International PhD Program in Neuropharmacology
XXVII Cycle

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

PhD Thesis

Giuseppe Grosso

Coordinator: Prof. Salvatore Salomone
Tutor: Prof. Fabio Galvano
Co-tutor: Prof. Claudio Bucolo
Co-tutor: Prof. Filippo Caraci


Department of Biomedical and Biotechonological Sciences
University of Catania – Medical School

*February 2015*

**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
*Burden of the disease*……...……...……..... 10
*Depression and diet, the association with fish consumption*…….....11
Hypothesized mechanisms of action…….……...15
*Neuro-endocrine modulation of omega-3 PUFA in depression*……..…….. 15
*Anti-inflammatory effects of omega-3 PUFA*……...…….…… 19
*Relation between omega-3 PUFA intake and structural changing of brain*……. 23
*Genetic aspects of association between omega-3 PUFA and depression*……..25
CHAPTER 1……..…….…….. 29
CHAPTER 2……...……... 81
DISCUSSION AND CONCLUSIONS……..…….. 117
REFERENCES……...……... 117
LIST OF PUBLICATIONS…….……..……... 137

**ACKNOWLEDGEMENTS**

I express my warm thanks to all professors and department members that helped me to complete my PhD project with their support and guidance.

I would also like to thank my project external guides in Poland and all the people who provided me with the facilities being required and conductive conditions for my PhD project.

Finally, I would like to thank my family and friends for their effort in supporting me during hard times.

## 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 β1
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 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 (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 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 synthetized 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 serotoninergic 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 inhibits 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 mobidity 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 anti-depressants 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 serotoninergic 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 ATP'ase) [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 serotoninergic 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, TNFα, 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 β1 (TGF-β1) [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-1β (IL-1β), and tumor necrosis factor-α (TNFα) [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-β1, 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 concerting 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 cyclo-oxygenase-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-1β, 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 κB 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-ß1 [126, 129]. Plasma TGF-ß1 levels are reduced in major depressed patients and show a significant negative correlation with the Hamilton Depression Rating Scale [130, 164, 165]. Interestingly, TGF-ß1 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-β1 [165]. Similarly, therapeutic concentrations of venlafaxine prevent microglial activation, reduce pro-inflammatory cytokine secretion, and finally increase the release of TGF-ß1 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-ß1 [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-β1 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-β1 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.

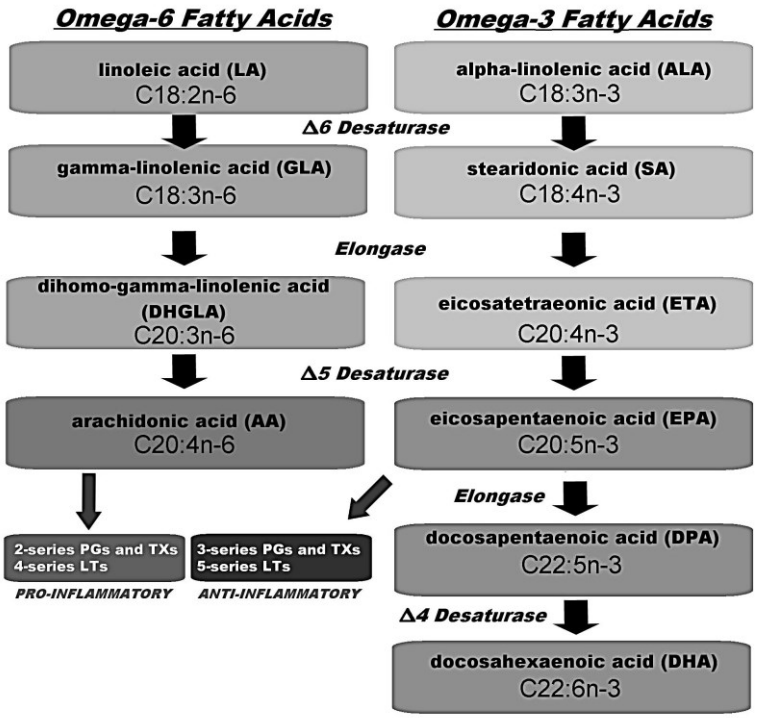
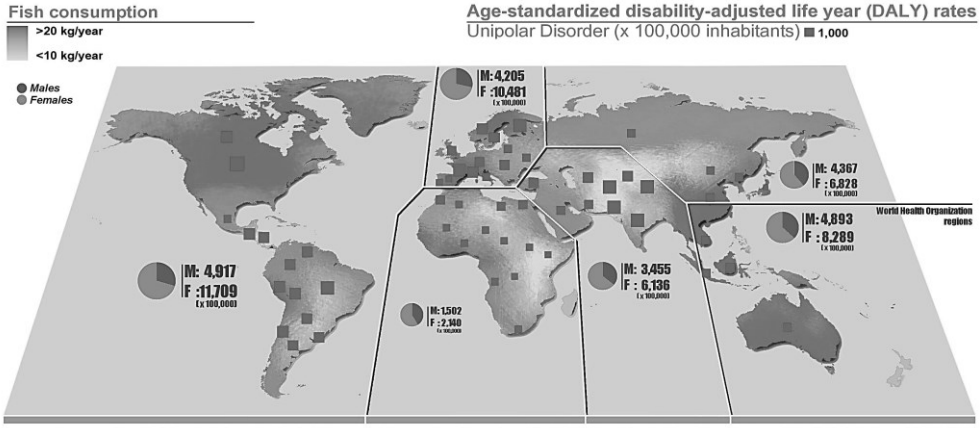
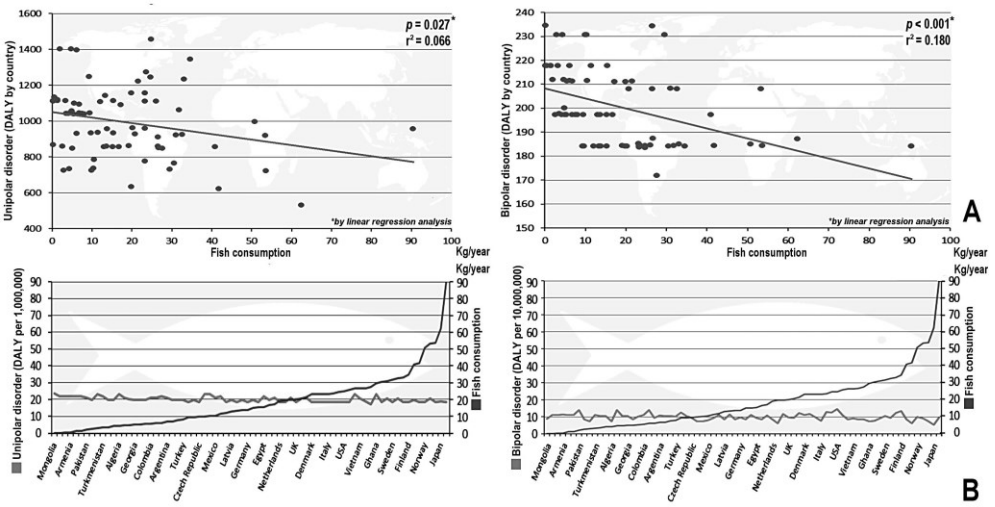



Figure 2. Per capita annual fish consumption and age-standardized disability-adjusted life year for unipolar disorder distribution across countries. DALY rates by gender are also reported per all World Health Organization regions (year 2004). High-income regions reported higher rates of DALY despite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



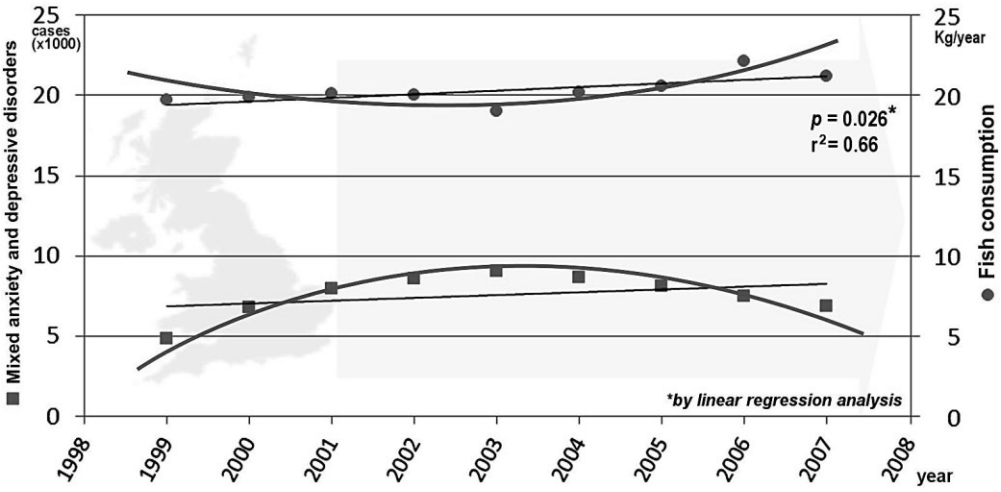

Source: *Consumption of Fish and Fishery Products*, Fishery and Aquaculture Department 2011, Food and Agriculture Organization of the United Nations (FAOSTAT); *The Global Burden of Disease: 2004 update*, World Health Organization, Geneva, 2008.

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.




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 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

*PLoS One. 2014 May 7;9(5):e96905. doi: 10.1371/journal.pone.0096905.*

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

Giuseppe Grosso[1], Andrzej Pajak[2], Stefano Marventano[3], Sabrina Castellano[1], Fabio Galvano[1], Claudio Bucolo[1], Filippo Drago[1], Filippo Caraci[4,5]

*[1]Department of Clinical and Molecular Biomedicine, Section of Pharmacology and Biochemistry, University of Catania, Catania, Italy; [2]Department of Epidemiology and Population Studies, Jagiellonian University Medical College, Krakow, Poland; [3]Department "G.F. Ingrassia", Section of Hygiene and Public Health, University of Catania, Catania, Italy; [4]Department of Educational Sciences, University of Catania, Catania, Italy; [5]IRCCS Associazione Oasi Maria S.S. – Institute for Research on Mental Retardation and Brain Aging, Troina, Enna, Italy*

## 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, justifing 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 risolved.

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, on-going 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 on-going 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 ≥30% [104]. Thus, the studies quality decreased when placebo response rates were not mantained 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 ≥ 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 maniacal 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-β1 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-alpha (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.

**References**


1. Kotwal S, Jun M, Sullivan D, Perkovic V, Neal B (2012) Omega 3 Fatty acids and cardiovascular outcomes: systematic review and meta-analysis. Circ Cardiovasc Qual Outcomes 5: 808-818.
2. Grosso G, Galvano F, Marventano S, Malaguarnera M, Bucolo C, Drago F, Caraci F (2014) Omega-3 Fatty Acids and Depression: Scientific evidence and Biological Mechanisms. Oxid Med Cell Longev 2014:313570.
3. Machado-Vieira R, Mallinger AG (2012) Abnormal function of monoamine oxidase-A in comorbid major depressive disorder and cardiovascular disease: pathophysiological and therapeutic implications (review). Mol Med Rep 6: 915-922.
4. Do DP, Dowd JB, Ranjit N, House JS, Kaplan GA (2010) Hopelessness, depression, and early markers of endothelial dysfunction in U.S. adults. Psychosom Med 72: 613-619.
5. Severus WE, Littman AB, Stoll AL (2001) Omega-3 fatty acids, homocysteine, and the increased risk of cardiovascular mortality in major depressive disorder. Harv Rev Psychiatry 9: 280-293.
6. Bourre JM (2004) Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing. J Nutr Health Aging 8: 163-174.
7. Hennebelle M, Balasse L, Latour A, Champeil-Potokar G, Denis S, et al. (2012) Influence of omega-3 fatty acid status on the way rats adapt to chronic restraint stress. PLoS One 7: e42142.
8. Maes M, Yirmyia R, Noraberg J, Brene S, Hibbeln J, et al. (2009) The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab Brain Dis 24: 27-53.

9. Rees AM, Austin MP, Owen C, Parker G (2009) Omega-3 deficiency associated with perinatal depression: case control study. Psychiatry Res 166: 254-259.
10. Schins A, Crijns HJ, Brummer RJ, Wichers M, Lousberg R, et al. (2007) Altered omega-3 polyunsaturated fatty acid status in depressed post-myocardial infarction patients. Acta Psychiatr Scand 115: 35-40.
11. Frasure-Smith N, Lesperance F, Julien P (2004) Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes. Biol Psychiatry 55: 891-896.
12. Parker GB, Heruc GA, Hilton TM, Olley A, Brotchie H, et al. (2006) Low levels of docosahexaenoic acid identified in acute coronary syndrome patients with depression. Psychiatry Res 141: 279-286.
13. Assies J, Pouwer F, Lok A, Mocking RJ, Bockting CL, et al. (2010) Plasma and erythrocyte fatty acid patterns in patients with recurrent depression: a matched case-control study. PLoS One 5: e10635.
14. Bloch MH, Hannestad J (2012) Omega-3 fatty acids for the treatment of depression: systematic review and meta-analysis. Mol Psychiatry 17: 1272-1282.
15. Martins JG, Bentsen H, Puri BK (2012) Eicosapentaenoic acid appears to be the key omega-3 fatty acid component associated with efficacy in major depressive disorder: a critique of Bloch and Hannestad and updated meta-analysis. Mol Psychiatry 17: 1144-1149; discussion 1163-1147.
16. Lin PY, Mischoulon D, Freeman MP, Matsuoka Y, Hibbeln J, et al. (2012) Are omega-3 fatty acids antidepressants or just mood-improving agents? The effect depends upon diagnosis, supplement preparation, and severity of depression. Mol Psychiatry 17: 1161-1163; author reply 1163-1167.

17. Association AP (2000) Diagnostic and Statistical Manual of Mental Disorders; 4th, editor. Washington, DC. 317-345 p.
18. Hamilton M (1967) Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol 6: 278-296.
19. Beck AT (1978) Depression Inventory. Center for Cognitive Therapy; Philadelphia.
20. Montgomery SA, Asberg M (1979) A new depression scale designed to be sensitive to change. Br J Psychiatry 134: 382-389.
21. Picardi A (2009) Rating scales in bipolar disorder. Curr Opin Psychiatry 22: 42-49.
22. Geddes JR, Miklowitz DJ (2013) Treatment of bipolar disorder. Lancet 381: 1672-1682.
23. Vesga-Lopez O, Blanco C, Keyes K, Olfson M, Grant BF, et al. (2008) Psychiatric disorders in pregnant and postpartum women in the United States. Arch Gen Psychiatry 65: 805-815.
24. Otto SJ, de Groot RH, Hornstra G (2003) Increased risk of postpartum depressive symptoms is associated with slower normalization after pregnancy of the functional docosahexaenoic acid status. Prostaglandins Leukot Essent Fatty Acids 69: 237-243.
25. Bylund DB, Reed AL (2007) Childhood and adolescent depression: why do children and adults respond differently to antidepressant drugs? Neurochem Int 51: 246-253.
26. Cox GR, Callahan P, Churchill R, Hunot V, Merry SN, et al. (2012) Psychological therapies versus antidepressant medication, alone and in combination for depression in children and adolescents. Cochrane Database Syst Rev 11: CD008324.
27. Appleton KM, Hayward RC, Gunnell D, Peters TJ, Rogers PJ, et al. (2006) Effects of n-3 long-chain polyunsaturated fatty acids on

depressed mood: systematic review of published trials. Am J Clin Nutr 84: 1308-1316.

28. Appleton KM, Rogers PJ, Ness AR (2010) Updated systematic review and meta-analysis of the effects of n-3 long-chain polyunsaturated fatty acids on depressed mood. Am J Clin Nutr 91: 757-770.

29. Sublette ME, Ellis SP, Geant AL, Mann JJ (2011) Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. J Clin Psychiatry 72: 1577-1584.

30. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, et al. (1996) Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials 17: 1-12.

31. Clayton EH, Hanstock TL, Hirneth SJ, Kable CJ, Garg ML, et al. (2009) Reduced mania and depression in juvenile bipolar disorder associated with long-chain omega-3 polyunsaturated fatty acid supplementation. Eur J Clin Nutr 63: 1037-1040.

