Review

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

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Background: Epidemiologic and case-control data suggest that increased dietary intake of omega-3 long-chain polyunsaturated fatty acids (ω3 LC-PUFAs) may be of benefit in depression. However, the results of randomized controlled trials are mixed and controversy exists as to whether either eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) or both are responsible for the reported benefits.

Objective: The aim of the current study was to provide an updated meta-analysis of all double-blind, placebo-controlled, randomized controlled trials examining the effect of ω3 LC-PUFA supplementation in which depressive symptoms were a reported outcome. The study also aimed to specifically test the differential effectiveness of EPA versus DHA through meta-regression and subgroup analyses.

Design: Studies were selected using the PubMed database on the basis of the following criteria: (1) randomized design; (2) placebo controlled; (3) use of an ω3 LC-PUFA preparation containing DHA, EPA, or both where the relative amounts of each fatty acid could be quantified; and (4) reporting sufficient statistics on scores of a recognizable measure of depressive symptoms.

Results: Two hundred forty-one studies were identified, of which 28 met the above inclusion criteria and were therefore included in the subsequent meta-analysis. Using a random effects model, overall standardized mean depression scores were reduced in response to ω3 LC-PUFA supplementation as compared with placebo (standardized mean difference = −0.291, 95% CI = −0.463 to −0.120, z = −3.327, $p = 0.001$). However, significant heterogeneity and evidence of publication bias were present. Meta-regression studies showed a significant effect of higher levels of baseline depression and lower supplement DHA:EPA ratio on therapeutic efficacy. Subgroup analyses showed significant effects for: (1) diagnostic category (bipolar disorder and major depression showing significant improvement with ω3 LC-PUFA supplementation versus mild-to-moderate depression, chronic fatigue and non-clinical populations not showing significant improvement); (2) therapeutic as opposed to preventive intervention; (3) adjunctive treatment as opposed to monotherapy; and (4) supplement type. Symptoms of depression were not significantly reduced in 3 studies using pure DHA (standardized mean difference 0.001, 95% CI −0.330 to 0.332, z = 0.004, $p = 0.997$) or in 4 studies using supplements containing greater than 50% DHA (standardized mean difference = 0.141, 95% CI = −0.195 to 0.477, z = 0.821, $p = 0.417$). In contrast, symptoms of depression were significantly reduced in 13 studies using supplements containing greater than 50% EPA (standardized mean difference = −0.446, 95% CI = −0.753 to −0.138, z = −2.843, $p = 0.005$) and in 8 studies using pure ethyl-EPA (standardized mean difference = −0.396, 95% CI = −0.650 to −0.141, z = −3.051, $p = 0.002$). However, further meta-regression studies showed significant inverse associations between efficacy and study methodological quality, study sample size, and duration, thus limiting the confidence of these findings.

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Abbreviations: EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, AA = arachidonic acid, LC-PUFA = long-chain polyunsaturated fatty acid, ω3 LC-PUFA = omega-3, UREML = unrestricted maximum likelihood method, HDRS = Hamilton Rating Scale for Depression, HDRS-SF = Hamilton Rating Scale for Depression-short form, BDI = Beck Depression Inventory, MADRS = Montgomery-Åsberg Depression Rating Scale, EPDS = Edinburgh Postnatal Depression Scale, SCID-IV = Structured Clinical Interview for DSM-IV, POMS = Profile of Mood States, CDI = Children's Depression Inventory, CDRS = Children's Depression Rating Scale, IDS-C = Inventory of Depressive Symptomatology, DASS = Depression Anxiety and Stress Scales, LPS = lipopolysaccharide, IL = interleukin, TNF-α = tumor necrosis factor-alpha, NF-κB = nuclear factor kappa-B.

Journal of the American College of Nutrition, Vol. 28, No. 5, 525–542 (2009)
Published by the American College of Nutrition

Conclusions: The current meta-analysis provides evidence that EPA may be more efficacious than DHA in treating depression. However, owing to the identified limitations of the included studies, larger, well-designed, randomized controlled trials of sufficient duration are needed to confirm these findings.

INTRODUCTION

Depression remains a serious public health problem with significant associated morbidity, mortality, and economic cost. Over the life course, major depression will affect 16.9% of individuals [1]. In addition to the risk of suicide, the commonest cause of death in individuals with depression, mortality is also increased as a result of the association with cardiovascular disease [2]. The estimated economic cost of depression, both direct in terms of cost of treatment and indirect through lost days at work and reduced productivity, is substantial [3]. In comparison with other causes of disease, the Global Burden of Disease Study has shown that, by 2030, unipolar depressive disorder and ischemic heart disease will be the major causes of disability in developed populations [4]. Given the increases in prevalence of depression [5] and cardiovascular disease, the latter especially among socio-economically disadvantaged groups [6] and young people with obesity [7], it appears likely that a common underlying environmental influence may account for these changes.

One theory that has been advanced to explain these changes, at least in part, is the significant shift over the last century in the dietary intake of long-chain polyunsaturated fatty acids (ω3 LC-PUFAs) toward an increase in saturated fat and an increase in the ratio of omega-6 to omega-3 fatty acids [8]. The pathophysiological basis of support for this theory comes from evidence that inflammatory processes such as excessive cytokine production [9] and glucocorticoid resistance [10] underpin major depression, coupled with evidence that ω3 LC-PUFAs produce both anti-inflammatory eicosanoids [11] that reduce levels of pro-inflammatory cytokines in depressed individuals [12] and have antidepressant protective actions as identified from the epidemiologic association of lower levels of depression with fish consumption [13–20], and the physiological association between reduced plasma or red blood cell (RBC) membrane ω3 LC-PUFAs and depressive symptoms [21–27] and suicide [28–30]. If true, this theory has substantial implications for both the prevention and treatment of depression, with the potential for large-scale impact through dietary interventions. Whilst it is acknowledged that many other factors may also be contributing to the rise in depression and that effective treatments already exist, such as antidepressant medication and cognitive behavioral therapy, even if the effect of dietary intervention is very small at the individual level, substantial benefits can result at the population level. This is because a population-based intervention, if capable of shifting the distribution curve of depressive symptoms in a positive direction, even by a small degree, can remove large numbers of individuals from the threshold of clinical disorder [31]. Furthermore, established antidepressant therapy is not effective in all cases, with less than 50% of patients showing full remission of symptoms [32].

In order to answer this important question of dietary influence in depression, Basant Puri's group in London became the first to demonstrate the efficacy of eicosapentaenoic acid (EPA) in a therapeutic case study of depression [33]. Subsequently, a number of double-blind, placebo-controlled trials have been conducted, some of which support the efficacy of ω3 LC-PUFAs in the adjunctive treatment of adult depression [34–42] and in the sole treatment of childhood depression [43] and adult depression [35,44], whereas other trials have not demonstrated effectiveness [45–59]. Some of these studies have been subjected to meta-analytic review by various authors. Freeman et al. [60] and Lin and Su [61] report overall benefit, whereas Appleton et al. [62] and, in an updated analysis of the same study, Rogers et al. [52] report negligible benefits. As a possible explanation for the variability in these findings, all of these meta-analytic studies acknowledge considerable between-study heterogeneity that may be explained by publication bias, severity of baseline depression, diagnostic variation, and variability in the nature of the ω3 LC-PUFA regime employed. Given the negative findings of a trial involving pure docosahexaenoic acid (DHA) [50], an additional meta-analysis conducted by Ross et al. [63] sought to examine whether this heterogeneity could be explained by variation in the ω3 LC-PUFA regime employed using random effects meta-regression. When this factor was taken into account, the between-study variance was substantially reduced. Specifically, the positive effect of ω3 LC-PUFAs on depressive symptoms appeared to be explained by the EPA rather than DHA content of the regime.

Because several new randomized controlled trials of ω3 LC-PUFA preparations in depressive disorders have been published in the last year, a further meta-analysis has been performed and is reported here. The specific aims of the current meta-analysis were to update the findings on overall efficacy and to provide further meta-regression analyses to either confirm or refute the original observation by Ross et al. [63] regarding the possibility that EPA and not DHA is responsible for the therapeutic effect of ω3 LC-PUFAs in depression.

METHODS

The PubMed MeSH database was searched using the following terms: ((''Psychiatry and Psychology Category''

[Mesh] OR ''Fatigue Syndrome, Chronic'' [Mesh]) AND (''Fatty Acids, Essential'' [Mesh] OR ''Fatty Acids, Omega-3'' [Mesh] OR ''Fish Oils'' [Mesh] OR ''Eicosapentaenoic Acid'' [Mesh] OR ''Docosahexaenoic Acids'' [Mesh])) AND ''Randomized Controlled Trial'' [Publication Type].

