Omega-3 and polyunsaturated fat for prevention of depression and anxiety symptoms: a systematic review and meta-analysis of randomised trials

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ABSTRACT 250 /250

Background: There is strong public belief that polyunsaturated fats protect against and ameliorate depression and anxiety.

Aims: To assess effects of increasing omega-3, omega-6 or total polyunsaturated fat on prevention and treatment of depression and anxiety symptoms.

Method: We searched widely (Central, Medline, Embase to April 2017, trials registers to September 2016, ongoing trials updated August 2019), including trials of adults with or without depression or anxiety, randomised to increased omega-3, omega-6 or total polyunsaturated fat for ≥24 weeks, excluding multi-factorial interventions. Inclusion, data extraction and risk of bias were assessed independently in duplicate, authors contacted for further data. We used random-effects meta-analysis, sensitivity analyses, subgrouping and GRADE assessment.

Results: We included 31 trials assessing effects of long-chain omega-3 (n=41,470), one of alpha-linolenic acid (n=4837), one of total polyunsaturated fat (n=4997), none of omega-6. Meta-analysis suggested increasing long-chain omega-3 probably has little or no effect on risk of depression symptoms (RR 1.01, 95% CI 0.92-1.10, I^2 0%, median dose 0.95g/d, duration 12 months) or anxiety symptoms (SMD 0.15, 95% CI 0.05-0.26, I^2 0%, median dose 1.1g/d, duration 6 months, both moderate-quality evidence). Evidence of effects on depression severity and remission in those with existing depression were unclear (very low-quality evidence). Results did not differ by risk of bias, omega-3 dose, duration or nutrients

replaced. Increasing alpha-linolenic acid by 2g/d may increase risk of depression symptoms very slightly over 40 months (number needed to harm=1000).

Conclusions: Long-chain omega-3 supplementation probably has little or no effect in preventing depression or anxiety symptoms.

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Keywords:

fatty acids omega-3, alpha-linolenic acid, docosahexaenoic acids, eicosapentaenoic acid, fatty acids omega-6, depression, anxiety, meta-analysis, randomized controlled trial

Relevance statement:

Many adults take omega-3 supplements to improve their mental health. Our comprehensive systematic review and meta-analysis included 31 trials (41470 participants) assessing long-term effects of long-chain omega-3 (LCn3). Meta-analysis suggested increasing LCn3 probably has little or no effect on risk of depression or anxiety symptoms (moderate-quality evidence). Results did not differ by risk of bias, omega-3 dose, duration or nutrients replaced. Physicians should not recommend omega-3 supplements for reducing depression or anxiety risk. Long-term utility of LCn3 in existing depression is unclear.

Background

There is a common belief that increasing omega-3 intake may prevent and treat both depression and anxiety, and in the US long-chain omega-3 intakes are greater from dietary supplements (0.72 g/d EPA and DHA) than foods (0.41 g/d).(1) Globally depressive disorders are the third most common cause of years lived with disability in women, and fifth in men, while anxiety disorders are eighth and fifteenth respectively.(2) Lifetime prevalence of anxiety disorders is 10-17%, mood disorders 10-16%,(3, 4) with higher rates in people with long-term conditions.(5-7)

The aetiological theories of depression and anxiety suggest concurrent alterations in brain chemistry, environmental stressors, and genetic predisposition. Polyunsaturated fatty acids (PUFAs), including long-chain omega-3 (LCn3, mostly from fish), alpha-linolenic acid (ALA, a plant-based omega-3) and omega-6 fatty acids (mostly from vegetable oils) have roles in the synthesis, release, reuptake, degradation and binding of neurotransmitters, and in neural structure and function.(8-10) Neuronal cell membranes contain high levels of docosahexaenoic acid (DHA, an LCn3). Observational research suggests correlations between low omega-3 or fish consumption and depression,(11, 12) while people with social anxiety disorder have lower erythrocyte membrane omega-3 and higher omega-6/omega-3 ratios than controls, and negative correlations between omega-3 levels and anxiety scores have been observed.(13) Thus, increasing omega-3 intake and/or reducing omega-6 intakes may have anti-depressant and anxiolytic effects,(9, 14) but reverse causation is highly feasible in that poor mental health may lead to lower quality dietary intake.