32. Ness AR, Gallacher JE, Bennett PD, Gunnell DJ, Rogers PJ, et al. (2003) Advice to eat fish and mood: a randomised controlled trial in men with angina. Nutr Neurosci 6: 63-65.

33. Beezhold BL, Johnston CS (2012) Restriction of meat, fish, and poultry in omnivores improves mood: a pilot randomized controlled trial. Nutr J 11: 9.

34. Marangell LB, Suppes T, Ketter TA, Dennehy EB, Zboyan H, et al. (2006) Omega-3 fatty acids in bipolar disorder: clinical and research considerations. Prostaglandins Leukot Essent Fatty Acids 75: 315-321.

35. Krauss-Etschmann S, Shadid R, Campoy C, Hoster E, Demmelmair H, et al. (2007) Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of

docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. Am J Clin Nutr 85: 1392-1400.

36. Mattes E, McCarthy S, Gong G, van Eekelen JA, Dunstan J, et al. (2009) Maternal mood scores in mid-pregnancy are related to aspects of neonatal immune function. Brain Behav Immun 23: 380-388.
37. Andreeva VA, Galan P, Torres M, Julia C, Hercberg S, et al. (2012) Supplementation with B vitamins or n-3 fatty acids and depressive symptoms in cardiovascular disease survivors: ancillary findings from the SUpplementation with FOLate, vitamins B-6 and B-12 and/or OMega-3 fatty acids (SU.FOL.OM3) randomized trial. Am J Clin Nutr 96: 208-214.
38. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, et al. (2010) Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. JAMA 304: 1675-1683.
39. Behan PO, Behan WM, Horrobin D (1990) Effect of high doses of essential fatty acids on the postviral fatigue syndrome. Acta Neurol Scand 82: 209-216.
40. Sohrabi N, Kashanian M, Ghafoori SS, Malakouti SK (2013) Evaluation of the effect of omega-3 fatty acids in the treatment of premenstrual syndrome: "a pilot trial". Complement Ther Med 21: 141-146.
41. Sampalis F, Bunea R, Pelland MF, Kowalski O, Duguet N, et al. (2003) Evaluation of the effects of Neptune Krill Oil on the management of premenstrual syndrome and dysmenorrhea. Altern Med Rev 8: 171-179.
42. Puolakka J, Makarainen L, Viinikka L, Ylikorkala O (1985) Biochemical and clinical effects of treating the premenstrual syndrome with prostaglandin synthesis precursors. J Reprod Med 30: 149-153.

43. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961) An inventory for measuring depression. Arch Gen Psychiatry 4: 561-571.

44. Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry 23: 56-62.

45. Giltay EJ, Gooren LJ, Toorians AW, Katan MB, Zock PL (2004) Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. Am J Clin Nutr 80: 1167-1174.

46. Grenyer BF, Crowe T, Meyer B, Owen AJ, Grigonis-Deane EM, et al. (2007) Fish oil supplementation in the treatment of major depression: a randomised double-blind placebo-controlled trial. Prog Neuropsychopharmacol Biol Psychiatry 31: 1393-1396.

47. da Silva TM, Munhoz RP, Alvarez C, Naliwaiko K, Kiss A, et al. (2008) Depression in Parkinson's disease: a double-blind, randomized, placebo-controlled pilot study of omega-3 fatty-acid supplementation. J Affect Disord 111: 351-359.

48. Jazayeri S, Tehrani-Doost M, Keshavarz SA, Hosseini M, Djazayery A, et al. (2008) Comparison of therapeutic effects of omega-3 fatty acid eicosapentaenoic acid and fluoxetine, separately and in combination, in major depressive disorder. Aust N Z J Psychiatry 42: 192-198.

49. Hallahan B, Hibbeln JR, Davis JM, Garland MR (2007) Omega-3 fatty acid supplementation in patients with recurrent self-harm. Single-centre double-blind randomised controlled trial. Br J Psychiatry 190: 118-122.

50. Freund-Levi Y, Basun H, Cederholm T, Faxen-Irving G, Garlind A, et al. (2008) Omega-3 supplementation in mild to moderate Alzheimer's disease: effects on neuropsychiatric symptoms. Int J Geriatr Psychiatry 23: 161-169.

51. Doornbos B, van Goor SA, Dijck-Brouwer DA, Schaafsma A, Korf J, et al. (2009) Supplementation of a low dose of DHA or DHA+AA does

not prevent peripartum depressive symptoms in a small population based sample. Prog Neuropsychopharmacol Biol Psychiatry 33: 49-52.

52. Fontani G, Corradeschi F, Felici A, Alfatti F, Bugarini R, et al. (2005) Blood profiles, body fat and mood state in healthy subjects on different diets supplemented with Omega-3 polyunsaturated fatty acids. Eur J Clin Invest 35: 499-507.

53. Rondanelli M, Giacosa A, Opizzi A, Pelucchi C, La Vecchia C, et al. (2011) Long chain omega 3 polyunsaturated fatty acids supplementation in the treatment of elderly depression: effects on depressive symptoms, on phospholipids fatty acids profile and on health-related quality of life. J Nutr Health Aging 15: 37-44.

54. Peet M, Horrobin DF (2002) A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. Arch Gen Psychiatry 59: 913-919.

55. Furukawa TA, Barbui C, Cipriani A, Brambilla P, Watanabe N (2006) Imputing missing standard deviations in meta-analyses can provide accurate results. J Clin Epidemiol 59: 7-10.

56. Warren G, McKendrick M, Peet M (1999) The role of essential fatty acids in chronic fatigue syndrome. A case-controlled study of red-cell membrane essential fatty acids (EFA) and a placebo-controlled treatment study with high dose of EFA. Acta Neurol Scand 99: 112-116.

57. Fux M, Benjamin J, Nemets B (2004) A placebo-controlled cross-over trial of adjunctive EPA in OCD. J Psychiatr Res 38: 323-325.

58. Hirashima F, Parow AM, Stoll AL, Demopulos CM, Damico KE, et al. (2004) Omega-3 fatty acid treatment and T(2) whole brain relaxation times in bipolar disorder. Am J Psychiatry 161: 1922-1924.

59. Keck PE, Jr., Mintz J, McElroy SL, Freeman MP, Suppes T, et al. (2006) Double-blind, randomized, placebo-controlled trials of ethyl-eicosapentanoate in the treatment of bipolar depression and rapid cycling bipolar disorder. Biol Psychiatry 60: 1020-1022.

60. Sinn N, Milte CM, Street SJ, Buckley JD, Coates AM, et al. (2012) Effects of n-3 fatty acids, EPA v. DHA, on depressive symptoms, quality of life, memory and executive function in older adults with mild cognitive impairment: a 6-month randomised controlled trial. Br J Nutr 107: 1682-1693.

61. Gracious BL, Chirieac MC, Costescu S, Finucane TL, Youngstrom EA, et al. (2010) Randomized, placebo-controlled trial of flax oil in pediatric bipolar disorder. Bipolar Disord 12: 142-154.

62. Bot M, Pouwer F, Assies J, Jansen EH, Beekman AT, et al. (2011) Supplementation with eicosapentaenoic omega-3 fatty acid does not influence serum brain-derived neurotrophic factor in diabetes mellitus patients with major depression: a randomized controlled pilot study. Neuropsychobiology 63: 219-223.

63. Chiu CC, Huang SY, Chen CC, Su KP (2005) Omega-3 fatty acids are more beneficial in the depressive phase than in the manic phase in patients with bipolar I disorder. J Clin Psychiatry 66: 1613-1614.

64. Peet M, Horrobin DF (2002) A dose-ranging exploratory study of the effects of ethyl-eicosapentaenoate in patients with persistent schizophrenic symptoms. J Psychiatr Res 36: 7-18.

65. Frangou S, Lewis M, McCrone P (2006) Efficacy of ethyl-eicosapentaenoic acid in bipolar depression: randomised double-blind placebo-controlled study. Br J Psychiatry 188: 46-50.

66. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Hwang BS, et al. (2012) Omega-3 supplementation lowers inflammation in

healthy middle-aged and older adults: a randomized controlled trial. Brain Behav Immun 26: 988-995.

67. van de Rest O, Geleijnse JM, Kok FJ, van Staveren WA, Hoefnagels WH, et al. (2008) Effect of fish-oil supplementation on mental well-being in older subjects: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 88: 706-713.

68. Mozurkewich EL, Clinton CM, Chilimigras JL, Hamilton SE, Allbaugh LJ, et al. (2013) The Mothers, Omega-3, and Mental Health Study: a double-blind, randomized controlled trial. Am J Obstet Gynecol 208: 313 e311-319.

69. Mozaffari-Khosravi H, Yassini-Ardakani M, Karamati M, Shariati-Bafghi SE (2013) Eicosapentaenoic acid versus docosahexaenoic acid in mild-to-moderate depression: a randomized, double-blind, placebo-controlled trial. Eur Neuropsychopharmacol 23: 636-644.

70. Lucas M, Asselin G, Merette C, Poulin MJ, Dodin S (2009) Ethyl-eicosapentaenoic acid for the treatment of psychological distress and depressive symptoms in middle-aged women: a double-blind, placebo-controlled, randomized clinical trial. Am J Clin Nutr 89: 641-651.

71. Sterne JA, Egger M, Smith GD (2001) Systematic reviews in health care: Investigating and dealing with publication and other biases in meta-analysis. BMJ 323: 101-105.

72. deeks JJ, Altman DG, bradbrun MJ (2001) Statistical methods for examining heterogeneity and combining results from several studies in metaanalysis. London. 285-312 p.

73. Higgins JP, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. Stat Med 21: 1539-1558.

74. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327: 557-560.

75. Stoll AL, Severus WE, Freeman MP, Rueter S, Zboyan HA, et al. (1999) Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. Arch Gen Psychiatry 56: 407-412.

76. Nemets B, Stahl Z, Belmaker RH (2002) Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. Am J Psychiatry 159: 477-479.

77. Marangell LB, Martinez JM, Zboyan HA, Kertz B, Kim HF, et al. (2003) A double-blind, placebo-controlled study of the omega-3 fatty acid docosahexaenoic acid in the treatment of major depression. Am J Psychiatry 160: 996-998.

78. Su KP, Huang SY, Chiu CC, Shen WW (2003) Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. Eur Neuropsychopharmacol 13: 267-271.

79. Silvers KM, Woolley CC, Hamilton FC, Watts PM, Watson RA (2005) Randomised double-blind placebo-controlled trial of fish oil in the treatment of depression. Prostaglandins Leukot Essent Fatty Acids 72: 211-218.

80. Frangou S, Lewis M, Wollard J, Simmons A (2007) Preliminary in vivo evidence of increased N-acetyl-aspartate following eicosapentanoic acid treatment in patients with bipolar disorder. J Psychopharmacol 21: 435-439.

81. Chiu CC, Su KP, Cheng TC, Liu HC, Chang CJ, et al. (2008) The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: a preliminary randomized double-blind placebo-controlled study. Prog Neuropsychopharmacol Biol Psychiatry 32: 1538-1544.

82. Su KP, Huang SY, Chiu TH, Huang KC, Huang CL, et al. (2008) Omega-3 fatty acids for major depressive disorder during pregnancy: results

from a randomized, double-blind, placebo-controlled trial. J Clin Psychiatry 69: 644-651.

83. Mischoulon D, Papakostas GI, Dording CM, Farabaugh AH, Sonawalla SB, et al. (2009) A double-blind, randomized controlled trial of ethyl-eicosapentaenoate for major depressive disorder. J Clin Psychiatry 70: 1636-1644.

84. Gertsik L, Poland RE, Bresee C, Rapaport MH (2012) Omega-3 fatty acid augmentation of citalopram treatment for patients with major depressive disorder. J Clin Psychopharmacol 32: 61-64.

85. Llorente AM, Jensen CL, Voigt RG, Fraley JK, Berretta MC, et al. (2003) Effect of maternal docosahexaenoic acid supplementation on postpartum depression and information processing. Am J Obstet Gynecol 188: 1348-1353.

86. Rogers PJ, Appleton KM, Kessler D, Peters TJ, Gunnell D, et al. (2008) No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. Br J Nutr 99: 421-431.

87. Carney RM, Freedland KE, Rubin EH, Rich MW, Steinmeyer BC, et al. (2009) Omega-3 augmentation of sertraline in treatment of depression in patients with coronary heart disease: a randomized controlled trial. JAMA 302: 1651-1657.

88. Antypa N, Van der Does AJ, Smelt AH, Rogers RD (2009) Omega-3 fatty acids (fish-oil) and depression-related cognition in healthy volunteers. J Psychopharmacol 23: 831-840.

89. Antypa N, Smelt AH, Strengholt A, Van der Does AJ (2012) Effects of omega-3 fatty acid supplementation on mood and emotional information processing in recovered depressed individuals. J Psychopharmacol 26: 738-743.

90. Fenton WS, Dickerson F, Boronow J, Hibbeln JR, Knable M (2001) A placebo-controlled trial of omega-3 fatty acid (ethyl eicosapentaenoic acid) supplementation for residual symptoms and cognitive impairment in schizophrenia. Am J Psychiatry 158: 2071-2074.

91. Zanarini MC, Frankenburg FR (2003) omega-3 Fatty acid treatment of women with borderline personality disorder: a double-blind, placebo-controlled pilot study. Am J Psychiatry 160: 167-169.

92. Amminger GP, Schafer MR, Papageorgiou K, Klier CM, Cotton SM, et al. (2010) Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. Arch Gen Psychiatry 67: 146-154.

93. Rees AM, Austin MP, Parker GB (2008) Omega-3 fatty acids as a treatment for perinatal depression: randomized double-blind placebo-controlled trial. Aust N Z J Psychiatry 42: 199-205.

94. Bot M, Pouwer F, Assies J, Jansen EH, Diamant M, et al. (2010) Eicosapentaenoic acid as an add-on to antidepressant medication for co-morbid major depression in patients with diabetes mellitus: a randomized, double-blind placebo-controlled study. J Affect Disord 126: 282-286.

95. Rondanelli M, Giacosa A, Opizzi A, Pelucchi C, La Vecchia C, et al. (2010) Effect of omega-3 fatty acids supplementation on depressive symptoms and on health-related quality of life in the treatment of elderly women with depression: a double-blind, placebo-controlled, randomized clinical trial. J Am Coll Nutr 29: 55-64.

96. Rizzo AM, Corsetto PA, Montorfano G, Opizzi A, Faliva M, et al. (2012) Comparison between the AA/EPA ratio in depressed and non depressed elderly females: omega-3 fatty acid supplementation correlates with improved symptoms but does not change immunological parameters. Nutr J 11: 82.

97. Tajalizadekhoob Y, Sharifi F, Fakhrzadeh H, Mirarefin M, Ghaderpanahi M, et al. (2011) The effect of low-dose omega 3 fatty acids on the treatment of mild to moderate depression in the elderly: a double-blind, randomized, placebo-controlled study. Eur Arch Psychiatry Clin Neurosci 261: 539-549.

98. Nemets H, Nemets B, Apter A, Bracha Z, Belmaker RH (2006) Omega-3 treatment of childhood depression: a controlled, double-blind pilot study. Am J Psychiatry 163: 1098-1100.

99. Freeman MP, Davis M, Sinha P, Wisner KL, Hibbeln JR, et al. (2008) Omega-3 fatty acids and supportive psychotherapy for perinatal depression: a randomized placebo-controlled study. J Affect Disord 110: 142-148.

100. Giltay EJ, Geleijnse JM, Kromhout D (2011) Effects of n-3 fatty acids on depressive symptoms and dispositional optimism after myocardial infarction. Am J Clin Nutr 94: 1442-1450.

101. DeFina LF, Marcoux LG, Devers SM, Cleaver JP, Willis BL (2011) Effects of omega-3 supplementation in combination with diet and exercise on weight loss and body composition. Am J Clin Nutr 93: 455-462.

102. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Glaser R (2011) Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. Brain Behav Immun 25: 1725-1734.

103. Barbui C, Cipriani A, Patel V, Ayuso-Mateos JL, van Ommeren M (2011) Efficacy of antidepressants and benzodiazepines in minor depression: systematic review and meta-analysis. Br J Psychiatry 198: 11-16, sup 11.

104. Iovieno N, Papakostas GI (2012) Correlation between different levels of placebo response rate and clinical trial outcome in major depressive disorder: a meta-analysis. J Clin Psychiatry 73: 1300-1306.