The overarching ''Psychiatry and Psychology Category'' was used to encompass not only the relevant terms for depression (''Depressive Disorder'' [Mesh], ''Depressive Disorder, Major'' [Mesh], ''Depression'' [Mesh], ''Bipolar Disorder'' [Mesh], ''Depression, Postpartum'' [Mesh], ''Dysthymic Disorder'' [Mesh], ''Seasonal Affective Disorder'' [Mesh]) but also terms for all other mental disorders and mood in normal subjects. This inclusive strategy was deemed necessary as some studies examining, for example, patients with schizophrenia also report changes in depressive symptoms. In addition, a number of studies have looked at the effect of ω3 LC-PUFA supplementation on mood in individuals without evidence of mental disorder. The term for chronic fatigue was also included, as there have been reports of the use of ω3 LC-PUFA supplementation in this disorder, which also is associated with considerable comorbid depressive symptoms. As of May 4, 2009, this strategy identified 288 potential studies for inclusion. Studies were then selected on the basis of the following criteria: (1) randomized design; (2) placebo controlled; (3) use of an ω3 LC-PUFA preparation containing DHA, EPA, or both where the relative amounts of each fatty acid could be quantified; and (4) reporting sufficient statistics on scores of a recognizable measure of depressive symptoms (in certain circumstances, the authors of studies were contacted directly for clarification of results).

This selection strategy resulted in 30 trials being identified that could be subjected to meta-analytic review. It is noteworthy that this selection strategy identified all of the studies included in the meta-analysis by Appleton et al. [62], who employed a rather more extensive search strategy of multiple databases. Therefore, despite the simplicity of the search strategy employed in the current study, it is unlikely that significant studies have been missed. One of the identified studies examined the effect of ω3 LC-PUFA supplementation in obsessive-compulsive disorder [64] and, although depressive symptoms were measured in this study using the Hamilton Depression Rating Scale, no outcome values were reported. Therefore, that study was excluded from further analysis, as it did not meet the selection criteria. The 2 previous meta-analyses by Appleton et al. [62] and Rogers et al. [52] included a study by Ness et al. [65] that was not included in the meta-analyses by Freeman et al. [60] and Lin and Su [61]. This study was based upon the assessment of mood in a trial of men with angina who had been randomized to receive advice to eat more fish or to receive no such advice, with those not tolerating fish being offered Maxepa fish oil supplementation instead. Given the absence of any possibility of placebo control or the ability to accurately quantify the relative amounts of DHA and EPA being consumed, it was felt that inclusion of this trial was inappropriate, as again, it did not meet the selection criteria.

Methodological quality was assessed using the Jadad score [66] plus 6 additional components: (1) whether similarities in baseline characteristics were adequately described; (2) whether attempts were made to conceal the fish taste of the active intervention; (3) whether the outcome assessors were adequately blinded; (4) whether data were analyzed according to intention-to-treat (ITT) methods; (5) whether compliance was assessed through measurement of RBC or plasma fatty acids; and (6) whether blinding success was evaluated. This gave a maximum possible quality score of 11. The characteristics of the final 28 included studies are shown in Table 1.

Standardized mean differences in depression scores were computed and analyzed using the program Comprehensive Meta-Analysis, identified as one of the best tools for this purpose in a recent systematic review [67]. One of the many advantages of this program is that effect sizes can be computed from a wide range of reporting methods, enabling studies to be included in the current analysis that were excluded by Appleton et al. [62]. The program also allows mean effect sizes to be computed in studies that use multiple outcome measures, for example, 2 questionnaire measures of depressive symptoms plus a categorical measure of clinical improvement, allowing all available data that relate to depressive symptoms to be included in this analysis. Some studies reported additional outcomes using generalized measures such as the Global Assessment of Functioning Scale or, in subjects with bipolar disorder, the Young Mania Rating Scale; these outcomes were excluded from the analysis to allow an exclusive focus on depressive symptoms.

The meta-analytic strategy employed was as follows: (1) to use random effects rather than fixed effects analyses, as it was evident that there was considerable variation in clinical populations studied, methodologies employed, and outcome measures used; (2) to examine overall effect sizes using forest plots; (3) to examine for possible publication bias using funnel plots with Duval and Tweedie's trim and fill method; (4) to assess heterogeneity using Cohen's Q, I^2, and τ^2, where I^2 represents the percentage of heterogeneity that can be attributed to true underlying differences in effect sizes between studies and τ^2 represents the extent of variance between studies; (5) on the basis of these findings, to conduct further sensitivity analyses on subpopulations of studies using random effects analysis of variance (ANOVA); and finally, (6) to conduct random effects meta-regression studies on relevant moderator variables using the unrestricted maximum likelihood (UREML) method, a method least likely to generate spurious findings.

Table 1. Characteristics of the 28 Included Studies Listed Chronologically according to Publication Date

Study [reference]	Clinical Group	Number, Total (ω3 LC-PUFA/placebo)	ω3 LC-PUFA Preparation	Daily Dosage Regime(s)	Treatment Status	Duration (days)	Outcome Measure(s)	Quality
Behan et al. 1990 [44]	Chronic fatigue	63 (39/24)	Efamol Marine	0.136 g EPA + 0.088 g DHA	Therapeutic, Monotherapy	90	Likert scale	6
Stoll et al. 1999 [38]	Bipolar disorder	30 (14/16)	Menhaden fish body oil concentrate	6.2 g EPA + 3.4 g DHA	Preventive, Adjunctive	112	HDRS-31	7
Warren et al. 1999 [55]	Chronic fatigue	50 (24/26)	Efamol Marine	0.136 g EPA + 0.088 g DHA	Therapeutic, Monotherapy	90	BDI	7
Fenton et al. 2001 [45]	Schizophrenia	87 (43/44)	Laxdale Ltd	3 g Ethyl-EPA	Therapeutic, Adjunctive	112	MADRS	8
Nemets et al. 2002 [36]	Major depression	20 (10/10)	Laxdale Ltd	2 g Ethyl-EPA	Therapeutic, Adjunctive	28	HDRS-24	9
Peet and Horrobin 2002 [37]	Major depression	70 (17, 18, 17/18)	Laxdale Ltd	1 g Ethyl-EPA *or* 2 g Ethyl-EPA *or* 4 g Ethyl-EPA	Therapeutic, Adjunctive	84	BDI, HDRS-17, MADRS	6
Zanarini and Frankenburg 2003 [54]	Borderline personality disorder	30 (20/10)	Laxdale Ltd	1 g Ethyl-EPA	Therapeutic, Monotherapy	56	MADRS	4
Llorente et al. 2003 [49]	Perinatal depression	89 (44/45)	DHASCO (Martek Biosciences Corporation)	0.2 g DHA	Preventive, Monotherapy	120	BDI, EPDS, SCID-IV	6
Marangell et al. 2003 [50]	Major depression	36 (18/18)	Pure DHA (no manufacturer stated)	2 g DHA	Therapeutic, Monotherapy	42	HDRS-28, MADRS	6
Su et al. 2003 [39]	Major depression	28 (14/14)	Menhaden fish body oil concentrate	4.4 g EPA + 2.2 g DHA	Therapeutic, Adjunctive	56	HDRS-21	5
Hirashima et al. 2004 [69]	Bipolar disorder	21 (6, 6/9)	Fish oil	5.0–5.2 g EPA + 3.0–3.4 g DHA *or* 1.3 g EPA + 0.7 g DHA	Therapeutic, Adjunctive	28	HDRS-23	2
Silvers et al. 2005 [53]	Major depression	77 (40/37)	DHA-enriched tuna fish oil (Clover Corporation PLC)	0.6 g EPA + 2.4 g DHA	Therapeutic, Adjunctive	84	BDI, HDRS-SF	10
Fontani et al. 2005 [68]	Non-clinical	33 (33/33)[1]	Fish oil	1.60 g EPA + 0.80 g DHA	Preventive, Monotherapy	70	POMS	5
Frangou et al. 2006 [34]	Bipolar disorder	75 (24, 25/26)	Laxdale Ltd.	1 g Ethyl-EPA *or* 2 g Ethyl-EPA	Therapeutic, Adjunctive	84	HDRS-17	9
Nemets et al. 2006 [43]	Major depression in childhood	28 (13/15)	Ocean Nutrition	0.4 g EPA + 0.2 g DHA	Therapeutic, Monotherapy	112	CDI, CDRS	6
Keck et al. 2006 [48]	Bipolar disorder	116 (59/57)	Laxdale Ltd	6 g Ethyl-EPA	Therapeutic, Adjunctive	120	IDS-C	6
Hallahan et al. 2007 [41]	Recurrent self-harm	49 (22/27)	EPAX 5500	1.22 g EPA + 0.908 g DHA	Therapeutic, Adjunctive	84	BDI, HDRS	8
Grenyer et al. 2007 [47]	Major depression	83 (40/43)	Tuna fish oil (Clover Corporation PLC)	0.6 g EPA + 2.2 g DHA	Therapeutic, Adjunctive	112	BDI, HDRS	10
Frangou et al. 2007 [46]	Bipolar disorder	14 (7/7)	Laxdale Ltd	2 g Ethyl-EPA	Therapeutic, Adjunctive	84	HDRS	6
Rogers et al. 2008 [52]	Mild-to-moderate depression	218 (109/109)	Minami Nutrition	0.63 g EPA + 0.85 g DHA	Therapeutic, Monotherapy	84	BDI, DASS	11
Jazayeri et al. 2008 [35]	Major depression	48 (32/16)	Minami Nutrition	1 g Ethyl-EPA	Therapeutic, Adjunctive	56	HDRS-17	6

Table 1. Continued.