We aimed to assess effects of increasing LCn3, ALA, omega-6 or total PUFA on depression and anxiety in randomised controlled trials of at least 6 months duration.

Method

This systematic review and meta-analysis is part of a series of systematic reviews commissioned by WHO assessing health effects of omega-3, omega-6 and total PUFA. (15-23) Its protocol was registered (*PROSPERO*: CRD42017056092). Specific methods for this review are discussed below, detailed methods for the review series are reported elsewhere, including detailed search strategies, list of variables data extracted, and the wider database of trials.(22)

Criteria for considering studies for this review

Types of studies

We included only randomised controlled trials (RCTs) of at least 6 months (24 weeks) duration. The 24 week cut-off reflects metabolic studies suggesting 6 months as the minimum duration of supplementation required to ensure equilibration of LCn3 into most body compartments, including the brain.(24)

Types of participants

Participants in included studies had to be adults (18+ years) who were not pregnant or seriously ill. Participants could have a current or previous diagnosis of clinical depression or anxiety, but this was not necessary.

Types of intervention and comparison

Studies were included where they compared higher with lower omega-3, omega-6 and/or total PUFA intakes. The intervention could consist of advice, foodstuffs or oral supplements (oil, capsules, or provided foodstuffs) that aimed to alter omega-3, omega-6 and/or total PUFA intake, or (if no specific aim was stated) achieved a change of ≥10% of baseline intake. Studies were excluded if they examined multiple risk factor interventions on lifestyle or dietary factors other than PUFA. Interventions had to be compared with usual diet, no advice, no supplementation or placebo (as appropriate) or compared raised versus lowered PUFA intake over ≥24 weeks.

Types of outcome measures

Included trials assessed at least one of the primary outcomes (even where these outcomes were not fully reported).

Primary outcomes:

- Risk of depression or anxiety symptoms assessed using formal diagnosis or an appropriate scale, dichotomised to give risk of depression or anxiety in participants without depression or anxiety at baseline
- Severity of depression or anxiety symptoms as a continuous scale in participants with or without existing depression.
- Severity of depression or anxiety, or relapse, in those with depression at baseline.

Assessment of depression or anxiety did not have to be the main study goal.

Secondary outcomes:

- Social participation
- Quality of life
- Carer stress
- Healthcare and patient costs
- Adherence
- Fidelity
- Adverse events
- Withdrawal rates
- Withdrawals due to non-compliance, lack of efficacy and/or side effects.
- Psychosis, suicidality, suicide and self-harm

Secondary outcomes were data extracted from included studies.

Search methods for identification of studies

We searched Cochrane Central, Medline and Embase to 27th April 2017, ClinicalTrials.com and the World Health Organization International Clinical Trials Registry Platform to September 2016, and reassessed all ongoing trials in August 2019. Searches were not limited by language or publication date. We checked included trials of relevant systematic reviews, and wrote to authors of included studies for additional studies and trial data (including unpublished summary outcome data). Full search methods and full text of electronic search strategies are reported in full in our methodology paper.(22)

Data collection

Study inclusion, data extraction and assessment of risk of bias were conducted independently in duplicate, disagreements resolved by discussion or a third reviewer.

Assessment of risk of bias in included studies

We assessed Cochrane risk of bias domains,(25) and also assessed risk from compliance problems and attention bias specifically for our reviews. (15-23) Included trials were judged at low summary risk of bias where randomisation, allocation concealment, blinding of participants, personnel and outcome assessors were adequate (all other trials were at moderate or high risk of bias).

Data synthesis

Main analyses assessed effects of increasing omega-6, LCn3, ALA and mixed PUFA on primary outcomes using random effects meta-analysis (as dietary interventions are heterogeneous by their nature (26)) with risk ratio or mean differences in Review Manager 5.3.(27) Where different scales could be combined the direction of scales was standardised (so lower scores signified lower levels of depression or anxiety) and combined using standardised mean differences.