105. Souery D, Papakostas GI, Trivedi MH (2006) Treatment-resistant depression. J Clin Psychiatry 67 Suppl 6: 16-22.

106. Freeman MP, Hibbeln JR, Wisner KL, Davis JM, Mischoulon D, et al. (2006) Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. J Clin Psychiatry 67: 1954-1967.

107. Lin PY, Su KP (2007) A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. J Clin Psychiatry 68: 1056-1061.

108. Ross BM, Seguin J, Sieswerda LE (2007) Omega-3 fatty acids as treatments for mental illness: which disorder and which fatty acid? Lipids Health Dis 6: 21.

109. Martins JG (2009) EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: evidence from a meta-analysis of randomized controlled trials. J Am Coll Nutr 28: 525-542.

110. Balanza-Martinez V, Fries GR, Colpo GD, Silveira PP, Portella AK, et al. (2011) Therapeutic use of omega-3 fatty acids in bipolar disorder. Expert Rev Neurother 11: 1029-1047.

111. Montgomery P, Richardson AJ (2008) Omega-3 fatty acids for bipolar disorder. Cochrane Database Syst Rev: CD005169.

112. Sarris J, Mischoulon D, Schweitzer I (2012) Omega-3 for bipolar disorder: meta-analyses of use in mania and bipolar depression. J Clin Psychiatry 73: 81-86.

113. Mocking RJ, Assies J, Bot M, Jansen EH, Schene AH, et al. (2012) Biological effects of add-on eicosapentaenoic acid supplementation in

diabetes mellitus and co-morbid depression: a randomized controlled trial. PLoS One 7: e49431.

114. Caraci F, Copani A, Nicoletti F, Drago F (2010) Depression and Alzheimer's disease: neurobiological links and common pharmacological targets. Eur J Pharmacol 626: 64-71.

115. Caraci F, Bosco P, Signorelli M, Spada RS, Cosentino FI, et al. (2012) The CC genotype of transforming growth factor-beta1 increases the risk of late-onset Alzheimer's disease and is associated with AD-related depression. Eur Neuropsychopharmacol 22: 281-289.

116. Salomone S, Caraci F, Leggio GM, Fedotova J, Drago F (2012) New pharmacological strategies for treatment of Alzheimer's disease: focus on disease modifying drugs. Br J Clin Pharmacol 73: 504-517.

117. Caraci F, Spampinato S, Sortino MA, Bosco P, Battaglia G, et al. (2012) Dysfunction of TGF-beta1 signaling in Alzheimer's disease: perspectives for neuroprotection. Cell Tissue Res 347: 291-301.

118. Caraci F, Battaglia G, Bruno V, Bosco P, Carbonaro V, et al. (2011) TGF-beta1 pathway as a new target for neuroprotection in Alzheimer's disease. CNS Neurosci Ther 17: 237-249.

119. Markhus MW, Skotheim S, Graff IE, Froyland L, Braarud HC, et al. (2013) Low omega-3 index in pregnancy is a possible biological risk factor for postpartum depression. PLoS One 8: e67617.

120. Arterburn LM, Hall EB, Oken H (2006) Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr 83: 1467S-1476S.

121. Calder PC (2006) n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 83: 1505S-1519S.

122. Brooks JD, Milne GL, Yin H, Sanchez SC, Porter NA, et al. (2008) Formation of highly reactive cyclopentenone isoprostane compounds

(A3/J3-isoprostanes) in vivo from eicosapentaenoic acid. J Biol Chem 283: 12043-12055.

123. Bhattacharya A, Sun D, Rahman M, Fernandes G (2007) Different ratios of eicosapentaenoic and docosahexaenoic omega-3 fatty acids in commercial fish oils differentially alter pro-inflammatory cytokines in peritoneal macrophages from C57BL/6 female mice. J Nutr Biochem 18: 23-30.

124. Zhao Y, Joshi-Barve S, Barve S, Chen LH (2004) Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. J Am Coll Nutr 23: 71-78.

125. Sierra S, Lara-Villoslada F, Comalada M, Olivares M, Xaus J (2008) Dietary eicosapentaenoic acid and docosahexaenoic acid equally incorporate as decosahexaenoic acid but differ in inflammatory effects. Nutrition 24: 245-254.

126. Umhau JC, Dauphinais KM, Patel SH, Nahrwold DA, Hibbeln JR, et al. (2006) The relationship between folate and docosahexaenoic acid in men. Eur J Clin Nutr 60: 352-357.

127. Gao F, Kiesewetter D, Chang L, Ma K, Rapoport SI, et al. (2009) Whole-body synthesis secretion of docosahexaenoic acid from circulating eicosapentaenoic acid in unanesthetized rats. J Lipid Res 50: 2463-2470.

128. Chang CY, Ke DS, Chen JY (2009) Essential fatty acids and human brain. Acta Neurol Taiwan 18: 231-241.

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

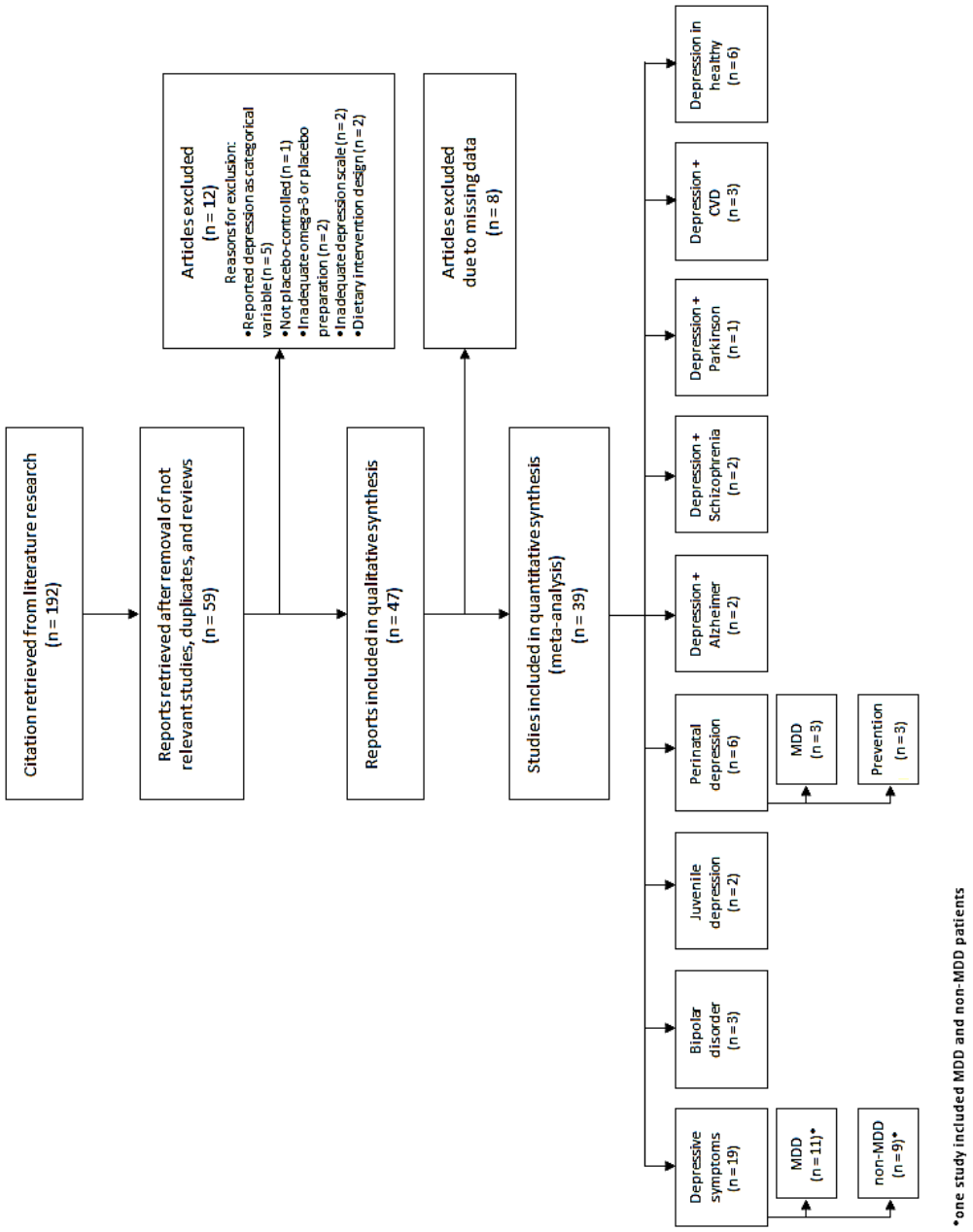


Figure 2. Forest plot showing individual and combined effect size estimates and 95% CIs for 19 trials grouped in those conducted on patients with a DSM-defined diagnosis of major depressive disorder (MDD group, n = 11) and those on patients with an assessment of depression but not rigorously diagnosed 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.

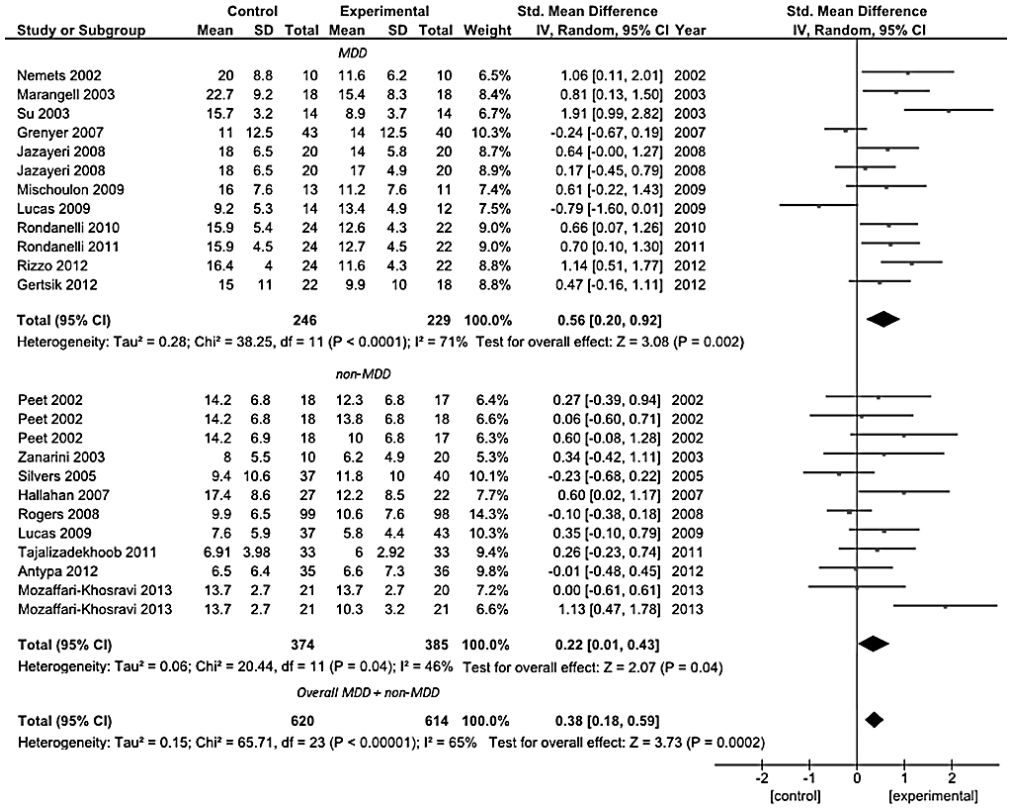


Figure 3. Funnel plot of effect size estimates for individual trials conducted on patients with depressive disorder without secondary comorbidities (MDD group and non-MDD group, n = 19).

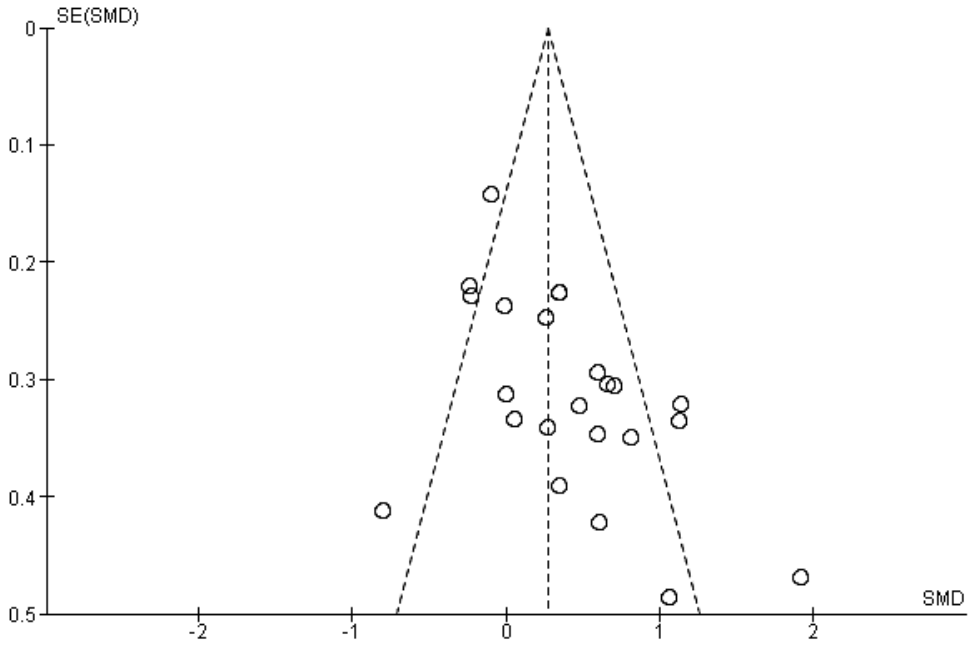


Figure 4. Forest plot examining the effect of the type of omega-3 PUFA supplementation employed on the reduction in depressive symptoms (MDD group and non-MDD group, n = 19).

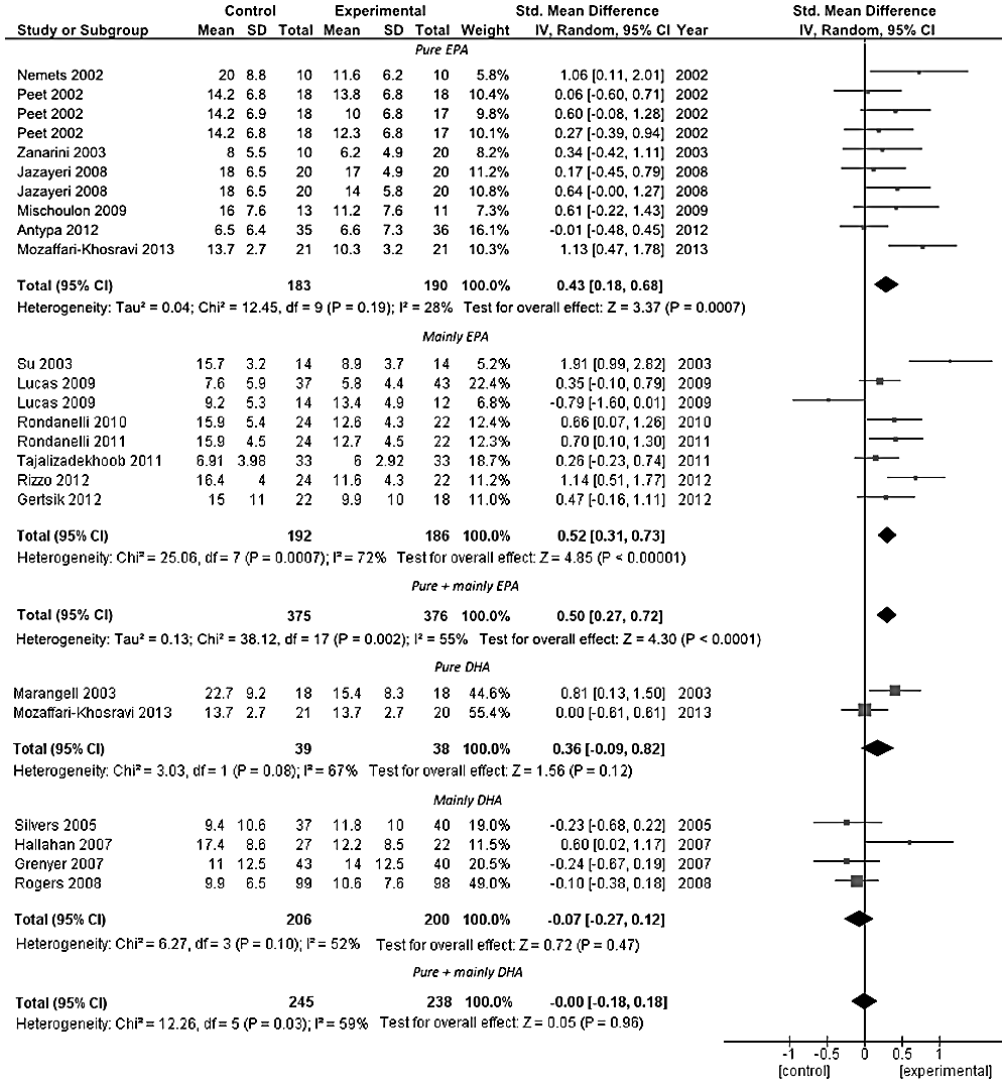


Figure 5. Forest plot showing individual and combined effect size estimates and 95% CIs for 3 trials conducted on patients with bipolar depression.