Study [reference]	Clinical Group	Number, Total (ω3 LC-PUFA/placebo)	ω3 LC-PUFA Preparation	Daily Dosage Regime(s)	Treatment Status	Duration (days)	Outcome Measure(s)	Quality
Rees et al. 2008 [51]	Perinatal depression	26 (13/13)	Fish oil	0.414 g EPA + 1.638 g DHA	Therapeutic, Monotherapy	42	EPDS, HDRS-17, MADRS	11
Su et al. 2008 [40]	Perinatal depression	33 (17/16)	Menhaden fish body oil concentrate	2.2 g EPA + 1.2 g DHA	Therapeutic, Monotherapy	56	BDI, EPDS, HDRS-21	9
Freeman et al. 2008 [57]	Perinatal depression	59 (31/28)	Not specified	1.1 g EPA + 0.8 g DHA	Therapeutic, Monotherapy[2]	56	EPDS, HDRS	5
van de Rest et al. 2008 [59]	Non-clinical	302 (100, 96/106)	Lipid Nutrition	0.226 g EPA + 0.176 g DHA *or* 1.093 g EPA + 0.847 g DHA	Preventive, Monotherapy	182	CES-D, GDS-15, MADRS	10
da Silva et al. 2008 [42]	Parkinson's disease	29 (6, 8/7, 8)	Not specified	0.720 g EPA + 0.480 g DHA as monotherapy *or* 0.720 g EPA + 0.480 g DHA + antidepressant	Therapeutic, Monotherapy & Adjunctive	84	BDI, MADRS	5
Lucas et al. 2009 [58]	Mild-to-moderate depression	120 (59/61)	Isodis Natura	1.05 g Ethyl-EPA + 0.15 g DHA	Therapeutic, Monotherapy	56	HDRS, HSCL-D-20	11
Doornbos et al. 2009 [56]	Perinatal depression	119[3] (42, 41/36)	Not specified	0.22 g DHA[4] *or* 0.22 g DHA + 0.22 g AA	Preventive, Monotherapy	252	EPDS	4

[1] Crossover design.

[2] This study used ω3 LC-PUFA supplementation as monotherapy, but adjunctive supportive psychotherapy was provided.

[3] 182 women were initially recruited, but 57 dropped out by the 36th week of pregnancy; data on the remaining 125 women are presented.

[4] Only data from the DHA group were entered into this meta-analysis.

BDI = Beck Depression Inventory, CDI = Children's Depression Inventory, CDRS = Children's Depression Rating Scale, CES-D = Center for Epidemiologic Studies of Depression Scale, DASS = Depression Anxiety and Stress Scales, EPDS = Edinburgh Postnatal Depression Scale, GDS-15 = Geriatric Depression Rating Scale, HDRS = Hamilton Rating Scale for Depression (SF refers to short form), HSCL-D-20 = 20-item Hopkins Symptom Checklist Depression Scale, IDS-C = Inventory of Depressive Symptomatology, MADRS = Montgomery-Åsberg Depression Rating Scale, POMS = Profile of Mood States, SCID-IV = Structured Clinical Interview for DSM-IV.

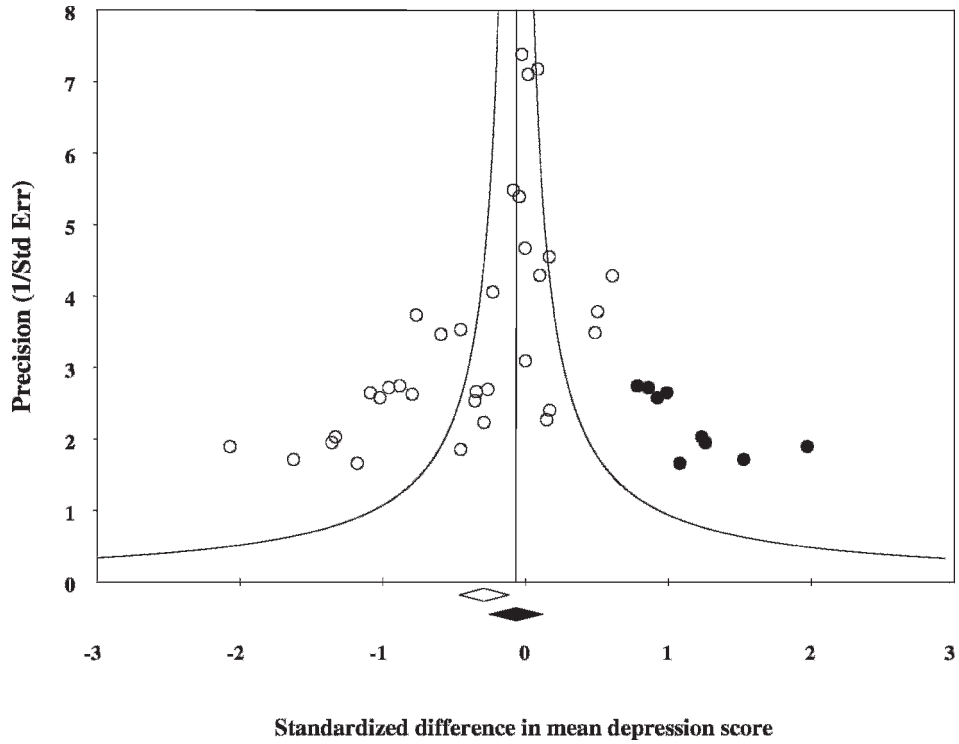

Fig. 1. Funnel plot for all 28 studies of precision (1/standard error) by standardized difference in mean depression scores. Observed studies are shown in open circles with the associated estimate in an open diamond. Imputed studies are shown in solid circles with the adjusted estimate in a solid diamond.

RESULTS

The overall effect using a random effects model showed a significant fall in depressive symptoms with ω3 LC-PUFA supplementation in the 28 studies; standardized difference in means −0.291 (95% CI = −0.463 to −0.120, z = −3.327, p = 0.001). However, significant heterogeneity was present; Q = 98.16, $p < 0.0001$ with an I^2 of 67.4 and $\tau^2 = 0.147$, indicating that the heterogeneity found represented an underlying true difference in effect sizes across studies. In addition, there was considerable evidence of publication bias as shown by the funnel plot in Fig. 1. Using Duval and Tweedie's trim and fill, 9 additional imputed studies (solid circles) are needed to correct the asymmetry of the observed studies (open circles), which reduces the estimate of standardized mean difference to a nonsignificant level of −0.066 (95% CI = −0.252 to 0.121).

Given that substantial heterogeneity was present, rather than assume that ω3 LC-PUFA supplementation in depression is ineffective, further analyses were undertaken to attempt to identify what supplement regimes and in what disorders ω3 LC-PUFA supplementation might show efficacy. In order to investigate possible sources of this heterogeneity, sensitivity and meta-regression analyses were performed. The most obvious source of possible heterogeneity relates to the differing clinical populations studied who are likely to have very different baseline levels of depression. For example, the pathophysiological processes of depressive symptoms involved in schizophrenia [45] are likely to be very different from those in a community sample of individuals with mild-to-moderate depression [52] or in healthy people with little or no depression [68]. To confirm this, a random effects meta-regression analysis was performed of standardized mean depression scores on baseline depression scores using the UREML method. Baseline scores were standardized according to published norms for all of the rating scales used in the study. However, 3 studies had to be excluded from this analysis because no baseline scores were published [48,68,69]. The analysis showed a significant relationship between baseline depression scores and efficacy, with the greater the baseline depression score, the more likely that ω3 LC-PUFA supplementation would reduce depressive symptoms (point estimate for slope = −1.692, 95% CI = −2.969 to −0.415, z = −2.597, p = 0.0094).