Sensitivity analyses

Pre-specified sensitivity analyses of primary outcomes included fixed effects meta-analysis, limiting analysis to studies at low summary risk of bias, limiting to studies at low risk for compliance issues, and limiting to trials randomising ≥100 participants.

Subgroup analysis and investigation of heterogeneity

Pre-specified subgroup analyses were conducted for primary outcomes with ≥8 included studies by intervention type, replacement, dose, duration, baseline depression risk (high risk - people with clinically diagnosed depression and/or anxiety using any diagnostic criteria, medium risk - with depression or anxiety risk factors such as a long-term conditions or low risk – all other populations) and anti-depressant or anti-anxiety medication use in ≥50% participants.(22) We planned to sub-group by severity of baseline depression and combined anxiety/ depression diagnosis, but only two trials included participants with diagnosed depression (28), and by baseline intake of omega-3, omega-6 or total PUFA, but this information was not available in most trials so was not attempted.

We assessed heterogeneity between trials using I^2, and small study bias using funnel plots, the Harbord and Peters (for dichotomous data) or Egger (for continuous data) tests (25, 29)

where there were ≥10 included trials, comparison of random and fixed effects analyses and knowledge of missing data.

Interpretation of findings

Effect sizes were interpreted as agreed with the WHO Nutrition Guidance Expert Advisory Group (NUGAG) Subgroup on Diet and Health (who commissioned this review as part of a set of work to underpin their dietary guidance)and pre-specified for this set of reviews.(22) RR <0.92 or >1.08 was considered a relevant clinical effect (RR 0.92 to 1.08 was considered "little or no effect"), while mean difference between arms of ≥10% of baseline was required for a relevant clinical effect for continuous measures. Outcome data were interpreted using GRADE assessment, drafted by LH then discussed and agreed with WHO NUGAG as elaborated elsewhere.(22) Where GRADE suggested data of very low-quality we did not interpret effect sizes. Where data were of low-quality we used the term "may", moderate-quality evidence warranted "probably" in describing effect sizes.

Results

The search strategy for the wider set of reviews found 364 RCTs (reported in 1020 papers) of omega-3, omega-6 or total PUFA with a duration of at least six months.(22) From this set 32 RCTs that assessed outcomes of interest were included in this review (Supplementary Figure 1, for more detail see methods paper).(22) Systematic review results, including sensitivity analyses and subgrouping, are provided briefly here and in more detail in Supplementary Text 1, in the Supplementary file.

Characteristics of included studies

Characteristics of included studies and risk of bias are detailed in Table 1 and in more detail in our database paper (20). Thirty one trials (41,470 participants) assessed effects of LCn3,(28, 30-61) one assessed effects of ALA (4837 participants),(30) and one assessed effects of higher total PUFA (4997 participants).(62) No trials assessed effects of omega-6 on depression or anxiety.

Participants were recruited with chronic illness or risk factors in 17 trials; memory deficit, cognitive impairment or Alzheimer's disease in six; mental health problems in four; and healthy participants in 5 trials.

Of the 31 LCn3 trials, most gave supplementary capsules or medicinal oils, two used supplemental foods (enriched margarine and fish sausages);(30, 40) one provided dietary advice;(57) and one a combination.(43) The ALA trial provided enriched margarine,(30) and the PUFA trial dietary advice plus nuts.(62) LCn3 doses ranged from 300-3360mg/d EPA+DHA,(28, 54) 12 trial arms assessed doses of ≤1000mg/d, 13 arms 1001-2000mg/d, and seven arms >2000mg/d EPA+DHA. Control groups received olive, corn or sunflower oils, other fats, other 'inert' or ill-defined substances, different dietary advice, foods without omega-3 enrichment, or nothing.