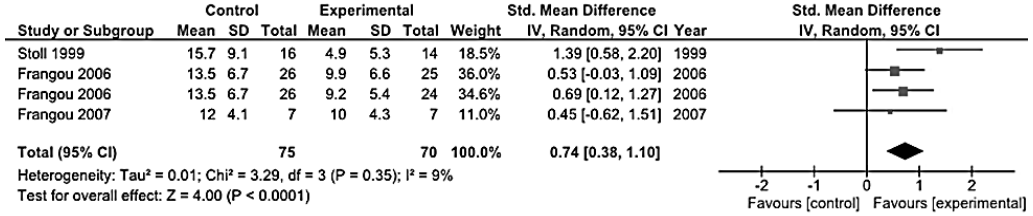



Figure 6. Forest plot showing individual and combined effect size estimates and 95% CIs for 3 trials conducted on pregnant women with major depressive disorder.

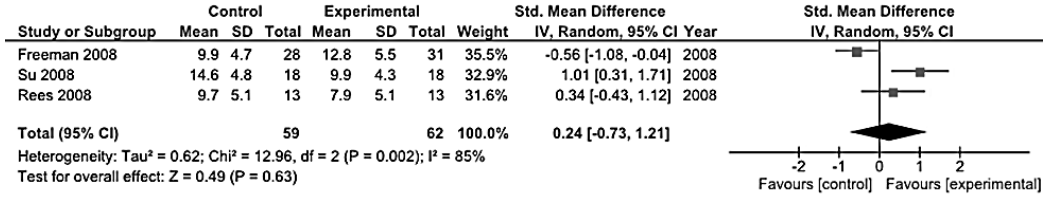



Figure 7. Forest plot showing individual and combined effect size estimates and 95% CIs for 3 trials conducted on healthy pregnant women for prevention of post-partum depression.

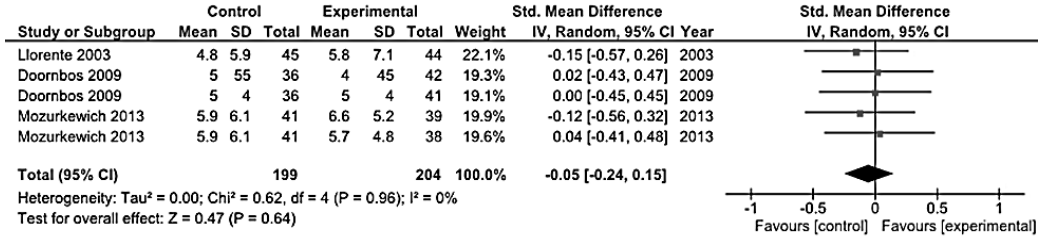



Figure 8. Forest plot showing individual and combined effect size estimates and 95% CIs for 2 trials conducted on patients with Alzheimer or mild cognitive impairment.

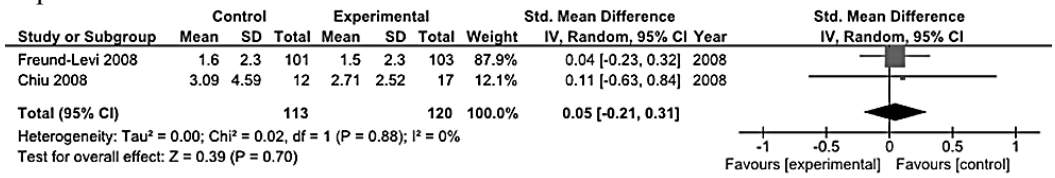


Figure 9. Forest plot showing individual and combined effect size estimates and 95% CIs for 2 trials conducted on patients with schizophrenia.

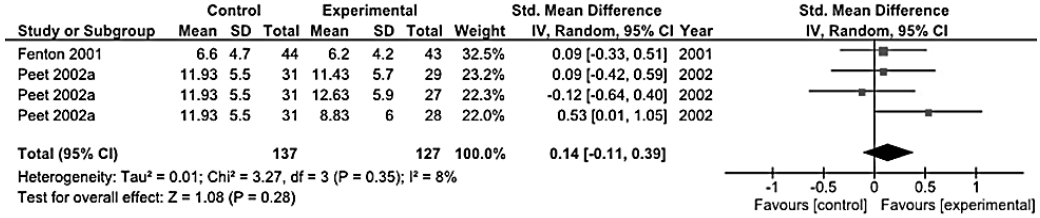



Figure 10. Forest plot showing individual and combined effect size estimates and 95% CIs for 3 trials conducted on patients with cardiovascular disease.

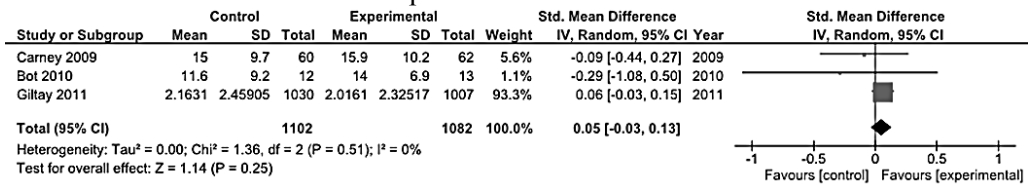



Figure 11. Forest plot showing individual and combined effect size estimates and 95% CIs for 6 trials conducted on healthy individuals for prevention of depressive symptoms.

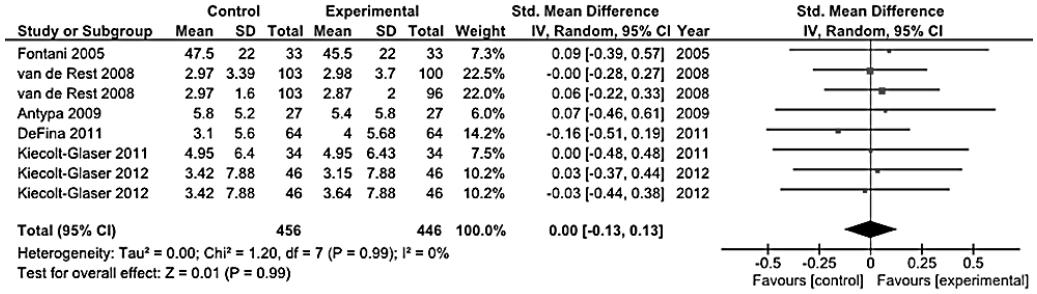


Table 1. Randomized controlled trials investigating effects of omega-3 polyunsaturated fatty acids (PUFAs) on depressed mood listed in chronological order by type of depressive disorder.

| Author | Year | Partecipating Group | Subjects, n (I/C) | Type of treatment | Intervention | Placebo | Daily dose | Duration (weeks) | Outcome measure | Study quality |
|---|---|---|---|---|---|---|---|---|---|---|
| *MDD* | | | | | | | | | | |
| 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 |
| *Non-MDD* | | | | | | | | | | |
| Behan [39] | 1990 | Pz with post viral fatigue | 63 (39/24) | NR | EPA + DHA | Liquid paraffin + 0.4 g LA | 0.14 g EPA + 0.09 g DHA | 13 | 4-point Linkert scale | 8 |
| Warren [56] | 1999 | Pz with chronic fatigue syndrome | 50 (24/26) | None | EPA + DHA | Sunflower oil | 0.14 g EPA + 0.9 g DHA | 13 | BDI | 8 |
| Peet [54] | 2002 | Pz treated for depression | 70 (17/18/17/ 18) | Mixed antidepressants | E-EPA | Liquid paraffin | 1g; 2g; 4 g | 12 | HDRS, MADRS, BDI | 7 |
| Zanarini [91] | 2003 | Pz with borderline personality disorder | 30 (20/10) | Heterogeneous | E-EPA | Mineral oil | 1 g | 8 | MADRS | 6 |
| Fux [57] | 2004 | Pz with obsessive compulsive disorder | 11 (11/11) (within-subjects crossover design) | Heterogeneous | E-EPA | Liquid paraffin | 2 g | 6 | HDRS | 6 |
| Silvers [79] | 2005 | Pz treated for depression | 77 (40/37) | Mixed antidepressants | EPA + DHA | Olive oil | 0.6 g EPA + 2.4 g DHA | 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 | 1.2 g EPA + 0.9 DHA | 12 | BDI, HDRS | 9 |
| Rogers [86] | 2008 | Untreated pz with mild-to-moderate depression | 218 (109/109) | None | EPA + DHA | Olive oil | 0.63 EPA 0.85 DHA | 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 |
| Tajalizadekhoob [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-mestrual syndrome | 139 (70/69) | 113 sedative, 26 none | EPA + DHA | NR | 0.24 EPA +0.36 DHA | 12 | VAS | 8 |
| *Bipolar disorder* | | | | | | | | | | |
| 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 |
| Keck [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 |
| *Depression or bipolar disorder in children and adolescents* | | | | | | | | | | |
| 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 psycosis | 81 (41/40) | Heterogeneous | EPA+DHA | Coconut oil | 0.70 g EPA + 0.48 g DHA | 48 | MADRS, SCID | 11 |
| *Peritanal MDD* | | | | | | | | | | |
| 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 |
| *Prevention of post-partum depression* | | | | | | | | | | |
| Llorente [85] | 2003 | Healthy pregnant women | 99 (44/45) | None | DHA | NR | 0.2 g | 16 | BDI | 10 |
| Doornbos [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 |
| *Depressive symptoms in pz with schizophrenia* | | | | | | | | | | |
| 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 |
| *MDD in pz with Parkinson's disease* | | | | | | | | | | |
| 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 |
| *Depressive symptoms in healthy subjects* | | | | | | | | | | |
| 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 | 26 | CES-D, MADRS, GDS-15 | 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 1g 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: etyl-eicosapentaenoic 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 Score.

# CHAPTER 2

*Epidemiology and Psychiatric Sciences 2015, UNDER REVIEW*

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

G. Grosso[1], S. Marventano[2], S. Castellano[1], F. Caraci[3,4], F. Drago[1], F. Galvano[1]

[1]*Department of Clinical and Molecular Biomedicine, Section of Pharmacology and Biochemistry, University of Catania, Catania, Italy*
[2]*Department "G.F. Ingrassia", Section of Hygiene and Public Health, University of Catania, Catania, Italy*
[3] *Department of Educational Sciences, University of Catania, Catania, Italy;*
[4] *IRCCS Associazione Oasi Maria S.S. – Institute for Research on Mental Retardation and Brain Aging, Troina, Enna, Italy*

## 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 studies 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, Kyrozis 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, Kyrozis 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, Kyrozis 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, Kyrozis 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 medicament 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 Myiake 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.

**References**

**Albanese E, Lombardo FL, Dangour AD, Guerra M, Acosta D, Huang Y, Jacob KS, Llibre Rodriguez Jde J, Salas A, Schonborn C, Sosa AL, Williams J, Prince MJ , Ferri CP** (2012). No association between fish intake and depression in over 15,000 older adults from seven low and middle income countries--the 10/66 study. *PLoS One* **7**, e38879.

**Appleton KM, Peters TJ, Hayward RC, Heatherley SV, Mcnaughton SA, Rogers PJ, Gunnell D, Ness AR , Kessler D** (2007a). Depressed mood and n-3 polyunsaturated fatty acid intake from fish: non-linear or confounded association? *Social Psychiatry and Psychiatric Epidemiology* **42**, 100-104.

**Appleton KM, Woodside JV, Yarnell JW, Arveiler D, Haas B, Amouyel P, Montaye M, Ferrieres J, Ruidavets JB, Ducimetiere P, Bingham A , Evans A** (2007b). Depressed mood and dietary fish intake: direct relationship or indirect relationship as a result of diet and lifestyle? *Journal of Affective Disorders* **104**, 217-223.

**Astorg P, Couthouis A, Bertrais S, Arnault N, Meneton P, Guesnet P, Alessandri JM, Galan P , Hercberg S** (2008). Association of fish and long-chain n-3 polyunsaturated fatty acid intakes with the occurrence of depressive episodes in middle-aged French men and women. *Prostaglandins, Leukotrienes & Essential Fatty Acids* **78**, 171-182.

**Barberger-Gateau P, Jutand MA, Letenneur L, Larrieu S, Tavernier B , Berr C** (2005). Correlates of regular fish consumption in French elderly community dwellers: data from the Three-City study. *European Journal of Clinical Nutrition* **59**, 817-825.

**Beydoun MA, Fanelli Kuczmarski MT, Beydoun HA, Hibbeln JR, Evans MK , Zonderman AB** (2013). Omega-3 fatty acid intakes are inversely related to elevated depressive symptoms among United States women. *Journal of Nutrition* **143**, 1743-1752.

**Bountziouka V, Polychronopoulos E, Zeimbekis A, Papavenetiou E, Ladoukaki E, Papairakleous N, Gotsis E, Metallinos G, Lionis C , Panagiotakos D** (2009). Long-term fish intake is associated with less severe depressive symptoms among elderly men and women: the MEDIS (MEDiterranean ISlands Elderly) epidemiological study. *Journal of Aging and Health* **21**, 864-880.

**Bourre JM** (2004). Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing. *The journal of nutrition, health & aging* **8**, 163-174.

**Caraci F, Copani A, Nicoletti F, Drago F** (2010). Depression and Alzheimer's disease: neurobiological links and common pharmacological targets. *European Journal of Pharmacology* **626**, 64-71.

**Chang CY, Ke DS , Chen JY** (2009). Essential fatty acids and human brain. *Acta Neurologica Taiwanica* **18**, 231-241.

**Colangelo LA, He K, Whooley MA, Daviglus ML , Liu K** (2009). Higher dietary intake of long-chain omega-3 polyunsaturated fatty acids is inversely associated with depressive symptoms in women. *Nutrition* **25**, 1011-1019.

**Da Rocha CM , Kac G** (2012). High dietary ratio of omega-6 to omega-3 polyunsaturated acids during pregnancy and prevalence of post-partum depression. *Maternal & Child Nutrition* **8,** 36-48.

**Daley C, Patterson A, Sibbritt D , Macdonald-Wicks L** (2014). Unsaturated fat intakes and mental health outcomes in young women from the Australian Longitudinal Study on Women's Heath. *Public Health Nutrition* ***9***, 1-8.

**De Vriese SR, Christophe AB , Maes M** (2003). Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. *Life Sciences* **73**, 3181-3187.

**Edwards R, Peet M, Shay J , Horrobin D** (1998). Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. *Journal of Affective Disorders* **48**, 149-155.

**Giles GE, Mahoney CR , Kanarek RB** (2013). Omega-3 fatty acids influence mood in healthy and depressed individuals. *Nutrition Reviews* **71**, 727-741.

**Golding J, Steer C, Emmett P, Davis JM , Hibbeln JR** (2009). High levels of depressive symptoms in pregnancy with low omega-3 fatty acid intake from fish. *Epidemiology* **20**, 598-603.

**Grosso G, Galvano F, Marventano S, Malaguarnera M, Bucolo C, Drago F , Caraci F** (2014a). Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. *Oxidative Medicine and Cellular Longevity* **2014**, 313570.

**Grosso G, Pajak A, Marventano S, Castellano S, Galvano F, Bucolo C, Drago F , Caraci F** (2014b). Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. *PLoS One* **9**, e96905.