To gain an understanding of the difference in likely effect size as a result of baseline depression levels and to assess changes in heterogeneity, the sample was split into 2 groups based upon insignificant and significant levels of baseline depression according to the cut-off cores for each rating scale: normal and mild as insignificant, and moderate and severe as significant. As expected, the group with significant baseline depression showed a substantial and highly significant reduction in standardized mean depression scores with ω3

Table 2. Model Statistics for the Random Effects Subgroup ANOVA Analyses Examining the Influence of Diagnostic Category, Preventive Versus Therapeutic Intervention, and ω3 LC-PUFA Monotherapy Versus ω3 LC-PUFA as an Adjunct to Antidepressant Therapy

Model	Number of Studies	Estimate	95% CI	z Value	*p* of z	Q	*p* of Q
Diagnostic category						76.10	<0.0001
Bipolar disorder	5	−0.364	−0.682 to −0.045	−2.239	0.0251	7.73	0.1720
Borderline personality disorder	1	−0.288	−1.169 to 0.593	−0.641	0.5218	0.00	1.0000
Chronic fatigue	2	−0.140	−1.366 to 1.086	−0.224	0.8229	10.14	0.0015
Major depression	8	−0.551	−1.059 to −0.043	−2.125	0.0336	46.23	<0.0001
Mild-to-moderate depression	2	−0.044	−0.257 to 0.170	−0.403	0.6870	0.071	0.7900
Nonclinical	2	0.016	−0.164 to 0.197	0.178	0.8587	1.24	0.5379
Parkinson's disease	1	−1.405	−2.229 to −0.580	−3.340	0.0008	0.28	0.5965
Perinatal depression	5	−0.071	−0.507 to 0.365	−0.318	0.7503	10.42	0.0339
Recurrent self harm	1	−0.954	−1.677 to −0.232	−2.588	0.0096	0.00	1.0000
Schizophrenia	1	0.000	−0.420 to 0.420	0.000	1.0000	0.00	1.0000
Preventive vs therapeutic						95.24	<0.0001
Preventive intervention	5	−0.060	−0.282 to 0.163	−0.525	0.5996	8.25	0.1432
Therapeutic intervention	23	−0.362	−0.578 to −0.147	−3.296	0.001	86.99	<0.0001
Mono- vs adjunctive therapy						93.56	<0.0001
Monotherapy	16	−0.130	−0.317 to 0.057	−1.360	0.174	33.47	0.004
Adjunctive therapy	13	−0.475	−0.780 to −0.169	−3.042	0.002	60.09	<0.0001

LC-PUFA supplementation (−0.605, 95% CI = −0.871 to −0.339, z = −4.451, $p < 0.0001$), whereas the groups with insignificant depression showed no significant reduction (−0.074, 95% CI = −0.317 to 0.169, z = −0.598, $p = 0.55$). However, despite overall heterogeneity falling by 16.2%, this remained significant (Q = 82.25, $p < 0.0001$). Hence, other factors apart from baseline depression need to be examined to account for the observed levels of heterogeneity.

To further explore the influence of subcategories of depression and other diagnostic groups, together with the influence of preventive versus therapeutic intervention, and ω3 LC-PUFA monotherapy versus ω3 LC-PUFA supplementation used as an adjunct to antidepressant therapy, further sensitivity analyses were performed. The results of these analyses are shown in Table 2. As some diagnostic categories contained only one representative study (borderline personality disorder, Parkinson's disease, recurrent self-harm, and schizophrenia), results are reported in these studies for comparative purposes only. However, when considering groups that contained 2 or more representative studies, these results would suggest that ω3 LC-PUFA supplementation may be ineffective for the treatment of depressive symptoms in chronic fatigue, mild-to-moderate depression, and in non-clinical populations. When considering groups that contained 5 or more representative studies, there was stronger evidence to suggest that ω3 LC-PUFA supplementation may be effective for the treatment of depressive symptoms in bipolar disorder and major depression but ineffective in perinatal depression. Overall, diagnostic variability accounts for 22.4% of the observed heterogeneity (Q = 76.12), which nevertheless remained significant ($p <$ 0.0001). Finally, studies examining therapeutic intervention (n = 23) as opposed to preventive intervention (n = 5), and studies examining adjunctive therapy (n = 13) as opposed to monotherapy (n = 16) showed significant benefit, with these factors accounting for only small proportions of the observed heterogeneity; Q = 95.2 (3%) and 93.56 (1.6%), respectively.

Having identified that baseline depression, diagnostic category, preventive versus therapeutic intervention, and monotherapy versus adjunctive therapy account for some of the observed heterogeneity, further random effects meta-regression analyses were conducted to identify possible sources of outstanding heterogeneity. As in the Ross et al. [63] study, variables associated with the dosages used of DHA and EPA and the DHA:EPA ratio were included alongside various indices of study characteristics, notably methodological quality as assessed by the modified Jadad score, study duration, and sample size. The results of these analyses are shown in Table 3.

These results showed that whilst the total dose of the ω3 LC-PUFA preparation was unrelated to efficacy, the purity of EPA within the preparation appeared to be influential. This was illustrated by the significant negative intercept for dose of DHA, suggesting that studies containing no DHA were more likely to show a fall in depressive symptoms, and the significant negative intercept and positive slope for the DHA:EPA ratio, suggesting that as the purity of EPA increased the more likely the studies were to show a fall in depressive symptoms. With respect to study characteristics, the significant negative intercept of the modified Jadad score suggested that studies of the lowest methodological quality were more likely

Table 3. UREML Random Effects Meta-Regression Analyses for Standardized Difference in Means of Depressive Symptoms

Variable	Point Estimate	95% CI	z Value	*p* Value
Total dose of ω3 LC-PUFA				
Slope	−0.050	−0.146 to 0.045	−1.037	0.2997
Intercept	−0.195	−0.476 to 0.086	−1.361	0.1736
Dose of DHA				
Slope	0.008	−0.206 to 0.221	0.070	0.9439
Intercept	−0.314	−0.555 to −0.074	−2.564	0.0103
Dose of EPA				
Slope	−0.077	−0.192 to 0.037	−1.322	0.1861
Intercept	−0.187	−0.440 to 0.066	−1.450	0.1471
DHA:EPA ratio				
Slope	0.005	0.0001 to 0.011	1.977	0.0480
Intercept	−0.495	−0.759 to −0.230	−3.669	0.0002
Modified Jadad score				
Slope	0.049	−0.031 to 0.129	1.190	0.2342
Intercept	−0.665	−1.288 to −0.043	−2.094	0.0363
Study duration				
Slope	0.003	−0.001 to 0.007	1.682	0.0926
Intercept	−0.603	−1.000 to −0.206	−2.981	0.0029
Sample size				
Slope	0.009	0.003 to 0.014	2.961	0.0031
Intercept	−0.617	−0.892 to −0.341	−4.387	<0.0001

to show a fall in depressive symptoms; the significant negative intercept for study duration suggested that studies of the shortest duration were more likely to show a fall in depressive symptoms; and the significant negative intercept and positive slope for sample size suggested that the smallest studies were more likely to report a fall in depressive symptoms but, as study sample size increased, no change in depressive symptoms was more likely to be reported.

On the basis of these meta-regression findings suggesting that EPA purity was a significant factor, a random effects subgroup ANOVA was performed as in the Ross et al. [63] study. However, in this case, the current studies were classified into 4 groups on the basis of whether they employed pure DHA, mainly DHA (>50%), mainly EPA (>50%), or pure ethyl-EPA. One of the included studies employed ethyl-EPA, but as a small quantity of DHA was also present, this study was classified as mainly EPA rather than pure ethyl-EPA [58]. The results of this analysis are shown in a forest plot (Fig. 2) and the associated Table 4.

These results appear to confirm the observation originally made by Ross et al. [63], that only ω3 LC-PUFA preparations with a predominant or pure EPA content show efficacy in treating depressive symptoms. In addition, the observed heterogeneity was now insignificant in the pure DHA (Q = 0.687, $p = 0.7093$), predominantly DHA (Q = 6.98, $p = 0.0726$), and pure ethyl-EPA (Q = 16.76, $p = 0.0799$) groups, with low levels of between-study variance ($\tau^2 = 0.000$, 0.064, and 0.069, respectively). However, the predominantly EPA group of studies still showed significant heterogeneity (Q = 62.78, $p < 0.0001$) and between-study variance ($\tau^2 = 0.248$), and overall heterogeneity fell by only 11.15% (Q = 87.2).