Risk of bias of included studies

Risk of bias is itemised by domain and study in Figure 1. Of the 32 RCTs (33 comparisons including 46,467 randomised participants) twelve were judged to be at low summary risk of bias,(30, 31, 34, 38, 43, 45, 48, 50, 51, 55, 56, 59, 60) including twelve LCn3 comparisons, and the single ALA assessment (Figure 1). Trial authors provided some response to attempted contact for 16 trials.

Effects of increasing omega-3, omega-6 or total PUFA on risk of depression symptoms

Thirteen RCTs (randomising 26,528 participants, reporting 1355 people developing depression symptoms, median dose 0.95g/d, range 0.4 to 3.4g/d, median duration 12 months, range 6 to 89 months) suggested little or no effect of increasing LCn3 on risk of depression symptoms (RR 1.01, 95% CI 0.92 to 1.10, I^2 0%, Figure 2). This did not differ in sensitivity analyses by summary risk of bias, fixed effects or study size, though retaining only trials with good compliance suggested increased depression risk with increased LCn3 (RR 1.16, 95% CI 0.99 to 1.36, I^2 0%, Supplementary Table 1). Over 90% of meta-analytic weight came from three trials that assessed depression symptoms dichotomously using the Center for Epidemiologic Studies Depression Scale (CESD, score ≥16),(31) Becks Depression Inventory (BDI-II, score ≥14),(50) and General Health Questionnaire (GHQ-30, ≥5).(51) In other trials depression events were based on Geriatric Depression scores (GDS-15, >10), reported as adverse events or were unclear. There was no suggestion of publication bias in visual inspection of the funnel plot, or using statistical tests (Harbord test p=0.27, Peters test p=0.29), and no suggestion of heterogeneity. Effects did not differ by intervention type, replacement nutrients, or LCn3 dose, but subgrouping suggested increased depression risk with LCn3 in healthy adults, and little or no effect in those with comorbid illnesses. One LCn3 trial recruited only participants with current depression in which ≥50% took antidepressants.(28) As pre-specified LCn3 dose subgroupings did not divide included trials effectively, post-hoc we re-ran even LCn3, EPA and DHA dose subgroupings. There was no suggestion of LCn3 dose effects (test for subgroup differences p=0.98), EPA (p=0.13) or DHA (p=0.87) effects, Supplementary Figures 2-4.

GRADE assessment suggests that increasing LCn3 probably has little or no effect on risk of depression symptoms (moderate-quality evidence, downgraded once for imprecision, Supplementary Table 2). This was confirmed in data on depression symptoms analysed as continuous data in 15 trials including participants not selected for depression at baseline (for details see Supplementary Text 1 and Supplementary Table 2).

Data were limited from trials of ALA and total PUFA on depression (Supplementary Tables 3 and 4). We found no data from trials of omega-6 (Figure 2). GRADE suggests that increasing ALA may increase risk of depression symptoms very slightly (NNH 1000, low-quality evidence, downgraded twice for imprecision) and effects of increasing total PUFA on

depression risk are unclear as the evidence is of very low-quality (downgraded once each for risk of bias, indirectness and inconsistency, Supplementary Tables 5 and 6).

Effects of increasing omega-3, omega-6 or total PUFA on depression severity and remission in those with existing depression

A single small trial assessed effects of LCn3 for 6 months in poor Iranian men with mild or moderate depression at baseline(28), and found that GDS score fell by >10% of baseline (suggesting reduced depression severity) in the higher vs lower LCn3 arm (MD -0.94, 95% CI -2.27 to 0.39, 61 participants). A further small study (n=24) included participants with Parkinson's Disease,(53) some of whom were depressed at baseline, and reported on remission, suggesting more remission in those on higher LCn3 (Supplementary Table 1). GRADE assessment suggests that effects of increasing LCn3 on risk of depression severity and risk of remission in those with existing depression are unclear as the evidence was of very low-quality (depression severity downgraded twice for risk of bias, once for inconsistency, risk of remission ARR 0.58, downgraded once for risk of bias and twice for indirectness, Supplementary Table 2).

No trials of ALA, omega-6 or total PUFA included participants with depression at baseline.

