95% CI: 0.55, 1.68), P for trend 0.95	0.17	0.25
Sontrop et al, 2008	Not specified	Sociodemographic, health and lifestyle variables.	Fish consumption (1/week vs. <1/week), B -0.2 (95% CI: -0.9, 0.4)		5
			Omega-3 (85 mg/day vs. <85 mg/day), B 0.1 (95% CI: -0.6, 0.8)	<85 mg/d	>85 mg/d
Golding et al, 2009	14%	Energy intake, maternal age, maternal education, maternal smoking, maternal ethnic background, housing tenure, crowding, childhood life events, recent life events, chronic stress (FAI), parity, and outcome of immediately preceding pregnancy.	Omega-3 (none vs. >1.5 g/week), OR 1.54 (95% CI: 1.25, 1.89), P for trend <0.001	0	>1.5 gr/week
Strom et al, 2009	PDD-admission 159 (0.3%)	Total energy intake, prepregnant BMI, maternal age, parity, alcohol intake, smoking, occupation, education, homeownership, marital status, social support, and history of previous depression.	PDD-admission - Fish consumption (lowest vs. highest quintile), OR 0.82 (95% CI: 0.42, 1.64), P for trend 0.50	<3 g/d	>30 g/d
	PDD-prescription 866 (1.6%)		PDD-prescription - Fish consumption (lowest vs. highest quintile), OR 1.46 (95% CI: 1.12, 1.90) P for trend 0.04		
			PDD-admission - Omega-3 (lowest vs. highest decile), OR 0.96 (95% CI: 0.53, 1.53), P for trend 0.61	9.1 mg/d	72.8 mg/d

			PDD-prescription - Omega-3 (lowest vs. highest decile), OR 1.24 (95% CI: 0.96, 1.61) P for trend 0.33	
Da Rocha et al, 2012	28 women	Age, schooling, pre-pregnancy BMI, time elapsed since delivery, lipids consumption.	Omega-6:omega-3 ratio (>9:1 vs. <9:1), HR: 2.50 (95% CI: 1.21, 5.14), P = 0.013	4

DISCUSSION AND CONCLUSIONS

The role of omega-3 on preventing psychiatric diseases, if acting through short-term anti-inflammatory effects or on the cerebral parenchyma itself through a long-term structural or functional action, remains to be clarified. It can be speculated that all types of action can occur simultaneously: on one hand, by maintaining and increasing the brain structures, and preserving their function by interacting with phospholipid metabolism and, hence, the modulation of signal transduction; on the other hand, preventing or decreasing the inflammatory status occurring during depression. However, the problem of how to correct the inadequate supply of omega-3 fatty acids in Westernized countries' diet is a priority in order to set food and health policies and dietary recommendations for individuals and population groups. Moreover, accompanying the increased dietary intake of omega-3 fatty acid, an omega-6/omega3 ratio maintained below 5 is highly desirable. If omega-3 PUFA will result effective for both the prevention and treatment of depression, substantial implication with large-scale impact through dietary interventions could be reached. Although many other factors may also contribute to the rise in depression and effective (although not efficient) treatments already exist, dietary recommendations suggesting proper intake of omega-3 PUFA and dietary interventions including omega-3 PUFA supplement can result in substantial benefits at the population level.

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LIST OF PUBLICATIONS

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