Despite these findings suggesting EPA efficacy, a note of caution is necessary here, both because the number of studies within each group was small, especially in the pure DHA and mainly DHA groups, and because meta-analytic and meta-regression findings are observational, so it is important not to assume causality on the basis of these findings. The reader will recall that the earlier UREML analyses (Table 3) showed that as the proportion of EPA in the ω3 LC-PUFA preparation rose, so did efficacy, but that in contrast, study sample size and study duration were inversely associated with efficacy. Therefore, the ''effect'' of EPA-containing preparations could simply be confounded by the fact that studies with small sample sizes and of short duration are more likely to show efficacy than are studies with large sample sizes and long duration. Against this possibility, however, is the finding of nonsignificant correlations between DHA:EPA ratio and both study duration (Kendall's tau = 0.127, z = 1.0869, $p = 0.2771$), and sample size (tau = 0.216, z = 1.8063, $p = 0.0709$).

Although the possibility of confounding relationships appeared to be minimal in the whole group of studies, within the pure ethyl-EPA group of studies, a number of anomalous and/or confounding relations were suggested by UREML meta-regression analyses (Table 5). Despite an overall beneficial effect of pure ethyl-EPA on depressive symptoms, a paradoxical inverse relationship between EPA dose and improvement in depressive symptoms was evident in this group as shown by a significant negative value for intercept and a significant positive value for slope. In addition, both study duration and sample size were inversely associated with improvement in depressive symptoms to a highly significant extent. Moreover, in this group of studies, a significant correlation was evident between the EPA dose itself and study duration (Kendall's tau = 0.4, z = 1.988, $p = 0.0468$), which could therefore indicate that the inverse relationship between EPA and efficacy is artefactual and simply a consequence of the inverse relationship between study duration and efficacy.

In contrast to these observations in the pure ethyl-EPA group of studies, when the mainly DHA and mainly EPA groups are considered independently (n = 17 studies), UREML meta-regression analyses shown in Fig. 3 demonstrated a significant dose-response relationship for EPA efficacy in reducing depressive symptoms (EPA point estimate for slope −0.195, 95% CI = −0.375 to −0.015, z = −2.123, $p = 0.0338$; and intercept −0.052, 95% CI = −0.398 to 0.294, z = −0.293, $p = 0.7692$), whereas DHA dose was unrelated to efficacy (DHA point estimate for slope −0.032, 95% CI =

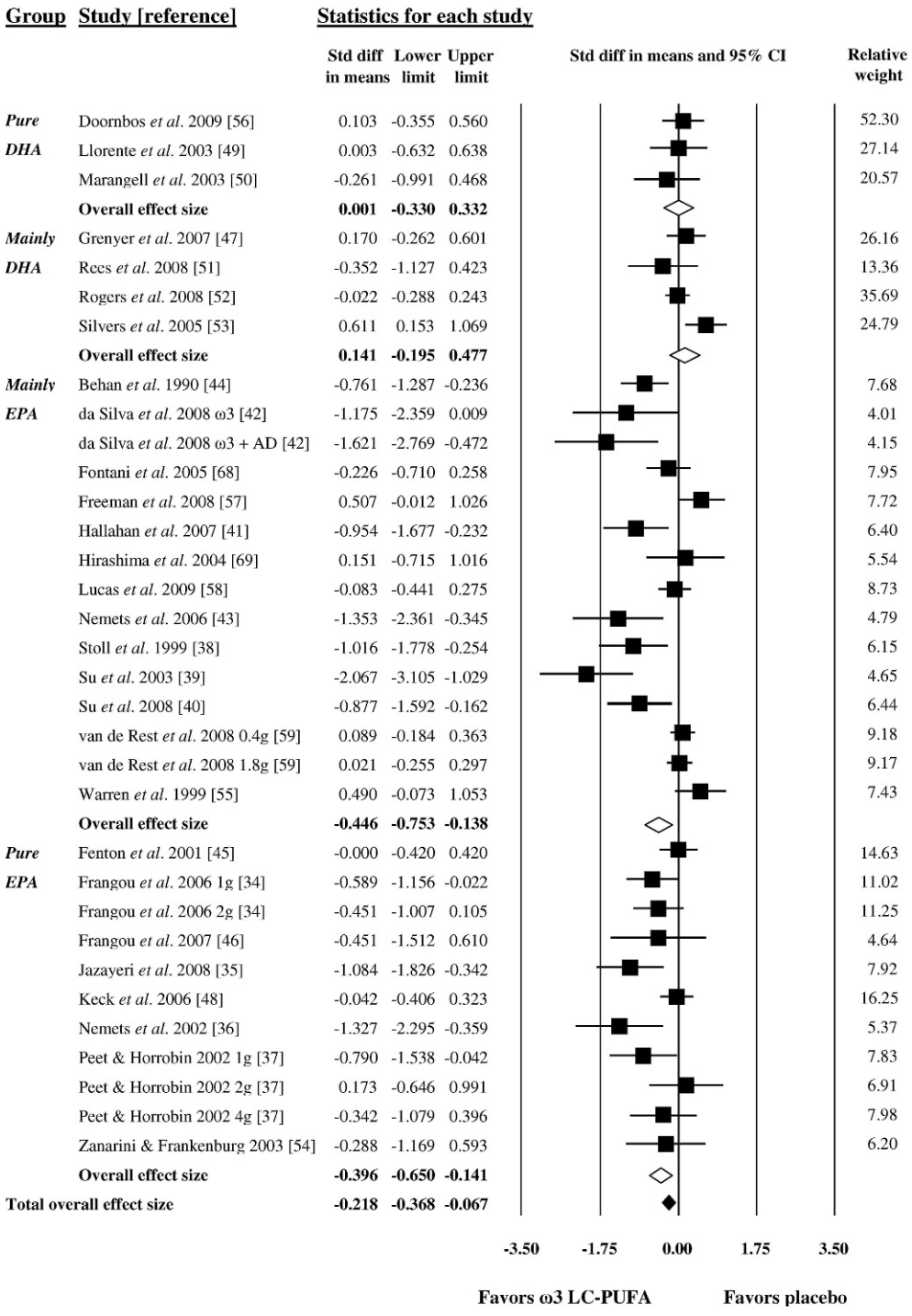

Fig. 2. Forest plot examining the effect of the type of ω3 LC-PUFA supplementation employed on the reduction in depressive symptoms.

−0.357 to 0.294, z = −0.191, p = 0.8488; and intercept −0.296, 95% CI = −0.756 to 0.165, z = −1.257, p = 0.2088). In addition, in these studies employing mixed ω3 LC-PUFA preparations, there appeared to be no evidence of confounding with study duration (point estimate for slope 0.002, 95% CI = −0.005 to 0.009, z = 0.517, p = 0.6052; and intercept −0.497, 95% CI = −1.201 to 0.208, z = −1.381, p = 0.1672), although studies with small sample sizes were still more likely to show a fall in depressive symptoms with supplementation (point estimate for slope 0.009, 95% CI =

Table 4. Model Statistics for the 4 Subgroup Random Effects ANOVA of ω3 LC-PUFA Preparation Type

Group	Number of Studies	Estimate	95% CI	z Value	*p* of z	Q	*p* of Q	I^2	τ^2
Pure DHA	3	0.001	−0.330 to 0.332	0.004	0.9965	0.687	0.7093	0.00	0.000
Mainly DHA	4	0.141	−0.195 to 0.477	0.821	0.4116	6.98	0.0726	57.01	0.064
Mainly EPA	13	−0.446	−0.753 to −0.138	−2.843	0.0045	62.80	0.0000	77.71	0.248
Pure EPA	8	−0.396	−0.650 to −0.141	−3.051	0.0023	16.76	0.0799	40.33	0.069

0.001 to 0.017, z = 2.223, $p = 0.0262$; and intercept −0.696, 95% CI = −1.130 to −0.261, z = −3.140, $p = 0.0017$). It is noteworthy that in these groups the ω3 LC-PUFA preparations were predominantly of natural origin, in contrast to artificially purified DHA or synthetic ethyl-EPA, with 3 out of the top 6 studies showing the strongest effect sizes employing the mainly EPA containing menhaden fish body oil.

DISCUSSION

The results of the current meta-analysis appear to confirm the original observation made by Ross et al. [63] that EPA and not DHA may be the responsible agent conferring benefit for the treatment of depressive symptoms with ω3 LC-PUFA supplementation (see Fig. 2 and Table 4). These results also demonstrate that it is inappropriate to assume that the effects of these 2 ω3 LC-PUFAs will be the same, either in randomized controlled trials or in meta-analytic studies examining the effect of ω3 LC-PUFA supplementation in a variety of disorders. It is noteworthy that the meta-analyses by Appleton et al. [62] and Rogers et al. [52], which largely concluded that ω3 LC-PUFA supplementation was ineffective in depression, did not consider the differential effects of EPA versus DHA. Moreover, these findings demonstrate that there is a significant relationship between baseline depression levels and efficacy, indicating that future studies examining the effects of ω3 LC-PUFA supplementation in depression should ensure that the population studied is actually suffering from clinically relevant levels of depressive symptomatology.