Effects of increasing omega-3, omega-6 or total PUFA on risk of anxiety symptoms, severity and remission

Data were limited from trials of LCn3 assessing anxiety symptoms (Supplementary Tables 1 and 2). One study provided data on effects of LCn3 on risk of anxiety (RR 1.00, 95% CI 0.32 to 3.10), none on remission. Five studies assessed effects of increasing LCn3 on anxiety symptoms using four different scales (SMD 0.15, 95% CI 0.05 to 0.26, I^2 0%, 1378 participants, and no included studies were at low summary risk of bias, Figure 3). No studies provided data on effects of ALA, omega-6 or total PUFA on anxiety incidence, remission or symptoms. GRADE assessment suggests that increasing LCn3 probably has little or no effect on anxiety symptoms (moderate-quality evidence, downgraded once for risk of bias, Supplementary Table 2).

Secondary outcomes

Data on secondary outcomes are reported in Supplementary Text 1 and Supplementary Tables 7 and 8. Data were found on quality of life, carer stress, suicidality, adverse events,

drop outs and drop outs due to adverse events, but data were sparse, often poorly reported and may suffer from reporting bias. We did not identify any clear harms or benefits of interventions for these outcomes. We have formally systematically reviewed effects of omega-3, omega-6 and total PUFA on cancer, diabetes, cognition, inflammatory bowel disease, cardiovascular disease, functional outcomes, mortality, adiposity and lipids in sister reviews, so these outcomes are not reported here. (15-23)

Discussion

GRADE assessment of our meta-analytic data suggests that increasing LCn3 probably has little or no effect on risk of depression or anxiety symptoms in those without depression or anxiety at baseline (moderate-quality evidence), but effects on depression severity and risk of remission in depression were unclear. Increasing ALA may increase risk of depression symptoms very slightly (1000 people would need to increase their ALA intake for one additional person to develop depression symptoms). Data on other outcomes and effects of increasing omega-6 and total PUFA were missing or of very low quality.

Strengths and Limitations

Strengths of this review include our very broad search of long-term trials that assessed effects of omega-3, omega-6 or total PUFA on any outcomes,(22) and contact with many trial authors enabling us to include previously unpublished data. Evidence for the lack of impact of LCn3 on risk of depression symptoms comes from a broad range of trials, across thousands of men and women with diverse health status and depression risk, including large, long-term trials with low summary risk of bias. The broad set of trials also allowed thorough assessment of publication bias. We have used subgrouping to assess potential effects of LCn3, EPA and DHA dose, study duration (much of our data came from large trials of 3 or more years duration) and replacement of other nutrients (including omega-6, monounsaturated and saturated fats) on depression symptoms. Increasing LCn3, EPA or DHA dose or trial duration or altering nutrients replaced by LCn3 do not improve effectiveness of LCn3 on risk of depression symptoms.

Limitations include lack of information within trials on baseline LCn3 intake. Baseline intake of LCn3 could alter effectiveness of LCn3 supplementation, as increasing LCn3 would be more likely to be effective in those with poor baseline intakes. However where trials

reported baseline LCn3 intake or status they did so in ways that are not comparable across trials (e.g. oily fish intake, erythrocyte membrane EPA, plasma LCn3), so we were unable to assess effects by baseline LCn3 status or intake. While available data did not allow us to assess effects by omega-3/omega-6 ratio there was no suggestion of greater effects when omega-3 replaced omega-6, downplaying the importance of this ratio in depression and anxiety. The variety of methods of assessment of depression and anxiety symptoms, and limited clinical diagnoses of depression or anxiety (relying on scales of symptoms) may also limit clinical interpretation. However, these are the best data available on prevention of depression and anxiety, there are no previous systematic reviews of prevention and our collation of a broad database of all long-term trials of omega-3, omega-6 and total PUFA has allowed assessment of effects that are otherwise unpublished and inaccessible.(22) We carried out SMD analysis and reported effects in the single most common scale.