**Hakkarainen R, Partonen T, Haukka J, Virtamo J, Albanes D , Lonnqvist J** (2004). Is low dietary intake of omega-3 fatty acids associated with depression? *The American Journal of Psychiatry* **161**, 567-569.

**Harris WS** (2006). The omega-6/omega-3 ratio and cardiovascular disease risk: uses and abuses. *Current Atherosclerosis Reports* **8**, 453–9.

**Hepgul N, Mondelli V , Pariante CM** (2010). Psychological and biological mechanisms of cytokine induced depression. *Epidemiologia e Psichiatria Sociale* **19**, 98-102.

**Hibbeln JR** (1998). Fish consumption and major depression. *Lancet* **351**, 1213.

**Hibbeln JR** (2002). Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression: a cross-national, ecological analysis. *Journal of Affective Disorders* **69,** 15-29.

**Hibbeln JR, Linnoila M, Umhau JC, Rawlings R, George DT , Salem N, Jr.** (1998). Essential fatty acids predict metabolites of serotonin and dopamine in cerebrospinal fluid among healthy control subjects, and early- and late-onset alcoholics. *Biological Psychiatry* **44**, 235-242.

**Hibbeln JR, Nieminen LR, Blasbalg TL, Riggs JA , Lands WE** (2006). Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. *The American Journal of Clinical Nutrition* **83**, 1483S-1493S.

**Hibbeln JR , Salem N, Jr.** (1995). Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy. *The American Journal of Clinical Nutrition* **62**, 1-9.

**Huffman JC, Celano CM, Beach SR, Motiwala SR, Januzzi JL** (2013). Depression and cardiac disease: epidemiology, mechanisms, and diagnosis. *Cardiovascular Psychiatry and Neurology* **2013**, 695925.

**Jacka FN, Pasco JA, Williams LJ, Meyer BJ, Digger R , Berk M** (2013). Dietary intake of fish and PUFA, and clinical depressive and anxiety disorders in women. *British Journal of Nutrition* **109**, 2059-2066.

**Kamphuis MH, Geerlings MI, Tijhuis MA, Kalmijn S, Grobbee DE , Kromhout D** (2006). Depression and cardiovascular mortality: a role

for n-3 fatty acids? *The American Journal of Clinical Nutrition* **84**, 1513-1517.

**Kesse-Guyot E, Touvier M, Andreeva VA, Jeandel C, Ferry M, Hercberg S , Galan P** (2012). Cross-sectional but not longitudinal association between n-3 fatty acid intake and depressive symptoms: results from the SU.VI.MAX 2 study. *American Journal of Epidemiology* **175**, 979-987.

**Kyrozis A, Psaltopoulou T, Stathopoulos P, Trichopoulos D, Vassilopoulos D , Trichopoulou A** (2009). Dietary lipids and geriatric depression scale score among elders: the EPIC-Greece cohort. *Journal of Psychiatric Research* **43**, 763-769.

**Li Y, Dai Q, Ekperi LI, Dehal A , Zhang J** (2011). Fish consumption and severely depressed mood, findings from the first national nutrition follow-up study. *Psychiatry Research* **190**, 103-109.

**Lin PY, Mischoulon D, Freeman MP, Matsuoka Y, Hibbeln J, Belmaker RH , Su KP** (2012). Are omega-3 fatty acids antidepressants or just mood-improving agents? The effect depends upon diagnosis, supplement preparation, and severity of depression. *Molecular Psychiatry* **17**, 1161-1163; author reply 1163-1167.

**Lucas M, Mirzaei F, O'reilly EJ, Pan A, Willett WC, Kawachi I, Koenen K , Ascherio A** (2011). Dietary intake of n-3 and n-6 fatty acids and the risk of clinical depression in women: a 10-y prospective follow-up study. *The American Journal of Clinical Nutrition* **93**, 1337-1343.

**Maes M, Yirmyia R, Noraberg J, Brene S, Hibbeln J, Perini G, Kubera M, Bob P, Lerer B, Maj M** (2009). The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metabolic Brain Disease* **24**, 27-53.

**Markhus MW, Skotheim S, Graff IE, Froyland L, Braarud HC, Stormark KM , Malde MK** (2013). Low omega-3 index in pregnancy is a possible biological risk factor for postpartum depression. *PLoS One* **8**, e67617.

**Miyake Y, Sasaki S, Yokoyama T, Tanaka K, Ohya Y, Fukushima W, Saito K, Ohfuji S, Kiyohara C , Hirota Y** (2006). Risk of postpartum depression in relation to dietary fish and fat intake in Japan: the Osaka Maternal and Child Health Study. *Psychological Medicine* **36**, 1727-1735.

**Murakami K, Miyake Y, Sasaki S, Tanaka K , Arakawa M** (2010). Fish and n-3 polyunsaturated fatty acid intake and depressive symptoms: Ryukyus Child Health Study. *Pediatrics* **126**, e623-630.

**Murakami K, Mizoue T, Sasaki S, Ohta M, Sato M, Matsushita Y , Mishima N** (2008). Dietary intake of folate, other B vitamins, and omega-3 polyunsaturated fatty acids in relation to depressive symptoms in Japanese adults. *Nutrition* **24**, 140-147.

**Noaghiul S , Hibbeln JR** (2003). Cross-national comparisons of seafood consumption and rates of bipolar disorders. *American Journal of Psychiatry* **160**, 2222-2227.

**Oddy WH, Hickling S, Smith MA, O'sullivan TA, Robinson M, De Klerk NH, Beilin LJ, Mori TA, Syrette J, Zubrick SR , Silburn SR** (2011). Dietary intake of omega-3 fatty acids and risk of depressive symptoms in adolescents. *Depression and Anxiety* **28**, 582-588.

**Parletta N, Milte CM , Meyer BJ** (2013). Nutritional modulation of cognitive function and mental health. *The Journal of Nutritional Biochemistry* **24**, 725-743.

**Peet M, Murphy B, Shay J , Horrobin D** (1998). Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biological Psychiatry* **43**, 315-319.

**Sanchez-Villegas A, Henriquez P, Figueiras A, Ortuno F, Lahortiga F , Martinez-Gonzalez MA** (2007). Long chain omega-3 fatty acids intake, fish consumption and mental disorders in the SUN cohort study. *European Journal of Nutrition* **46**, 337-346.

**Sanchez-Villegas A , Martinez-Gonzalez MA** (2013). Diet, a new target to prevent depression? *BMC Medicine* **11**, 3.

**Sontrop J, Avison WR, Evers SE, Speechley KN , Campbell MK** (2008). Depressive symptoms during pregnancy in relation to fish consumption and intake of n-3 polyunsaturated fatty acids. *Paediatric and Perinatal Epidemiology* **22**, 389-399.

**Strom M, Mortensen EL, Halldorsson TI, Thorsdottir I , Olsen SF** (2009). Fish and long-chain n-3 polyunsaturated fatty acid intakes during pregnancy and risk of postpartum depression: a prospective study based on a large national birth cohort. *The American Journal of Clinical Nutrition* **90**, 149-155.

**Suominen-Taipale AL, Partonen T, Turunen AW, Mannisto S, Jula A , Verkasalo PK** (2010). Fish consumption and omega-3

polyunsaturated fatty acids in relation to depressive episodes: a cross-sectional analysis. *PLoS One* **5**, e10530.

**Taha AY, Cheon Y, Faurot KF, Macintosh B, Majchrzak-Hong SF, Mann JD, Hibbeln JR, Ringel A , Ramsden CE** (2014). Dietary omega-6 fatty acid lowering increases bioavailability of omega-3 polyunsaturated fatty acids in human plasma lipid pools. *Prostaglandins, Leukotrienes & Essential Fatty Acids* **90**, 151-157.

**Tanskanen A, Hibbeln JR, Tuomilehto J, Uutela A, Haukkala A, Viinamaki H, Lehtonen J , Vartiainen E** (2001). Fish consumption and depressive symptoms in the general population in Finland. *Psychiatric Services* **52**, 529-531.

**Timonen M, Horrobin D, Jokelainen J, Laitinen J, Herva A , Rasanen P** (2004). Fish consumption and depression: the Northern Finland 1966 birth cohort study. *Journal of Affective Disorders* **82**, 447-452.

**Vaz JS, Kac G, Nardi AE , Hibbeln JR** (2014). Omega-6 fatty acids and greater likelihood of suicide risk and major depression in early pregnancy. *Journal of Affective Disorders* **152-154**, 76-82.

Figure 1. Process of studies identification and inclusion.

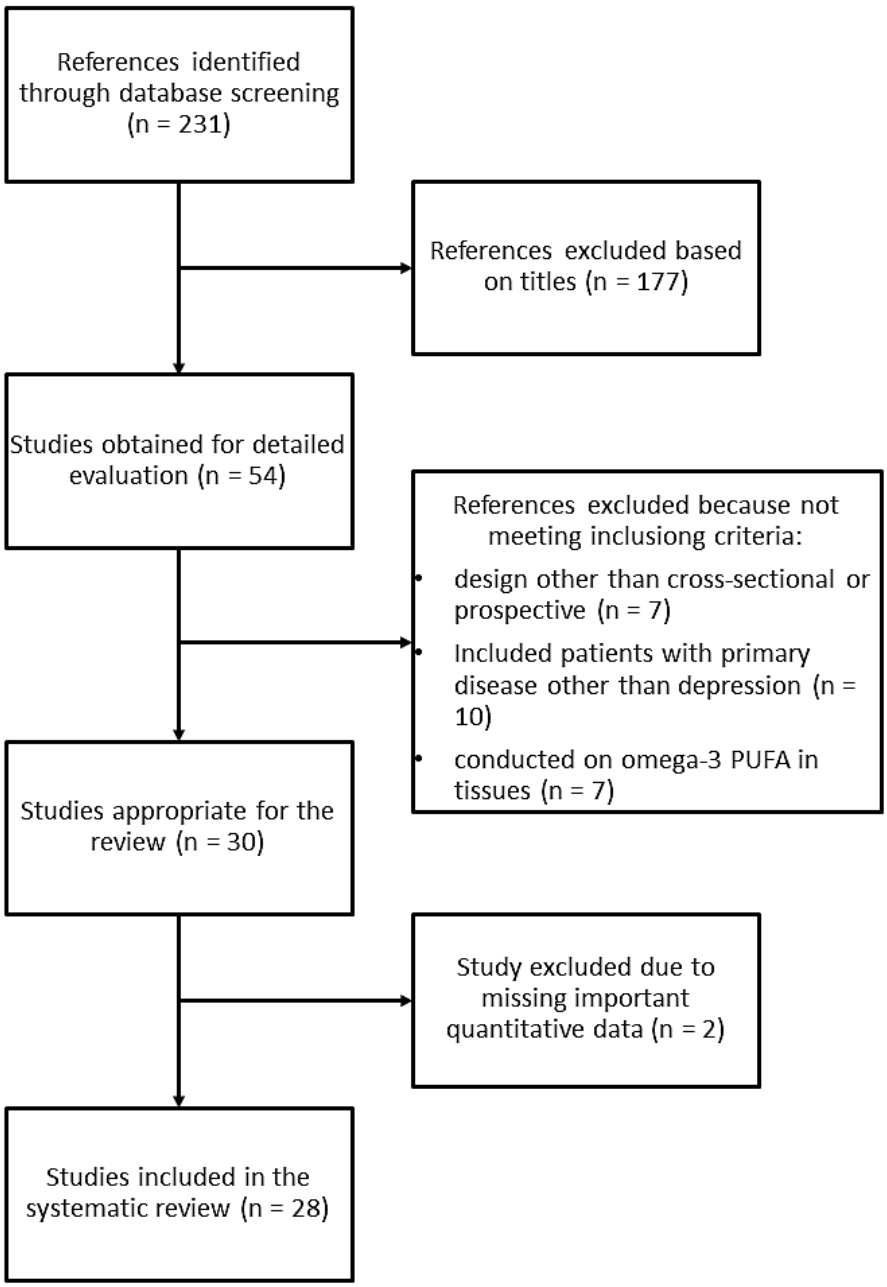


Table 1. Characteristics of the epidemiological studies conducted on fish and/or omega-3 PUFA consumption and depression.

| 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 (Zutphen 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 medicament 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 (schoolstudents) | 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 | Edimburgh 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 | - |

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 | <59 mg/d | >156 mg/d | 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 leasure 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 | 36.5 g/d | 176.9 g/d | 4 |
| | | | Omega-3 (highest vs. lowest quintile), OR 1.04 (95% CI: 0.78, 1.40), P for trend 0.376 | 0.39 g/d | 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 |
| | | | Omega-3 (highest vs. lowest tertile), OR 0.79 (95% CI: 0.58, 1.15) | 0.075% energy | 0.135% energy | |
| Murakami et al, 2008 | 112 men and 76 women | Age, BMI, work place, marital status, occupational physical activity, leasure-time physical activity, smoking status, alcohol drinking, and job stress score. | Men - Omega-3 (higest vs. lowest quartile), OR 0.58 (95% CI: 0.28, 1.19) , P for trend 0.13 | 0.91% energy | 1.71% energy | 3 |
| | | | Women - Omega-3 (highest vs. lowest quartile), OR 1.46 (95% CI: 0.57, 3.76), P for trend 0.54 | 1.09% energy | 1.87% energy | |
| | | | Men - alpha-Lynolenic acid (highest vs. lowest quartile), OR 0.37 (95% CI: 0.17, 0.83), P for trend 0.058 | | | |
| | | | Women - alpha-Lynolenic acid (highest vs. lowest quartile), OR 0.83 (95% CI: 0.32, 2.12), P for trend 0.59 | | | |
| 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 | 9.1 g/1000 kcal | 29.1 g/1000 kcal | 5 |
| | | | Females - Fish consumption (highest vs. lowest quintile), OR 1.01 (95% CI: 0.80, 1.28), P for trend 0.79 | 9.2 g/1000 kcal | 30 g/1000 kcal | |
| | | | Males - Omega-3 (highest vs. lowest quintile), OR 0.72 (95% CI: 0.55, 0.98), P for trend 0.08 | 0.073% energy | 0.297% energy | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | 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 |
| | | | Females - Fish consumption (<1/week vs. >1 week), OR 1.15 (95% CI: 0.83, 1.59), P for trend 0.40 | | | |
| 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, | 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 | 8 |
| | | | 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, alchol 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 (higest vs. lowest tertile), OR 1.31 (95% CI: 1.64, 2.69) | 210 mg/d | 480 mg/d | 4 |
| | | | DHA (higest vs. lowest tertile), OR 1.44 (95% CI: 0.73, 2.83) | | | |
| 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) | 0.41% energy | 1.14% energy | 6 |
| | | | Women - Omega-3 (highest vs. lowest tertile), OR 0.51 (95% CI: 0.27, 0.95) | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | 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 | 4 |
| Strom et al, 2009 | PPD-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 | 5 |
| | PPD-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: | 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.

## REFERENCES

1. A. P. Simopoulos, "The importance of the ratio of omega-6/omega-3 essential fatty acids," Biomed Pharmacother, vol. 56, no. 8, pp. 365-379, 2002.
2. J. N. Din, D. E. Newby, A. D. Flapan, "Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment," BMJ, vol. 328, no. 7430, pp. 30-35, 2004.
3. A. P. Simopoulos, "Executive summary of the International Conference on Genetic Variation and Nutrition," World Rev Nutr Diet, vol. 63, no. pp. 1-13, 1990.
4. A. P. Simopoulos and J. Ordovas, "Nutrigenetics and nutrigenomics," vol. 93, Karger, Washington D.C, 2004.