Table 5. UREML Meta-Regression Statistics for Standardized Difference in Means of Depressive Symptoms in the Ethyl-EPA Group of Studies

Variable	Point Estimate	95% CI	z Value	*p* Value
Dose of EPA				
Slope	0.122	0.026 to 0.218	2.484	0.0130
Intercept	−0.694	−1.040 to −0.347	−3.924	0.0001
Modified Jadad score				
Slope	−0.055	−0.212 to 0.102	−0.686	0.4928
Intercept	0.002	−1.124 to 1.127	0.003	0.9979
Study duration				
Slope	0.013	0.005 to 0.021	3.346	0.0008
Intercept	−1.535	−2.270 to −0.801	−4.097	0.0000
Sample size				
Slope	0.014	0.004 to 0.024	2.720	0.0065
Intercept	−0.790	−1.174 to −0.405	−4.028	0.0001

For example, in the large, excellently designed study by Rogers et al. [52] baseline Beck Depression Inventory (BDI) scores were 13.9 in both study groups. According to the published norms for the BDI, a score of 0–13 is regarded as indicative of minimal or no significant depression, 14–19 as indicative of mild depression, 20–28 as indicative of moderate depression, and 29–63 as indicative of severe depression [70]. This would suggest that, despite the recruitment strategy having been designed to select individuals with mild-to-moderate depression, the population studied by Rogers et al. [52] was not suffering from clinically relevant depression and, notwithstanding the mainly DHA-containing regime used in this study, these 2 factors could explain why no clinical effect was demonstrated.

In addition, the subgroup analyses presented in Table 2 provide further evidence that ω3 LC-PUFA supplementation may be most efficacious in clinical populations. Notably, the 2 studies in non-clinical populations failed to demonstrate efficacy [59,68] and ω3 LC-PUFA supplementation was most effective in bipolar disorder and major depression, as a therapeutic as opposed to a preventive intervention, and as an adjunctive treatment rather than as monotherapy. The increased efficacy of ω3 LC-PUFA supplementation as an adjunctive treatment is consistent with the observational study of Féart et al. [24], which found that higher plasma EPA concentration was associated with lower depressive symptoms, especially in patients taking antidepressants. Overall, these analyses lend further support to the conclusion that ω3 LC-PUFA supplementation is most efficacious in clinical populations. However, the point prevalence of major depression is low at 2.6% [71], meaning that in order to identify whether ω3 LC-PUFA supplementation is effective in non-clinical populations for the prevention of new episodes of depression, much larger sample sizes, studied over an extended period of time, may be required to demonstrate efficacy.

Although the current meta-analysis identified that diagnostic variation, baseline depression severity, and ω3 LC-PUFA regime type accounted for some of the observed heterogeneity, significant heterogeneity remained. A possible

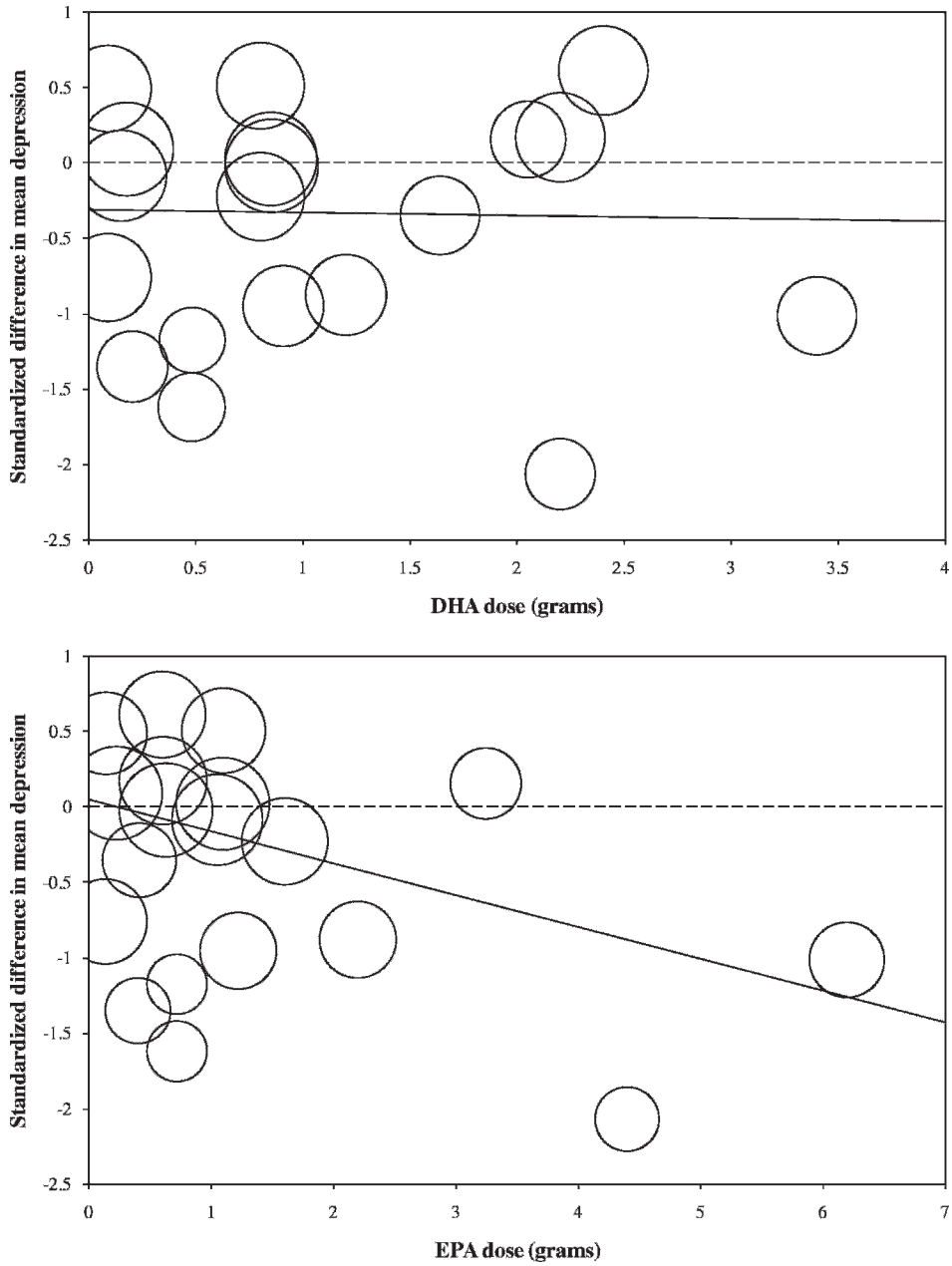

Fig. 3. UREML meta-regression of DHA and EPA dose on standardized difference in mean depression scores in the mainly DHA and mainly EPA groups of studies. The size of the circles is proportional to the statistical weighting attached to each study.

outstanding source of this heterogeneity is the influence of sex, which, however, could not be assessed in the current meta-analysis, as none of the included studies reported outcomes for depressive symptoms stratified by sex. Despite documented epidemiologic associations between fish intake and reduced risk of depression [13–19], one study that analyzed results by sex found that low consumption of fish was associated with depression only in women [20]. In addition, metabolic studies indicate that, despite identical dietary intake, women show higher levels of plasma DHA than men [72]. This increase in DHA in women appears to be sensitive to estrogen and may therefore represent a biological preparedness for the large demands of the fetus and neonate for DHA during pregnancy and lactation [73]. This, in turn, may cause women to be more vulnerable to dietary deficiencies of ω3 LC-PUFAs and may also explain, at least in part, why women are at greater risk for the development of depression [74]. It may also explain why studies examining perinatal depression in the current meta-analysis failed to show efficacy, as supplemented ω3 LC-PUFAs may have been diverted to the demands of the developing fetus rather than contributing to therapeutic efficacy for depressive symptoms. Subsequent studies of ω3

LC-PUFA supplementation in depression should therefore report outcomes in both males and females.

Despite these conclusions concerning EPA efficacy, a number of concerns remain regarding the robustness of the findings concerning the effectiveness of EPA in depressive symptoms identified in the current meta-analysis. These concerns relate to the potential influence of specific methodological characteristics of the included studies; publication bias; statistical power; anomalous findings with respect to the influence of the proportion of EPA versus the absolute amounts of EPA between differing subgroups of studies; and the quantitative assessment of study methodological quality, sample size, and duration.