Comparison with other research

The MooDFOOD trial randomised participants to 1.4g/d LCn3 plus additional micronutrients or placebo and found no effect on diagnosis of major depressive disorder after 1 year in 1025 overweight adults with subsyndromal depressive symptoms.(63) This trial is not included in our systematic review as the intervention was multifactorial (effects of LCn3 cannot be separated out) but confirms our review findings that LCn3 supplementation does not help to prevent depression. We found no previous systematic reviews of randomised controlled trials on effects of omega-6 or total PUFA, none separated out effects of ALA, and none assessed effects on prevention of depression. Systematic reviews on anxiety have included trials of very short duration and without controls.(64)

Given that humans require at least 6 months to equilibrate fatty acids throughout our bodies when changes to LCn3 intake occur,(24) we were surprised to find only two small trials of LCn3 with a duration of at least 24 weeks that included participants with depression at baseline, to enable assessment of effects on depression severity and remission. As depression and anxiety are commonly recurring illnesses, longer term health effects are crucial to understand, and we assumed we would find trials of polyunsaturated fats alongside effective antidepressants or anxiolytics compared with placebo and the same effective antidepressant or anxiolytic. None of our included trials clearly assessed dietary

fats in combination with medications for depression or anxiety, which could potentiate effectiveness.

Shorter term trials of omega-3 fats have been extensively reviewed. For example, a previous high quality Cochrane systematic review of shorter trials of LCn3 in people with depression suggested small to modest non-clinically beneficial effects but queried risk of bias and publication bias in this dataset. (14) However, another systematic review of trials in major depression suggested efficacy at higher EPA doses and alongside antidepressants. (65) Like the Cochrane review, which also used GRADE assessment, (14) we found that evidence of effects of LCn3 on depression severity and remission were of very low-quality. Other recent systematic reviews of effects of omega-3 in people with existing depression have concluded that there were "mixed findings" in older adults, suggesting that more high-quality, large-scale RCTs are needed (66), in call for trials in people with diagnosed depression and of longer duration (67), and with a suggestion that combined EPA and DHA are of (non-significant) benefit in women (based on fewer than 400 participants) (68).

LCn3 was mainly provided in supplementary form, so while there was no suggestion of different effects in trials of dietary advice or where oily fish was provided to participants compared with trials of LCn3 supplements, effects of dietary fish may differ (as dietary fish replaces other foods, and includes a wide range of additional nutrients including protein, selenium, iodine, calcium and magnesium).

While LCn3 and ALA may protect against depression and anxiety in select individuals due to specific genetic, dietary and/or metabolic characteristics, LCn3 and ALA will be harmful in other selected individuals. This systematic review suggests that any such benefits and harms are balanced, and that there will be no overall benefits on depression and anxiety symptoms of increasing LCn3 in general populations.

Implications for practice

Many adults take omega-3 supplements to improve their mental health. Our comprehensive systematic review and meta-analysis suggested that taking LCn3 supplements probably has little or no effect on risk of depression or anxiety symptoms (moderate-quality evidence). Results did not differ by risk of bias, omega-3 dose, duration or nutrients replaced. Effects on depression severity and remission were unclear (very low-

quality evidence). Physicians should not recommend omega-3 supplements for reducing depression or anxiety risk, and evidence of effectiveness in existing depression is of very low-quality.

Research Implications

Further methodologically strong long-term trials (that focus on robust randomisation, allocation concealment, and blinding of participants, trial staff and outcome assessors, as well as adequately checking compliance in both the intervention and control arms) are needed to drive practice in people with existing depression and anxiety.