5. A. P. Simopoulos, "Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases," Biomed Pharmacother, vol. 60, no. 9, pp. 502-507, 2006.
6. A. J. Sinclair, D. Begg, M. Mathai, R. S. Weisinger, "Omega 3 fatty acids and the brain: review of studies in depression," Asia Pac J Clin Nutr, vol. 16 Suppl 1, no. pp. 391-397, 2007.
7. C. J. Murray, A. D. Lopez, "Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study," Lancet, vol. 349, no. 9063, pp. 1436-1442, 1997.
8. S. Rivelli, W. Jiang, "Depression and ischemic heart disease: what have we learned from clinical trials?," Curr Opin Cardiol, vol. 22, no. 4, pp. 286-291, 2007.
9. World Health Organization, "The global burden of disease: 2004 update," Geneva, 2008.
10. R. C. Kessler, P. Berglund, O. Demler et al., "The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R)," JAMA, vol. 289, no. 23, pp. 3095-3105, 2003.
11. L. Andrade, J. J. Caraveo-Anduaga, P. Berglund et al., "The epidemiology of major depressive episodes: results from the International Consortium of Psychiatric Epidemiology (ICPE) Surveys," Int J Methods Psychiatr Res, vol. 12, no. 1, pp. 3-21, 2003.
12. D. S. Hasin, R. D. Goodwin, F. S. Stinson, B. F. Grant, "Epidemiology of major depressive disorder: results from the National Epidemiologic Survey on Alcoholism and Related Conditions," Arch Gen Psychiatry, vol. 62, no. 10, pp. 1097-1106, 2005.
13. S. B. Patten, "Accumulation of major depressive episodes over time in a prospective study indicates that retrospectively assessed lifetime prevalence estimates are too low," BMC Psychiatry, vol. 9, no. pp. 19, 2009.
14. World Health Organization, "A public health approach to mental health," Geneva, 2001.
15. P. Sobocki, B. Jonsson, J. Angst, C. Rehnberg, "Cost of depression in Europe," J Ment Health Policy Econ, vol. 9, no. 2, pp. 87-98, 2006.
16. J. Tiihonen, J. Lonnqvist, K. Wahlbeck et al., "No mental health without physical health," Lancet, vol. 377, no. 9766, pp. 611, 2011.
17. M. Prince, V. Patel, S. Saxena et al., "No health without mental health," Lancet, vol. 370, no. 9590, pp. 859-877, 2007.
18. A. Sanchez-Villegas, M. A. Martinez-Gonzalez, "Diet, a new target to prevent depression?," BMC Med, vol. 11, no. pp. 3, 2013.

19. R. Machado-Vieira, A. G. Mallinger, "Abnormal function of monoamine oxidase-A in comorbid major depressive disorder and cardiovascular disease: pathophysiological and therapeutic implications (review)," Mol Med Rep, vol. 6, no. 5, pp. 915-922, 2012.
20. D. P. Do, J. B. Dowd, N. Ranjit, J. S. House, G. A. Kaplan, "Hopelessness, depression, and early markers of endothelial dysfunction in U.S. adults," Psychosom Med, vol. 72, no. 7, pp. 613-619, 2010.
21. W. E. Severus, A. B. Littman, A. L. Stoll, "Omega-3 fatty acids, homocysteine, and the increased risk of cardiovascular mortality in major depressive disorder," Harv Rev Psychiatry, vol. 9, no. 6, pp. 280-293, 2001.
22. H. Ito, R. Kawashima, S. Awata et al., "Hypoperfusion in the limbic system and prefrontal cortex in depression: SPECT with anatomic standardization technique," J Nucl Med, vol. 37, no. 3, pp. 410-414, 1996.
23. T. A. Kimbrell, T. A. Ketter, M. S. George et al., "Regional cerebral glucose utilization in patients with a range of severities of unipolar depression," Biol Psychiatry, vol. 51, no. 3, pp. 237-252, 2002.
24. N. J. Stapelberg, D. L. Neumann, D. H. Shum, H. McConnell, I. Hamilton-Craig, "A topographical map of the causal network of mechanisms underlying the relationship between major depressive disorder and coronary heart disease," Aust N Z J Psychiatry, vol. 45, no. 5, pp. 351-369, 2011.
25. B. K. Puri, "Cardiovascular disease and depression: the PUFA connection," Int J Clin Pract, vol. 62, no. 3, pp. 355-357, 2008.
26. P. H. Cui, N. Petrovic, M. Murray, "The omega-3 epoxide of eicosapentaenoic acid inhibits endothelial cell proliferation by p38 MAP kinase activation and cyclin D1/CDK4 down-regulation," Br J Pharmacol, vol. 162, no. 5, pp. 1143-1155, 2011.
27. F. Pifferi, M. Jouin, J. M. Alessandri et al., "n-3 Fatty acids modulate brain glucose transport in endothelial cells of the blood-brain barrier," Prostaglandins Leukot Essent Fatty Acids, vol. 77, no. 5-6, pp. 279-286, 2007.
28. F. Pifferi, M. Jouin, J. M. Alessandri et al., "n-3 long-chain fatty acids and regulation of glucose transport in two models of rat brain endothelial cells," Neurochem Int, vol. 56, no. 5, pp. 703-710, 2010.
29. A. Ximenes da Silva, F. Lavialle, G. Gendrot et al., "Glucose transport and utilization are altered in the brain of rats deficient in n-3 polyunsaturated fatty acids," J Neurochem, vol. 81, no. 6, pp. 1328-1337, 2002.

30. F. Pifferi, F. Roux, B. Langelier et al., "(n-3) polyunsaturated fatty acid deficiency reduces the expression of both isoforms of the brain glucose transporter GLUT1 in rats," J Nutr, vol. 135, no. 9, pp. 2241-2246, 2005.
31. A. P. Simopoulos, "The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases," Exp Biol Med (Maywood), vol. 233, no. 6, pp. 674-688, 2008.
32. J. R. Hibbeln, N. Salem, Jr., "Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy," Am J Clin Nutr, vol. 62, no. 1, pp. 1-9, 1995.
33. T. L. Blasbalg, J. R. Hibbeln, C. E. Ramsden, S. F. Majchrzak, R. R. Rawlings, "Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century," Am J Clin Nutr, vol. 93, no. 5, pp. 950-962, 2011.
34. P. M. Kris-Etherton, W. S. Harris, L. J. Appel, C. American Heart Association. Nutrition, "Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease," Circulation, vol. 106, no. 21, pp. 2747-2757, 2002.
35. World Health Organization, “Diet, nutrition and the prevention of chronic diseases. Report of the joint WHO/FAO expert consultation,” Geneva, 2003.
36. N. H. a. M. R. Council, "Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes," NHMRC, vol. no. pp. 2006.
37. A. Tanskanen, J. R. Hibbeln, J. Tuomilehto et al., "Fish consumption and depressive symptoms in the general population in Finland," Psychiatr Serv, vol. 52, no. 4, pp. 529-531, 2001.
38. V. Bountziouka, E. Polychronopoulos, A. Zeimbekis et al., "Long-term fish intake is associated with less severe depressive symptoms among elderly men and women: the MEDIS (MEDiterranean ISlands Elderly) epidemiological study," J Aging Health, vol. 21, no. 6, pp. 864-880, 2009.
39. M. Timonen, D. Horrobin, J. Jokelainen et al., "Fish consumption and depression: the Northern Finland 1966 birth cohort study," J Affect Disord, vol. 82, no. 3, pp. 447-452, 2004.
40. A. L. Suominen-Taipale, T. Partonen, A. W. Turunen et al., "Fish consumption and omega-3 polyunsaturated fatty acids in relation to depressive episodes: a cross-sectional analysis," PLoS One, vol. 5, no. 5, pp. e10530, 2010.
41. K. M. Appleton, J. V. Woodside, J. W. Yarnell et al., "Depressed mood and dietary fish intake: direct relationship or indirect relationship as a

result of diet and lifestyle?," J Affect Disord, vol. 104, no. 1-3, pp. 217-223, 2007.
42. K. Murakami, Y. Miyake, S. Sasaki, K. Tanaka, M. Arakawa, "Fish and n-3 polyunsaturated fatty acid intake and depressive symptoms: Ryukyus Child Health Study," Pediatrics, vol. 126, no. 3, pp. e623-630, 2010.
43. Y. Li, Q. Dai, L. I. Ekperi, A. Dehal, J. Zhang, "Fish consumption and severely depressed mood, findings from the first national nutrition follow-up study," Psychiatry Res, vol. 190, no. 1, pp. 103-109, 2011.
44. A. Sanchez-Villegas, P. Henriquez, A. Figueiras et al., "Long chain omega-3 fatty acids intake, fish consumption and mental disorders in the SUN cohort study," Eur J Nutr, vol. 46, no. 6, pp. 337-346, 2007.
45. P. Astorg, A. Couthouis, S. Bertrais et al., "Association of fish and long-chain n-3 polyunsaturated fatty acid intakes with the occurrence of depressive episodes in middle-aged French men and women," Prostaglandins Leukot Essent Fatty Acids, vol. 78, no. 3, pp. 171-182, 2008.
46. S. Noaghiul, J. R. Hibbeln, "Cross-national comparisons of seafood consumption and rates of bipolar disorders," Am J Psychiatry, vol. 160, no. 12, pp. 2222-2227, 2003.
47.
48. K. Walters, G. Rait, M. Griffin, M. Buszewicz, I. Nazareth, "Recent trends in the incidence of anxiety diagnoses and symptoms in primary care," PLoS One, vol. 7, no. 8, pp. e41670, 2012.
49. T. N. Akbaraly, S. Sabia, M. J. Shipley, G. D. Batty, M. Kivimaki, "Adherence to healthy dietary guidelines and future depressive symptoms: evidence for sex differentials in the Whitehall II study," Am J Clin Nutr, vol. 97, no. 2, pp. 419-427, 2013.
50. T. N. Akbaraly, E. J. Brunner, J. E. Ferrie et al., "Dietary pattern and depressive symptoms in middle age," Br J Psychiatry, vol. 195, no. 5, pp. 408-413, 2009.
51. F. N. Jacka, P. J. Kremer, M. Berk et al., "A prospective study of diet quality and mental health in adolescents," PLoS One, vol. 6, no. 9, pp. e24805, 2011.
52. F. N. Jacka, A. Mykletun, M. Berk, I. Bjelland, G. S. Tell, "The association between habitual diet quality and the common mental disorders in community-dwelling adults: the Hordaland Health study," Psychosom Med, vol. 73, no. 6, pp. 483-490, 2011.

53. F. N. Jacka, J. A. Pasco, A. Mykletun et al., "Association of Western and traditional diets with depression and anxiety in women," Am J Psychiatry, vol. 167, no. 3, pp. 305-311, 2010.
54. K. A. Skarupski, C. C. Tangney, H. Li, D. A. Evans, M. C. Morris, "Mediterranean diet and depressive symptoms among older adults over time," J Nutr Health Aging, vol. 17, no. 5, pp. 441-445, 2013.
55. A. Sanchez-Villegas, M. Delgado-Rodriguez, A. Alonso et al., "Association of the Mediterranean dietary pattern with the incidence of depression: the Seguimiento Universidad de Navarra/University of Navarra follow-up (SUN) cohort," Arch Gen Psychiatry, vol. 66, no. 10, pp. 1090-1098, 2009.
56. J. Rienks, A. J. Dobson, G. D. Mishra, "Mediterranean dietary pattern and prevalence and incidence of depressive symptoms in mid-aged women: results from a large community-based prospective study," Eur J Clin Nutr, vol. 67, no. 1, pp. 75-82, 2013.
57. A. Hodge, O. P. Almeida, D. R. English, G. G. Giles, L. Flicker, "Patterns of dietary intake and psychological distress in older Australians: benefits not just from a Mediterranean diet," Int Psychogeriatr, vol. 25, no. 3, pp. 456-466, 2013.
58. M. Luciano, R. Mottus, J. M. Starr et al., "Depressive symptoms and diet: their effects on prospective inflammation levels in the elderly," Brain Behav Immun, vol. 26, no. 5, pp. 717-720, 2012.
59. G. Antonogeorgos, D. B. Panagiotakos, C. Pitsavos et al., "Understanding the role of depression and anxiety on cardiovascular disease risk, using structural equation modeling; the mediating effect of the Mediterranean diet and physical activity: the ATTICA study," Ann Epidemiol, vol. 22, no. 9, pp. 630-637, 2012.
60. A. Sanchez-Villegas, P. Henriquez, M. Bes-Rastrollo, J. Doreste, "Mediterranean diet and depression," Public Health Nutr, vol. 9, no. 8A, pp. 1104-1109, 2006.
61. J. Sontrop, W. R. Avison, S. E. Evers, K. N. Speechley, M. K. Campbell, "Depressive symptoms during pregnancy in relation to fish consumption and intake of n-3 polyunsaturated fatty acids," Paediatr Perinat Epidemiol, vol. 22, no. 4, pp. 389-399, 2008.
62. K. Murakami, T. Mizoue, S. Sasaki et al., "Dietary intake of folate, other B vitamins, and omega-3 polyunsaturated fatty acids in relation to depressive symptoms in Japanese adults," Nutrition, vol. 24, no. 2, pp. 140-147, 2008.

63. W. H. Oddy, S. Hickling, M. A. Smith et al., "Dietary intake of omega-3 fatty acids and risk of depressive symptoms in adolescents," Depress Anxiety, vol. 28, no. 7, pp. 582-588, 2011.
64. K. M. Appleton, T. J. Peters, R. C. Hayward et al., "Depressed mood and n-3 polyunsaturated fatty acid intake from fish: non-linear or confounded association?," Soc Psychiatry Psychiatr Epidemiol, vol. 42, no. 2, pp. 100-104, 2007.
65. D. B. Panagiotakos, E. Mamplekou, C. Pitsavos et al., "Fatty acids intake and depressive symptomatology in a Greek sample: an epidemiological analysis," J Am Coll Nutr, vol. 29, no. 6, pp. 586-594, 2010.
66. L. A. Colangelo, K. He, M. A. Whooley, M. L. Daviglus, K. Liu, "Higher dietary intake of long-chain omega-3 polyunsaturated fatty acids is inversely associated with depressive symptoms in women," Nutrition, vol. 25, no. 10, pp. 1011-1019, 2009.
67. R. Hakkarainen, T. Partonen, J. Haukka et al., "Is low dietary intake of omega-3 fatty acids associated with depression?," Am J Psychiatry, vol. 161, no. 3, pp. 567-569, 2004.
68. F. N. Jacka, J. A. Pasco, M. J. Henry et al., "Dietary omega-3 fatty acids and depression in a community sample," Nutr Neurosci, vol. 7, no. 2, pp. 101-106, 2004.
69. A. Kyrozis, T. Psaltopoulou, P. Stathopoulos et al., "Dietary lipids and geriatric depression scale score among elders: the EPIC-Greece cohort," J Psychiatr Res, vol. 43, no. 8, pp. 763-769, 2009.
70. M. Strom, E. L. Mortensen, T. I. Halldorsson, I. Thorsdottir, S. F. Olsen, "Fish and long-chain n-3 polyunsaturated fatty acid intakes during pregnancy and risk of postpartum depression: a prospective study based on a large national birth cohort," Am J Clin Nutr, vol. 90, no. 1, pp. 149-155, 2009.
71. J. Golding, C. Steer, P. Emmett, J. M. Davis, J. R. Hibbeln, "High levels of depressive symptoms in pregnancy with low omega-3 fatty acid intake from fish," Epidemiology, vol. 20, no. 4, pp. 598-603, 2009.
72. C. M. da Rocha, G. Kac, "High dietary ratio of omega-6 to omega-3 polyunsaturated acids during pregnancy and prevalence of post-partum depression," Matern Child Nutr, vol. 8, no. 1, pp. 36-48, 2012.
73. M. Lucas, F. Mirzaei, E. J. O'Reilly et al., "Dietary intake of n-3 and n-6 fatty acids and the risk of clinical depression in women: a 10-y prospective follow-up study," Am J Clin Nutr, vol. 93, no. 6, pp. 1337-1343, 2011.