First, the studies were of mixed methodological quality according to the Jadad system: in 12 out of the 28 studies, the method of randomization was not adequately described [39–43,45,48,50,54,57,68,69]; in 6 studies the nature of the placebo intervention was not adequately described [42,50,54,56,57,69]; and in a further 4 studies dropouts were not adequately described [44,46,68,69]. Furthermore, in 2 studies dropout rates were very high. In the negative study by Doornbos et al. [56], examining the effect of low dose DHA for the prevention of perinatal depression, 57 out of the 182 individuals dropped out of the study during pregnancy. In the negative study by Keck et al. [48], examining pure ethyl-EPA for the adjunctive treatment of bipolar disorder, 54% of the participants dropped out prior to completion.

Regarding the use of ITT analyses, only 1 out of the 3 pure DHA studies used ITT [50]; all 4 of the mainly DHA studies used ITT [47,51–53]; only 2 out of the 13 mainly EPA studies used ITT [40,41]; and 5 out of the 8 pure ethyl-EPA studies used ITT [34,36,37,45,48]. Given that the mainly EPA group of studies showed the largest effect size, the relative absence of ITT analyses in this group may have biased the results in favor of EPA supplementation.

Concerning whether the outcome assessors were blinded to treatment group, this could not be ascertained with certainty from descriptions given in 2 out of the 3 pure DHA studies [49,50], in 4 out of the 13 mainly EPA studies [38,39,68,69], and in 3 out of the 8 pure ethyl-EPA studies [37,48,54]. Of note, all of the mainly DHA studies had clear descriptions of outcome assessor blinding [47,51–53]. It is possible, therefore, that a relative reduction in outcome assessor blinding observed in the studies employing EPA could have biased the results in favor of EPA supplementation.

Regarding baseline comparisons, 1 pure DHA study [56], 5 mainly EPA studies [42–44,68,69], and 3 pure ethyl-EPA studies [35,37,48] did not provide adequate descriptions of baseline characteristics between supplement and placebo groups. In 2 of the pure ethyl-EPA studies, baseline measurements were provided, but either no baseline variance [37] or appropriate labeling [35] of this variance was indicated. It is possible, therefore, that the relative absence of information regarding differences in baseline characteristics among studies employing EPA supplementation could have led to over- or underestimation of the efficacy of EPA. With respect to the use of pure DHA, in one of these studies, baseline Hamilton Depression Rating Scale measures were significantly greater in the placebo group compared with the DHA group [50], potentially biasing the results against demonstrating efficacy for DHA supplementation.

Concerning the attempt to conceal differences in the perception of fish taste between ω3 LC-PUFA supplementation and placebo groups, none of the pure DHA studies employed this strategy; 2 out of the 4 mainly DHA studies added orange oil [52] or peppermint oil [51] to both active and placebo preparations; 5 out of the 13 mainly EPA studies either stated that the capsules were indistinguishable with respect to taste [68]; or added 0.2% fish oil [58], 1% fish oil [57], or 1% EPA/DHA mixture [41] to the placebo capsules; and 1 out of the 8 pure ethyl-EPA studies stated that the capsules were indistinguishable by aftertaste [48]. It is possible, therefore, that a greater number of individuals in the pure ethyl-EPA group of studies were able to perceive that they were in receipt of ω3 LC-PUFA supplementation rather than placebo, biasing the results in favor of efficacy for EPA-containing preparations. However, none of the pure DHA groups made any attempt to conceal fish taste and, in one of these studies, a fish aftertaste was reported in 14 out of 35 participants, which might have disrupted blinding and biased the result in favor of the DHA group [50]. Moreover, the need to conceal fish taste may be more important for mixed preparations than for purified ethyl-EPA. In the study by Stoll et al. [38] employing mainly EPA without concealment, 86% of the ω3 LC-PUFA group correctly guessed their group allocation compared with 63% in the placebo group, whereas in the study by Nemets et al. [36] employing pure ethyl-EPA without concealment, neither patients nor clinicians were able to guess group allocation correctly. In addition, studies employing mixed preparations, which conducted both concealment and the assessment of blinding success, confirmed that individuals were unable to guess group allocation [51,58]. Therefore, it is unlikely that this factor significantly biased the overall results presented in this meta-analysis, as concealment was undertaken with equal frequency between the mainly DHA and mainly EPA groups of studies.

Regarding the assessment of compliance using RBC membrane or plasma phospholipid analyses, all 3 pure DHA studies conducted these analyses, showing significant changes in RBC membrane DHA levels as a result of supplementation [49,50,56]; all 4 mainly DHA studies conducted these analyses, showing significant increases in RBC membrane EPA and DHA [47,53], plasma EPA and DHA [52], and plasma total ω3 LC-PUFA levels [51]; 8 out of the 13 mainly

EPA studies conducted these analyses, showing either a significant increase in RBC membrane EPA and DHA [42,44,58], a significant decrease in plasma arachidonic acid (AA)/EPA ratio [68], a significant increase in RBC DHA but not EPA [40], or no significant changes in RBC membrane EPA or DHA [39,55]; and only 1 out of the 8 pure ethyl-EPA studies conducted these analyses, which nevertheless showed a significant increase in RBC membrane EPA content [45]. The lack of assessment of compliance in all but one of the pure ethyl-EPA group of studies suggests that the reported benefits on depressive symptoms in this group of studies cannot therefore be definitively attributed to the EPA content of the supplementation regime. In addition, the studies by Su et al. [39,40], which contributed substantially to the overall efficacy results of EPA-containing preparations, showed no significant change in RBC membrane EPA or DHA in the first study [39], and a significant change in RBC membrane DHA but not EPA in the second study [40]. Given that these studies employed EPA doses of 4.4 g and 2.2 g respectively, these findings are surprising, as other studies employing much lower loses of EPA, ranging from 80 mg to 600 mg, have shown significant increases in RBC membrane EPA [75–78]. This could suggest that the reported benefits on depressive symptoms in the studies by Su et al. [39,40] may have occurred as a result of factors other than EPA supplementation.

Outstanding methodological issues related to the exclusion of placebo responders during 1-week single-blind run-in phases in the Su et al. [39,40] studies, where 4 out of 34 patients in the first study [39] and 4 out of 40 in the second study [40] were excluded on the basis of a >20% reduction in HDRS scores. Whilst inclusion of this strategy undoubtedly compromises study generalizability, it has not, in fact, been shown to result in magnified treatment versus placebo group differences or to have any discernable impact on differential response rates [79]. Thus, in a meta-analysis of 34 trials examining 3047 patients receiving a selective serotonin-reuptake inhibitor for depression versus 3740 patients receiving placebo, there was no statistically significant difference in effect size between the clinical trials that had a placebo run-in phase followed by withdrawal of placebo responders and those trials that did not [80]. Therefore, it is unlikely that inclusion of the Su et al. [39,40] studies in the current meta-analysis has resulted in overestimation of ω3 LC-PUFA supplementation treatment effects.

The second concern regarding the robustness of these findings of EPA efficacy relates to the considerable evidence of publication bias evident from both the funnel plot (see Fig. 1) and the imputation of 9 further studies required to correct this bias from the Duval and Tweedie trim and fill analysis. Therefore, it is possible that had these ''missing'' studies been available for inclusion in the current meta-analysis, the preferential effect of EPA versus DHA might no longer be evident.

Third, the number of studies included in the analysis of subgroups was small, especially in the pure DHA and mainly DHA groups, which contained only 3 and 4 studies, respectively. Thus, only 7 studies examined DHA in contrast with 21 studies examining mainly EPA or pure EPA. With a larger number of studies in the DHA subgroups, it is possible that the preferential effect of EPA might become less evident.

Fourth, although the overall meta-regression analyses (see Table 3) suggested that, as the proportion of EPA increases within the preparation so does efficacy, analysis of the ethyl-EPA subgroup of studies suggested an inverse relationship between absolute EPA dose and efficacy (see Table 5). This latter finding is consistent with Peet and Horrobin's [37] dose-ranging study that identified 1 g/d of EPA as being more effective than either 2 or 4 g/d. It is also consistent with the same research group's hypothesis that the balance between EPA and AA within neuronal membranes may be important for optimal neurologic functioning. For example, in a study of EPA supplementation in schizophrenia, RBC membrane AA but not EPA correlated with clinical improvement [81], suggesting that excessive dosages of EPA may deplete neuronal AA, which may not be beneficial. However, against the conclusion that only low-dose EPA is efficacious is the finding from the current meta-analysis that in the larger group of 17 studies employing mixed EPA and DHA supplements, a significant positive relationship between absolute EPA dose and efficacy was demonstrated. Taken together with the significant correlation between EPA dose and study duration in the ethyl-EPA group of studies (Kendall's tau = 0.4), this would suggest that the inverse dose-response relationship with efficacy in the ethyl-EPA group of studies is a chance artefactual finding. However, further research will be required to finally resolve this question.