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Contributions:

LH conceived this review and gained funding, KHOD wrote the first draft of the protocol, SH submitted the protocol draft to PROSPERO; LH drafted the searches which were developed, refined, run and de-duplicated by the Cochrane Heart Group. KHOD, OFJ, PB, SH, ASA and LH screened titles and abstracts; KHOD, PB, SH, ASA and LH assessed full text papers for inclusion; LH and SH searched trials registers and assessed entries for inclusion; PB, LH and ASA located full texts, ASA and LH managed assessment and collection of titles, abstracts and full texts, data extraction and risk of bias assessment; KHOD, OFJ, PB, AO'B, SH, ASA and LH carried out data extraction and assessed risk of bias. CF advised on depression and anxiety. LH & KHOD designed risk of bias assessment; KHOD, ASA and LH wrote to study authors; LH, KHOD, and ASA carried out data checks; LH & ASA tabulated intake and status data. KHOD, ASA & LH provided methodological support. KHOD and LH entered data into RevMan and ran meta-analyses, carried out sensitivity analyses and subgrouping. KHOD wrote the first draft of the review, LH wrote the WHO report. KHOD and LH carried out GRADE assessment and interpretation. All authors critically read and commented on the final draft, and agreed it for submission.

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Data Availability: All authors have ongoing access to the study data within a shared database. The database for this set of reviews is available in our accepted methods and database paper **(22).**

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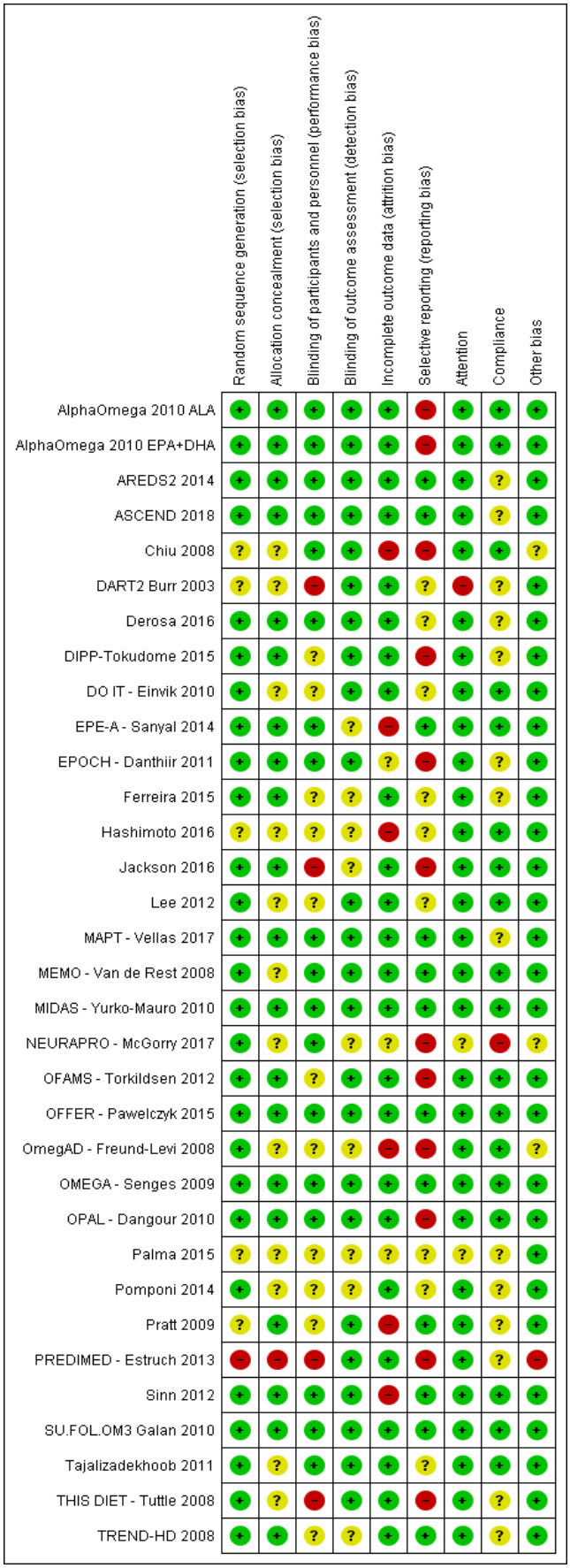