74. E. Kesse-Guyot, M. Touvier, V. A. Andreeva et al., "Cross-sectional but not longitudinal association between n-3 fatty acid intake and depressive symptoms: results from the SU.VI.MAX 2 study," Am J Epidemiol, vol. 175, no. 10, pp. 979-987, 2012.
75. Y. Li, J. Zhang, R. E. McKeown, "Cross-sectional assessment of diet quality in individuals with a lifetime history of attempted suicide," Psychiatry Res, vol. 165, no. 1-2, pp. 111-119, 2009.
76. A. Nanri, T. Mizoue, K. Poudel-Tandukar et al., "Dietary patterns and suicide in Japanese adults: the Japan Public Health Center-based Prospective Study," Br J Psychiatry, vol. 203, no. 6, pp. 422-427, 2013.
77. J. Zhang, Y. Li, M. E. Torres, "How does a suicide attempter eat differently from others? Comparison of macronutrient intakes," Nutrition, vol. 21, no. 6, pp. 711-717, 2005.
78. K. Poudel-Tandukar, A. Nanri, M. Iwasaki et al., "Long chain n-3 fatty acids intake, fish consumption and suicide in a cohort of Japanese men and women--the Japan Public Health Center-based (JPHC) prospective study," J Affect Disord, vol. 129, no. 1-3, pp. 282-288, 2011.
79. M. P. Freeman, J. R. Hibbeln, K. L. Wisner et al., "Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry," J Clin Psychiatry, vol. 67, no. 12, pp. 1954-1967, 2006.
80. P. Y. Lin, K. P. Su, "A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids," J Clin Psychiatry, vol. 68, no. 7, pp. 1056-1061, 2007.
81. K. M. Appleton, R. C. Hayward, D. Gunnell et al., "Effects of n-3 long-chain polyunsaturated fatty acids on depressed mood: systematic review of published trials," Am J Clin Nutr, vol. 84, no. 6, pp. 1308-1316, 2006.
82. P. J. Rogers, K. M. Appleton, D. Kessler et al., "No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial," Br J Nutr, vol. 99, no. 2, pp. 421-431, 2008.
83. P. Y. Lin, D. Mischoulon, M. P. Freeman et al., "Are omega-3 fatty acids antidepressants or just mood-improving agents? The effect depends upon diagnosis, supplement preparation, and severity of depression," Mol Psychiatry, vol. 17, no. 12, pp. 1161-1163; author reply 1163-1167, 2012.
84. B. M. Ross, J. Seguin, L. E. Sieswerda, "Omega-3 fatty acids as treatments for mental illness: which disorder and which fatty acid?," Lipids Health Dis, vol. 6, no. pp. 21, 2007.

85. J. G. Martins, "EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: evidence from a meta-analysis of randomized controlled trials," J Am Coll Nutr, vol. 28, no. 5, pp. 525-542, 2009.
86. M. E. Sublette, S. P. Ellis, A. L. Geant, J. J. Mann, "Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression," J Clin Psychiatry, vol. 72, no. 12, pp. 1577-1584, 2011.
87. R. Massart, R. Mongeau, L. Lanfumey, "Beyond the monoaminergic hypothesis: neuroplasticity and epigenetic changes in a transgenic mouse model of depression," Philos Trans R Soc Lond B Biol Sci, vol. 367, no. 1601, pp. 2485-2494, 2012.
88. P. C. Calder, P. Yaqoob, D. J. Harvey, A. Watts, E. A. Newsholme, "Incorporation of fatty acids by concanavalin A-stimulated lymphocytes and the effect on fatty acid composition and membrane fluidity," Biochem J, vol. 300 ( Pt 2), no. pp. 509-518, 1994.
89. C. R. Lee, M. W. Hamm, "Effect of dietary fat and cholesterol supplements on glucagon receptor binding and adenylate cyclase activity of rat liver plasma membrane," J Nutr, vol. 119, no. 4, pp. 539-546, 1989.
90. S. N. Ahmad, B. S. Alma, S. Q. Alam, "Dietary omega-3 fatty acids increase guanine nucleotide binding proteins and adenylate cyclase activity in rat salivary glands," FASEB J, vol. 3, no. pp. A948, 1989.
91. R. A. Bowen, M. T. Clandinin, "Dietary low linolenic acid compared with docosahexaenoic acid alter synaptic plasma membrane phospholipid fatty acid composition and sodium-potassium ATPase kinetics in developing rats," J Neurochem, vol. 83, no. 4, pp. 764-774, 2002.
92. V. V. Vaidyanathan, K. V. Rao, P. S. Sastry, "Regulation of diacylglycerol kinase in rat brain membranes by docosahexaenoic acid," Neurosci Lett, vol. 179, no. 1-2, pp. 171-174, 1994.
93. J. J. Mann, K. M. Malone, "Cerebrospinal fluid amines and higher-lethality suicide attempts in depressed inpatients," Biol Psychiatry, vol. 41, no. 2, pp. 162-171, 1997.
94. J. R. Hibbeln, M. Linnoila, J. C. Umhau et al., "Essential fatty acids predict metabolites of serotonin and dopamine in cerebrospinal fluid among healthy control subjects, and early- and late-onset alcoholics," Biol Psychiatry, vol. 44, no. 4, pp. 235-242, 1998.
95. A. Vines, A. M. Delattre, M. M. Lima et al., "The role of 5-HT(1)A receptors in fish oil-mediated increased BDNF expression in the rat

hippocampus and cortex: a possible antidepressant mechanism," Neuropharmacology, vol. 62, no. 1, pp. 184-191, 2012.
96. S. Delion, S. Chalon, D. Guilloteau, J. C. Besnard, G. Durand, "alpha-Linolenic acid dietary deficiency alters age-related changes of dopaminergic and serotoninergic neurotransmission in the rat frontal cortex," J Neurochem, vol. 66, no. 4, pp. 1582-1591, 1996.
97. R. K. McNamara, J. Able, Y. Liu et al., "Omega-3 fatty acid deficiency during perinatal development increases serotonin turnover in the prefrontal cortex and decreases midbrain tryptophan hydroxylase-2 expression in adult female rats: dissociation from estrogenic effects," J Psychiatr Res, vol. 43, no. 6, pp. 656-663, 2009.
98. R. K. McNamara, R. Jandacek, T. Rider et al., "Omega-3 fatty acid deficiency increases constitutive pro-inflammatory cytokine production in rats: relationship with central serotonin turnover," Prostaglandins Leukot Essent Fatty Acids, vol. 83, no. 4-6, pp. 185-191, 2010.
99. C. A. Heidbreder, I. C. Weiss, A. M. Domeney et al., "Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome," Neuroscience, vol. 100, no. 4, pp. 749-768, 2000.
100. A. Zangen, D. H. Overstreet, G. Yadid, "Increased catecholamine levels in specific brain regions of a rat model of depression: normalization by chronic antidepressant treatment," Brain Res, vol. 824, no. 2, pp. 243-250, 1999.
101. L. Zimmer, S. Vancassel, S. Cantagrel et al., "The dopamine mesocorticolimbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids," Am J Clin Nutr, vol. 75, no. 4, pp. 662-667, 2002.
102. L. Zimmer, S. Delion-Vancassel, G. Durand et al., "Modification of dopamine neurotransmission in the nucleus accumbens of rats deficient in n-3 polyunsaturated fatty acids," J Lipid Res, vol. 41, no. 1, pp. 32-40, 2000.
103. S. Chalon, S. Delion-Vancassel, C. Belzung et al., "Dietary fish oil affects monoaminergic neurotransmission and behavior in rats," J Nutr, vol. 128, no. 12, pp. 2512-2519, 1998.
104. N. Muller, M. J. Schwarz, "The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression," Mol Psychiatry, vol. 12, no. 11, pp. 988-1000, 2007.
105. Q. S. Yan, M. E. Reith, P. C. Jobe, J. W. Dailey, "Dizocilpine (MK-801) increases not only dopamine but also serotonin and norepinephrine transmissions in the nucleus accumbens as measured by microdialysis in freely moving rats," Brain Res, vol. 765, no. 1, pp. 149-158, 1997.

106. P. Martin, M. L. Carlsson, S. Hjorth, "Systemic PCP treatment elevates brain extracellular 5-HT: a microdialysis study in awake rats," Neuroreport, vol. 9, no. 13, pp. 2985-2988, 1998.
107. A. Latour, B. Grintal, G. Champeil-Potokar et al., "Omega-3 fatty acids deficiency aggravates glutamatergic synapse and astroglial aging in the rat hippocampal CA1," Aging Cell, vol. 12, no. 1, pp. 76-84, 2013.
108. B. Grintal, G. Champeil-Potokar, M. Lavialle et al., "Inhibition of astroglial glutamate transport by polyunsaturated fatty acids: evidence for a signalling role of docosahexaenoic acid," Neurochem Int, vol. 54, no. 8, pp. 535-543, 2009.
109. J. D. Moreira, L. Knorr, M. Ganzella et al., "Omega-3 fatty acids deprivation affects ontogeny of glutamatergic synapses in rats: relevance for behavior alterations," Neurochem Int, vol. 56, no. 6-7, pp. 753-759, 2010.
110. L. A. Speizer, M. J. Watson, L. L. Brunton, "Differential effects of omega-3 fish oils on protein kinase activities in vitro," Am J Physiol, vol. 261, no. 1 Pt 1, pp. E109-114, 1991.
111. O. Holian, R. Nelson, "Action of long-chain fatty acids on protein kinase C activity: comparison of omega-6 and omega-3 fatty acids," Anticancer Res, vol. 12, no. 3, pp. 975-980, 1992.
112. C. L. Raison, A. H. Miller, "When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders," Am J Psychiatry, vol. 160, no. 9, pp. 1554-1565, 2003.
113. T. W. Pace, F. Hu, A. H. Miller, "Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression," Brain Behav Immun, vol. 21, no. 1, pp. 9-19, 2007.
114. B. E. Murphy, "Steroids and depression," J Steroid Biochem Mol Biol, vol. 38, no. 5, pp. 537-559, 1991.
115. J. C. Nelson, J. M. Davis, "DST studies in psychotic depression: a meta-analysis," Am J Psychiatry, vol. 154, no. 11, pp. 1497-1503, 1997.
116. C. Song, X. Y. Zhang, M. Manku, "Increased phospholipase A2 activity and inflammatory response but decreased nerve growth factor expression in the olfactory bulbectomized rat model of depression: effects of chronic ethyl-eicosapentaenoate treatment," J Neurosci, vol. 29, no. 1, pp. 14-22, 2009.
117. M. Hennebelle, L. Balasse, A. Latour et al., "Influence of omega-3 fatty acid status on the way rats adapt to chronic restraint stress," PLoS One, vol. 7, no. 7, pp. e42142, 2012.

118. A. C. Ferraz, A. M. Delattre, R. G. Almendra et al., "Chronic omega-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol," Behav Brain Res, vol. 219, no. 1, pp. 116-122, 2011.
119. C. Song, X. Li, B. E. Leonard, D. F. Horrobin, "Effects of dietary n-3 or n-6 fatty acids on interleukin-1beta-induced anxiety, stress, and inflammatory responses in rats," J Lipid Res, vol. 44, no. 10, pp. 1984-1991, 2003.
120. H. Murck, C. Song, D. F. Horrobin, M. Uhr, "Ethyl-eicosapentaenoate and dexamethasone resistance in therapy-refractory depression," Int J Neuropsychopharmacol, vol. 7, no. 3, pp. 341-349, 2004.
121. F. Holsboer, "The corticosteroid receptor hypothesis of depression," Neuropsychopharmacology, vol. 23, no. 5, pp. 477-501, 2000.
122. A. M. Karssen, O. C. Meijer, I. C. van der Sandt et al., "Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain," Endocrinology, vol. 142, no. 6, pp. 2686-2694, 2001.
123. C. M. Pariante, A. Makoff, S. Lovestone et al., "Antidepressants enhance glucocorticoid receptor function in vitro by modulating the membrane steroid transporters," Br J Pharmacol, vol. 134, no. 6, pp. 1335-1343, 2001.
124. C. M. Pariante, A. H. Miller, "Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment," Biol Psychiatry, vol. 49, no. 5, pp. 391-404, 2001.
125. B. L. Mason, C. M. Pariante, "The effects of antidepressants on the hypothalamic-pituitary-adrenal axis," Drug News Perspect, vol. 19, no. 10, pp. 603-608, 2006.
126. C. Navarro, I. Gonzalez-Alvarez, M. Gonzalez-Alvarez et al., "Influence of polyunsaturated fatty acids on Cortisol transport through MDCK and MDCK-MDR1 cells as blood-brain barrier in vitro model," Eur J Pharm Sci, vol. 42, no. 3, pp. 290-299, 2011.
127. F. Caraci, A. Copani, F. Nicoletti, F. Drago, "Depression and Alzheimer's disease: neurobiological links and common pharmacological targets," Eur J Pharmacol, vol. 626, no. 1, pp. 64-71, 2010.
128. V. Krishnan, E. J. Nestler, "Linking molecules to mood: new insight into the biology of depression," Am J Psychiatry, vol. 167, no. 11, pp. 1305-1320, 2010.

129. K. Wager-Smith, A. Markou, "Depression: a repair response to stress-induced neuronal microdamage that can grade into a chronic neuroinflammatory condition?," Neurosci Biobehav Rev, vol. 35, no. 3, pp. 742-764, 2011.
130. M. Maes, R. Yirmyia, J. Noraberg et al., "The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression," Metab Brain Dis, vol. 24, no. 1, pp. 27-53, 2009.
131. A. M. Myint, B. E. Leonard, H. W. Steinbusch, Y. K. Kim, "Th1, Th2, and Th3 cytokine alterations in major depression," J Affect Disord, vol. 88, no. 2, pp. 167-173, 2005.
132. V. H. Perry, C. Cunningham, C. Holmes, "Systemic infections and inflammation affect chronic neurodegeneration," Nat Rev Immunol, vol. 7, no. 2, pp. 161-167, 2007.
133. S. L. Tilley, T. M. Coffman, B. H. Koller, "Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes," J Clin Invest, vol. 108, no. 1, pp. 15-23, 2001.
134. P. M. Kidd, "Omega-3 DHA and EPA for cognition, behavior, and mood: clinical findings and structural-functional synergies with cell membrane phospholipids," Altern Med Rev, vol. 12, no. 3, pp. 207-227, 2007.
135. P. C. Calder, "n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases," Am J Clin Nutr, vol. 83, no. 6 Suppl, pp. 1505S-1519S, 2006.
136. M. Peet, B. Murphy, J. Shay, D. Horrobin, "Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients," Biol Psychiatry, vol. 43, no. 5, pp. 315-319, 1998.
137. R. Edwards, M. Peet, J. Shay, D. Horrobin, "Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients," J Affect Disord, vol. 48, no. 2-3, pp. 149-155, 1998.
138. M. Maes, A. Christophe, J. Delanghe et al., "Lowered omega3 polyunsaturated fatty acids in serum phospholipids and cholesteryl esters of depressed patients," Psychiatry Res, vol. 85, no. 3, pp. 275-291, 1999.
139. P. B. Adams, S. Lawson, A. Sanigorski, A. J. Sinclair, "Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression," Lipids, vol. 31 Suppl, no. pp. S157-161, 1996.
140. M. Maes, R. Smith, A. Christophe et al., "Fatty acid composition in major depression: decreased omega 3 fractions in cholesteryl esters and

increased C20: 4 omega 6/C20:5 omega 3 ratio in cholesteryl esters and phospholipids," J Affect Disord, vol. 38, no. 1, pp. 35-46, 1996.
141. H. Tiemeier, H. R. van Tuijl, A. Hofman, A. J. Kiliaan, M. M. Breteler, "Plasma fatty acid composition and depression are associated in the elderly: the Rotterdam Study," Am J Clin Nutr, vol. 78, no. 1, pp. 40-46, 2003.
142. J. K. Kiecolt-Glaser, M. A. Belury, K. Porter et al., "Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults," Psychosom Med, vol. 69, no. 3, pp. 217-224, 2007.
143. A. M. Rizzo, P. A. Corsetto, G. Montorfano et al., "Comparison between the AA/EPA ratio in depressed and non depressed elderly females: omega-3 fatty acid supplementation correlates with improved symptoms but does not change immunological parameters," Nutr J, vol. 11, no. pp. 82, 2012.
144. A. M. Rees, M. P. Austin, C. Owen, G. Parker, "Omega-3 deficiency associated with perinatal depression: case control study," Psychiatry Res, vol. 166, no. 2-3, pp. 254-259, 2009.
145. A. Schins, H. J. Crijns, R. J. Brummer et al., "Altered omega-3 polyunsaturated fatty acid status in depressed post-myocardial infarction patients," Acta Psychiatr Scand, vol. 115, no. 1, pp. 35-40, 2007.
146. N. Frasure-Smith, F. Lesperance, P. Julien, "Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes," Biol Psychiatry, vol. 55, no. 9, pp. 891-896, 2004.
147. G. B. Parker, G. A. Heruc, T. M. Hilton et al., "Low levels of docosahexaenoic acid identified in acute coronary syndrome patients with depression," Psychiatry Res, vol. 141, no. 3, pp. 279-286, 2006.
148. M. E. Sublette, J. R. Hibbeln, H. Galfalvy, M. A. Oquendo, J. J. Mann, "Omega-3 polyunsaturated essential fatty acid status as a predictor of future suicide risk," Am J Psychiatry, vol. 163, no. 6, pp. 1100-1102, 2006.
149. S. Riemer, M. Maes, A. Christophe, W. Rief, "Lowered omega-3 PUFAs are related to major depression, but not to somatization syndrome," J Affect Disord, vol. 123, no. 1-3, pp. 173-180, 2010.
150. G. Mamalakis, M. Tornaritis, A. Kafatos, "Depression and adipose essential polyunsaturated fatty acids," Prostaglandins Leukot Essent Fatty Acids, vol. 67, no. 5, pp. 311-318, 2002.
151. G. Mamalakis, N. Kalogeropoulos, N. Andrikopoulos et al., "Depression and long chain n-3 fatty acids in adipose tissue in adults from Crete," Eur J Clin Nutr, vol. 60, no. 7, pp. 882-888, 2006.