Fifth, the meta-regression analyses on all 28 studies showed that studies with the lowest methodological quality score, sample size, and duration were more likely to show efficacy, whereas studies with large sample sizes were least likely to show efficacy (see Table 3). These findings could indicate that the positive efficacy of EPA in ameliorating depressive symptoms found in the current meta-analysis might be confounded by indices of study quality and therefore be a chance finding rather than a real effect. These concerns appeared to be most extreme in the ethyl-EPA group of studies where a highly significant inverse relationship between efficacy and study duration and sample size was demonstrated (see Table 5). Whilst this inverse association could indicate that EPA has only a temporary effect in depression that diminishes over time, the inverse relationship between efficacy and both absolute EPA dose (as discussed above) and sample size (see Table 5) would again tend to support the notion that both the purported preferential effect of low dose ethyl-EPA, and the purported temporary effect of ethyl-EPA, are

artifactual chance findings. It is noteworthy that there was no association between methodological quality and efficacy in the ethyl-EPA group of studies, as generally these studies were conducted to a high standard. The relative cost of ethyl-EPA as opposed to fish oil or enriched natural preparations may have limited sample size and duration, and thus be a possible explanation for the exacerbation of chance associations in this group of studies. However, against the conclusion that the efficacy of EPA in depression is a chance finding is the fact that in the whole group of studies the correlation between DHA:EPA ratio and study duration was insignificant; and, in the mainly DHA and mainly EPA group of studies, the largest grouping of studies in this analysis, a significant positive association between absolute EPA dose and efficacy was found that was not confounded by study duration (see Fig. 3).

Despite the methodological problems outlined in the previous sections, the evidence from 17 studies indicating a dose-response relationship between EPA dose and efficacy for depressive symptoms would suggest that EPA in the form of natural or enriched preparations may be more beneficial than pure ethyl-EPA. It is noteworthy that, in the 6 studies showing the greatest efficacy, 3 of them employed the mainly EPA containing menhaden fish body oil with an EPA:DHA ratio of approximately 65%. Further studies are required of sufficient sample size, duration, and methodological quality to compare pure ethyl-EPA with natural EPA preparations containing EPA:DHA ratios ranging from 60% through to 100% to resolve the question of what constitutes an optimal ω3 LC-PUFA formulation in the treatment of depression.

DHA is the most abundant LC-PUFA present in brain cell membranes in contrast to EPA, which is present at levels several hundred-fold lower than DHA [82,83]. Consequently, the rationale for supplementation with DHA has historically rested upon the assumption that increasing the nutritional availability of a major structural component of neuronal membranes would have beneficial effects on brain function, including the amelioration of depression. However, rather than providing a structural substrate, evidence is accumulating that ω3 LC-PUFAs may instead exert their effects through cell signaling mechanisms as outlined below. This may explain why EPA, present at very low levels in the brain as compared with DHA, may have beneficial effects in depression.

Regarding the possible reasons why EPA and not DHA may be more effective in depression, there is increasing evidence that DHA supplementation may have damaging effects on the nervous system. DHA, the most highly unsaturated ω3 LC-PUFA in the body, is very susceptible to lipid peroxidation and can damage DNA [84], increase production of reactive oxygen species in glial cells [85], and worsen neurologic state in rat perfusion-injury models [86]. Highly reactive A_4/J_4 neuroprostanes produced from DHA *in vivo* under conditions of oxidative stress [87] may explain some of the above negative findings. In addition, although retro-conversion of DHA to EPA can occur to a certain extent, DHA is at the end of the biosynthetic pathway of ω3 LC-PUFAs and therefore supplementation may boost DHA to levels that cannot be adequately handled by metabolic pathways, which in turn, may further exacerbate production of damaging reactive derivatives. It is noteworthy that under normal circumstances, the rate of conversion of dietary α-linolenic acid to DHA is about 1% [88] and daily turnover of DHA in the adult human brain is only 4.6 mg [89], suggesting that it may not be desirable to boost DHA levels to such an extent as occurs during supplementation [82].

In contrast to the above findings with respect to DHA, there are many lines of evidence to indicate why EPA might be beneficial in depression. First, EPA has neuroprotective actions on lipopolysaccharide (LPS)-induced hippocampal dysfunction via the prevention of LPS-induced phosphorylation of c-Jun N-terminal kinase, c-Jun and Bcl-2, which in turn prevents the secretion of interleukin 1β (IL-1β), prevents increases in mitochondrial membrane permeability, prevents release of cytochrome C, and prevents neuronal apoptosis [90]. Second, oxidized derivatives of EPA, unlike the A_4/J_4 neuroprostanes derived from DHA as described above, have beneficial anti-inflammatory effects [91], in addition to the well-documented anti-inflammatory effects of EPA-derived eicosanoids [11]. Third, with respect to the inflammatory hypothesis of depression, EPA appears to have the following effects: (1) EPA is more effective than DHA at reducing the inflammatory cytokines tumor necrosis factor-α (TNF-α), IL-6, and IL-1β [92], an action that occurs via the mechanism of EPA inhibition of the activity of nuclear factor kappa-B (NF-κB), an important nuclear regulator of the inflammatory response [93]; (2) although dietary EPA and DHA incorporate into cell membranes equally as DHA, dietary EPA is more effective at reducing inflammation *in vivo* [94]; and (3) dietary DHA may not be beneficial in depression owing to the fact that it appears to induce a T helper cell type 1–like immune response with a raised interferon-γ to IL-10 ratio, whereas EPA does not induce this effect [95]. For this reason, the authors of this latter study argue that highly purified EPA, free of any DHA, should be used in the treatment of depression.

Given that the available evidence discussed above suggests that pure EPA, in contrast to DHA, may be beneficial for depressive symptoms, this might appear at odds with the epidemiologic evidence linking fish consumption, a source of mixed fatty acids, with reduced risk of depression. However, the fatty acid content of fish varies substantially by species, method of farming, and season. For example, the percentage wet weight of EPA versus DHA is, respectively, 15.7 versus 0.7 for anchovy oil, 11.0 versus 9.1 for Atlantic menhaden oil, and 6.2 versus 9.1 for Atlantic salmon oil (see Table 2.2 on page 25 of reference 96). In addition, seasonal variation in

fatty acid content is largely determined by spawning. Thus, fish accumulate EPA and DHA in muscle tissue before the reproductive season and transfer these fatty acids to the gonads during spawning when levels of LC-PUFA in hard roe can reach 3–4 times higher than those in muscle tissue [97]. These natural variations in fatty acid levels may explain, in part, the mixed findings obtained from epidemiologic studies of fish consumption and intervention studies employing fish oils for therapeutic benefit in depression. If possible, further studies should examine whether individuals consuming predominantly EPA-containing fish are at lower risk for the development of depression compared with those consuming fish with a higher DHA content. If confirmed, this might have important implications with respect to dietary advice regarding the prevention of depression.

In conclusion, there is substantive evidence both from the current meta-analysis, and from the studies outlined above, that EPA and not DHA may be effective in depressive disorders. However, further studies are required of sufficient methodological quality, duration, and sample size to confirm these findings. A direct comparative trial of EPA versus DHA should be conducted, preferably also comparing these fatty acids in triglyceride versus ethyl ester forms to identify whether the method of fatty acid delivery influences efficacy. If EPA superiority is confirmed, further studies are needed to answer the following outstanding issues: (1) Are pure EPA preparations better than those containing a maximum of 40% DHA? (2) If DHA-free EPA preparations are found to be more effective, is highly purified ethyl-EPA better than enriched natural products containing triglycerides? and (3) If a U-shaped dose-response curve and short-term effect of EPA in depression is replicated, could this be due to increasing AA depletion as supplementation progresses? If so, could the addition of γ-linolenic acid to the supplement regime prolong the effect of EPA and allow larger doses of EPA to be tolerated by both increasing the synthesis of AA and by increasing the production of dihomo-γ-linolenic acid derived anti-inflammatory eicosanoids?

ACKNOWLEDGMENT

The author wishes to thank Mr Peter Avakian, Director of the Academy of Nutritional Medicine, for his unfailing support and encouragement during the preparation of this manuscript.

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Received September 19, 2008; revision accepted July 13, 2009.