152. G. Mamalakis, E. Jansen, H. Cremers et al., "Depression and adipose and serum cholesteryl ester polyunsaturated fatty acids in the survivors of the seven countries study population of Crete," Eur J Clin Nutr, vol. 60, no. 8, pp. 1016-1023, 2006.
153. G. Mamalakis, M. Kiriakakis, G. Tsibinos et al., "Lack of an association of depression with n-3 polyunsaturated fatty acids in adipose tissue and serum phospholipids in healthy adults," Pharmacol Biochem Behav, vol. 89, no. 1, pp. 6-10, 2008.
154. G. Mamalakis, M. Kiriakakis, G. Tsibinos, A. Kafatos, "Depression and adipose polyunsaturated fatty acids in an adolescent group," Prostaglandins Leukot Essent Fatty Acids, vol. 71, no. 5, pp. 289-294, 2004.
155. O. J. Schiepers, M. C. Wichers, M. Maes, "Cytokines and major depression," Prog Neuropsychopharmacol Biol Psychiatry, vol. 29, no. 2, pp. 201-217, 2005.
156. M. Wichers, M. Maes, "The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans," Int J Neuropsychopharmacol, vol. 5, no. 4, pp. 375-388, 2002.
157. Y. Dowlati, N. Herrmann, W. Swardfager et al., "A meta-analysis of cytokines in major depression," Biol Psychiatry, vol. 67, no. 5, pp. 446-457, 2010.
158. P. C. Calder, "n-3 fatty acids, inflammation and immunity: new mechanisms to explain old actions," Proc Nutr Soc, vol. 72, no. 3, pp. 326-336, 2013.
159. D. Y. Lu, Y. Y. Tsao, Y. M. Leung, K. P. Su, "Docosahexaenoic acid suppresses neuroinflammatory responses and induces heme oxygenase-1 expression in BV-2 microglia: implications of antidepressant effects for omega-3 fatty acids," Neuropsychopharmacology, vol. 35, no. 11, pp. 2238-2248, 2010.
160. Y. Park, H. J. Moon, S. H. Kim, "N-3 polyunsaturated fatty acid consumption produces neurobiological effects associated with prevention of depression in rats after the forced swimming test," J Nutr Biochem, vol. 23, no. 8, pp. 924-928, 2012.
161. A. Moranis, J. C. Delpech, V. De Smedt-Peyrusse et al., "Long term adequate n-3 polyunsaturated fatty acid diet protects from depressive-like behavior but not from working memory disruption and brain cytokine expression in aged mice," Brain Behav Immun, vol. 26, no. 5, pp. 721-731, 2012.

162. C. N. Serhan, "Novel eicosanoid and docosanoid mediators: resolvins, docosatrienes, and neuroprotectins," Curr Opin Clin Nutr Metab Care, vol. 8, no. 2, pp. 115-121, 2005.
163. A. A. Farooqui, L. A. Horrocks, T. Farooqui, "Modulation of inflammation in brain: a matter of fat," J Neurochem, vol. 101, no. 3, pp. 577-599, 2007.
164. S. M. Prescott, W. F. Stenson, "Fish oil fix," Nat Med, vol. 11, no. 6, pp. 596-598, 2005.
165. Y. Kim, C. Lee, "The gene encoding transforming growth factor beta 1 confers risk of ischemic stroke and vascular dementia," Stroke, vol. 37, no. 11, pp. 2843-2845, 2006.
166. L. Sutcigil, C. Oktenli, U. Musabak et al., "Pro- and anti-inflammatory cytokine balance in major depression: effect of sertraline therapy," Clin Dev Immunol, vol. 2007, no. pp. 76396, 2007.
167. P. Vollmar, A. Haghikia, R. Dermietzel, P. M. Faustmann, "Venlafaxine exhibits an anti-inflammatory effect in an inflammatory co-culture model," Int J Neuropsychopharmacol, vol. 11, no. 1, pp. 111-117, 2008.
168. M. Hida, H. Fujita, K. Ishikura et al., "Eicosapentaenoic acid inhibits PDGF-induced mitogenesis and cyclin D1 expression via TGF-beta in mesangial cells," J Cell Physiol, vol. 196, no. 2, pp. 293-300, 2003.
169. A. Thienprasert, S. Samuhaseneetoo, K. Popplestone et al., "Fish oil n-3 polyunsaturated fatty acids selectively affect plasma cytokines and decrease illness in Thai schoolchildren: a randomized, double-blind, placebo-controlled intervention trial," J Pediatr, vol. 154, no. 3, pp. 391-395, 2009.
170. S. Krauss-Etschmann, D. Hartl, P. Rzehak et al., "Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-beta levels after fish oil supplementation of pregnant women," The Journal of allergy and clinical immunology, vol. 121, no. 2, pp. 464-470 e466, 2008.
171. J. M. Bourre, "Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing," The journal of nutrition, health & aging, vol. 8, no. 3, pp. 163-174, 2004.
172. L. Svennerholm, "Distribution and fatty acid composition of phosphoglycerides in normal human brain," Journal of lipid research, vol. 9, no. 5, pp. 570-579, 1968.
173. S. J. Carlson, E. M. Fallon, B. T. Kalish, K. M. Gura, M. Puder, "The role of the omega-3 fatty acid DHA in the human life cycle," JPEN. Journal of parenteral and enteral nutrition, vol. 37, no. 1, pp. 15-22, 2013.

174. J. T. Brenna, "Animal studies of the functional consequences of suboptimal polyunsaturated fatty acid status during pregnancy, lactation and early post-natal life," Maternal & child nutrition, vol. 7 Suppl 2, no. pp. 59-79, 2011.
175. P. Guesnet, J. M. Alessandri, "Docosahexaenoic acid (DHA) and the developing central nervous system (CNS) - Implications for dietary recommendations," Biochimie, vol. 93, no. 1, pp. 7-12, 2011.
176. J. C. McCann, B. N. Ames, "Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals," The American journal of clinical nutrition, vol. 82, no. 2, pp. 281-295, 2005.
177. V. Krishnan, E. J. Nestler, "The molecular neurobiology of depression," Nature, vol. 455, no. 7215, pp. 894-902, 2008.
178. G. Rajkowska, J. J. Miguel-Hidalgo, J. Wei et al., "Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression," Biological psychiatry, vol. 45, no. 9, pp. 1085-1098, 1999.
179. R. M. Sapolsky, "The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death," Biological psychiatry, vol. 48, no. 8, pp. 755-765, 2000.
180. P. Fossati, A. Radtchenko, P. Boyer, "Neuroplasticity: from MRI to depressive symptoms," European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology, vol. 14 Suppl 5, no. pp. S503-510, 2004.
181. J. K. Virtanen, D. S. Siscovick, W. T. Longstreth, Jr., L. H. Kuller, D. Mozaffarian, "Fish consumption and risk of subclinical brain abnormalities on MRI in older adults," Neurology, vol. 71, no. 6, pp. 439-446, 2008.
182. G. L. Bowman, L. C. Silbert, D. Howieson et al., "Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging," Neurology, vol. 78, no. 4, pp. 241-249, 2012.
183. Z. S. Tan, W. S. Harris, A. S. Beiser et al., "Red blood cell omega-3 fatty acid levels and markers of accelerated brain aging," Neurology, vol. 78, no. 9, pp. 658-664, 2012.
184. W. C. Drevets, "Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders," Current opinion in neurobiology, vol. 11, no. 2, pp. 240-249, 2001.

185. J. P. Hamilton, M. Siemer, I. H. Gotlib, "Amygdala volume in major depressive disorder: a meta-analysis of magnetic resonance imaging studies," Molecular psychiatry, vol. 13, no. 11, pp. 993-1000, 2008.
186. T. Suslow, C. Konrad, H. Kugel et al., "Automatic mood-congruent amygdala responses to masked facial expressions in major depression," Biological psychiatry, vol. 67, no. 2, pp. 155-160, 2010.
187. C. Windischberger, R. Lanzenberger, A. Holik et al., "Area-specific modulation of neural activation comparing escitalopram and citalopram revealed by pharmaco-fMRI: a randomized cross-over study," NeuroImage, vol. 49, no. 2, pp. 1161-1170, 2010.
188. V. R. Venna, D. Deplanque, C. Allet, K. Belarbi, M. Hamdane, R. Bordet, "PUFA induce antidepressant-like effects in parallel to structural and molecular changes in the hippocampus," Psychoneuroendocrinology, vol. 34, no. 2, pp. 199-211, 2009.
189. C. Samieri, P. Maillard, F. Crivello et al., "Plasma long-chain omega-3 fatty acids and atrophy of the medial temporal lobe," Neurology, vol. 79, no. 7, pp. 642-650, 2012.
190. S. M. Conklin, P. J. Gianaros, S. M. Brown et al., "Long-chain omega-3 fatty acid intake is associated positively with corticolimbic gray matter volume in healthy adults," Neuroscience letters, vol. 421, no. 3, pp. 209-212, 2007.
191. D. Cao, K. Kevala, J. Kim et al., "Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function," Journal of neurochemistry, vol. 111, no. 2, pp. 510-521, 2009.
192. E. Castren, T. Rantamaki, "Role of brain-derived neurotrophic factor in the aetiology of depression: implications for pharmacological treatment," CNS drugs, vol. 24, no. 1, pp. 1-7, 2010.
193. E. Shimizu, K. Hashimoto, N. Okamura et al., "Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants," Biological psychiatry, vol. 54, no. 1, pp. 70-75, 2003.
194. J. S. Rao, R. N. Ertley, H. J. Lee et al., "n-3 polyunsaturated fatty acid deprivation in rats decreases frontal cortex BDNF via a p38 MAPK-dependent mechanism," Molecular psychiatry, vol. 12, no. 1, pp. 36-46, 2007.
195. W. Kou, D. Luchtman, C. Song, "Eicosapentaenoic acid (EPA) increases cell viability and expression of neurotrophin receptors in retinoic

acid and brain-derived neurotrophic factor differentiated SH-SY5Y cells," European journal of nutrition, vol. 47, no. 2, pp. 104-113, 2008.
196. H. S. Bhatia, R. Agrawal, S. Sharma, Y. X. Huo, Z. Ying, F. Gomez-Pinilla, "Omega-3 fatty acid deficiency during brain maturation reduces neuronal and behavioral plasticity in adulthood," PloS one, vol. 6, no. 12, pp. e28451, 2011.
197. B. M. Ross, "Omega-3 fatty acid deficiency in major depressive disorder is caused by the interaction between diet and a genetically determined abnormality in phospholipid metabolism," Medical hypotheses, vol. 68, no. 3, pp. 515-524, 2007.
198. C. U. Pae, H. S. Yu, J. J. Kim et al., "BanI polymorphism of the cytosolic phospholipase A2 gene and mood disorders in the Korean population," Neuropsychobiology, vol. 49, no. 4, pp. 185-188, 2004.
199. J. Covault, H. Pettinati, D. Moak, T. Mueller, H. R. Kranzler, "Association of a long-chain fatty acid-CoA ligase 4 gene polymorphism with depression and with enhanced niacin-induced dermal erythema," American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics, vol. 127B, no. 1, pp. 42-47, 2004.
200. G. N. Papadimitriou, D. G. Dikeos, D. Souery et al., "Genetic association between the phospholipase A2 gene and unipolar affective disorder: a multicentre case-control study," Psychiatric genetics, vol. 13, no. 4, pp. 211-220, 2003.
201. D. G. Dikeos, G. N. Papadimitriou, D. Souery et al., "Lack of genetic association between the phospholipase A2 gene and bipolar mood disorder in a European multicentre case-control study," Psychiatric genetics, vol. 16, no. 4, pp. 169-171, 2006.
202. M. Plourde, M. C. Vohl, M. Vandal, P. Couture, S. Lemieux, S. C. Cunnane, "Plasma n-3 fatty acid response to an n-3 fatty acid supplement is modulated by apoE epsilon4 but not by the common PPAR-alpha L162V polymorphism in men," The British journal of nutrition, vol. 102, no. 8, pp. 1121-1124, 2009.
203. S. Liang, L. M. Steffen, B. T. Steffen et al., "APOE genotype modifies the association between plasma omega-3 fatty acids and plasma lipids in the Multi-Ethnic Study of Atherosclerosis (MESA)," Atherosclerosis, vol. 228, no. 1, pp. 181-187, 2013.

204. C. Samieri, C. Feart, C. Proust-Lima et al., "omega-3 fatty acids and cognitive decline: modulation by ApoEepsilon4 allele and depression," Neurobiology of aging, vol. 32, no. 12, pp. 2317 e2313-2322, 2011.
205. K. Kitajka, A. J. Sinclair, R. S. Weisinger et al., "Effects of dietary omega-3 polyunsaturated fatty acids on brain gene expression," Proceedings of the National Academy of Sciences of the United States of America, vol. 101, no. 30, pp. 10931-10936, 2004.

**LIST OF PUBLICATIONS**

Marventano S, Grosso G, Mistretta A, Bogusz-Czerniewicz M, Ferranti R, Nolfo F, Giorgianni G, Rametta S, Drago F, Basile F, Biondi A. Evaluation of four comorbidity indices and Charlson comorbidity index adjustment for colorectal cancer patients. Int J Colorectal Dis. 2014 Sep;29(9):1159-69.

Grosso G, Pajak A, Marventano S, Castellano S, Galvano F, Bucolo C, Drago F, Caraci F. Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. PLoS One. 2014 May 7;9(5):e96905.

Grosso G, Galvano F, Marventano S, Malaguarnera M, Bucolo C, Drago F, Caraci F. Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. Oxid Med Cell Longev. 2014;2014:313570.

Grosso G, Biondi A, Galvano F, Mistretta A, Marventano S, Buscemi S, Drago F, Basile F. Factors associated with colorectal cancer in the context of the Mediterranean diet: a case-control study. Nutr Cancer. 2014;66(4):558-65.

Grosso G, Pajak A, Mistretta A, Marventano S, Raciti T, Buscemi S, Drago F, Scalfi L, Galvano F. Protective role of the Mediterranean diet on several cardiovascular risk factors: evidence from Sicily, southern Italy. Nutr Metab Cardiovasc Dis. 2014 Apr;24(4):370-7.

Grosso G, Mistretta A, Marventano S, Purrello A, Vitaglione P, Calabrese G, Drago F, Galvano F. Beneficial effects of the mediterranean diet on metabolic syndrome. Curr Pharm Des. 2014;20(31):5039-44.

Nolfo F, Rametta S, Marventano S, Grosso G, Mistretta A, Drago F, Gangi S, Basile F, Biondi A. Pharmacological and dietary prevention for colorectal cancer. BMC Surg. 2013;13 Suppl 2:S16.

Grosso G, Buscemi S, Galvano F, Mistretta A, Marventano S, La Vela V, Drago F, Gangi S, Basile F, Biondi A. Mediterranean diet and cancer: epidemiological evidence and mechanism of selected aspects. BMC Surg. 2013;13 Suppl 2:S14.

Grosso G, Galvano F, Mistretta A, Marventano S, Nolfo F, Calabrese G, Buscemi S, Drago F, Veronesi U, Scuderi A. Red orange: experimental models and epidemiological evidence of its benefits on human health. Oxid Med Cell Longev. 2013;2013:157240.