Figure 1: Itemised risk of bias for included RCTs

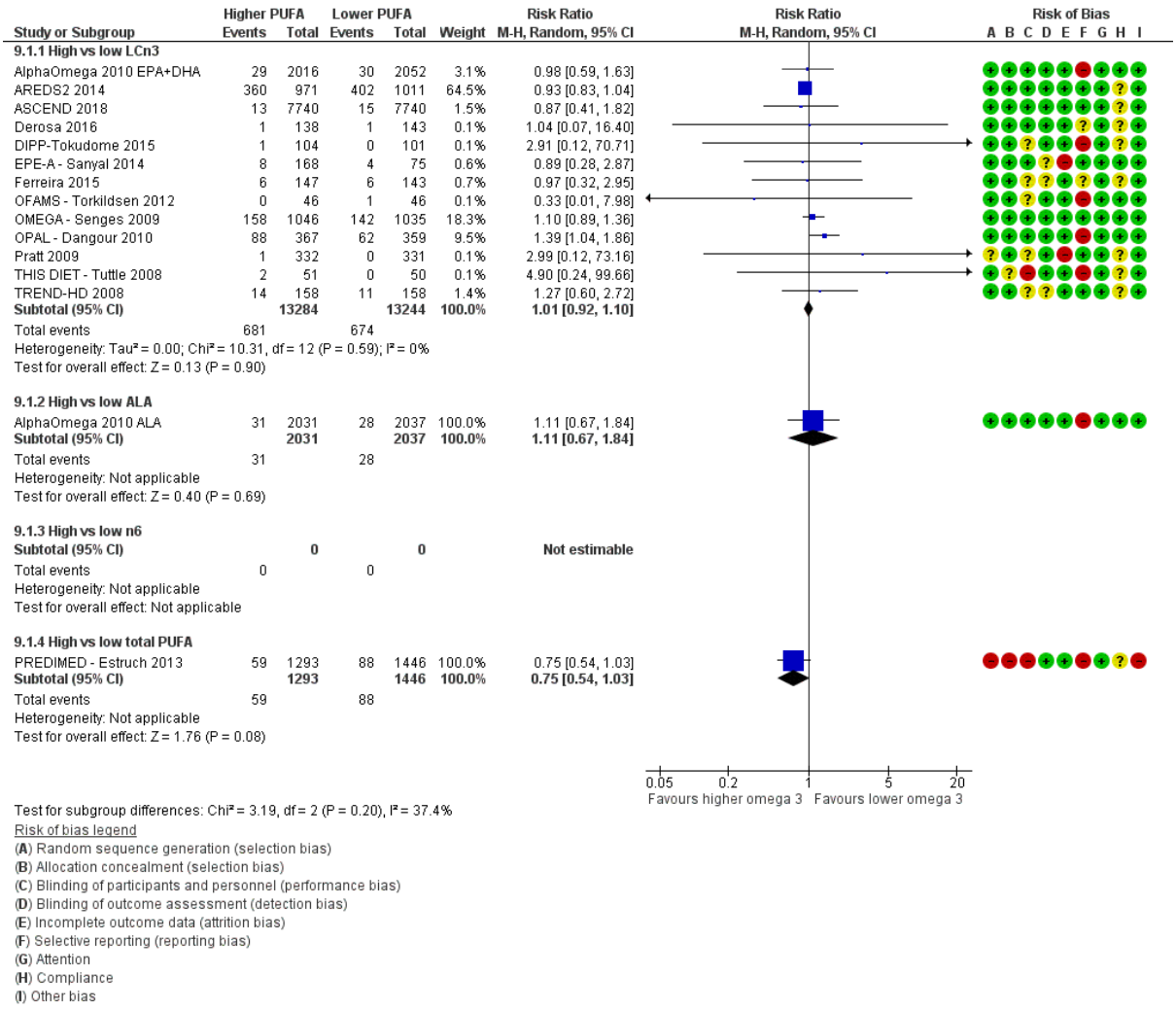

Figure 2. Meta-analysis of trials randomising to higher vs lower LCn3, ALA, omega-3 and total PUFA intake and reporting risk of depression symptoms

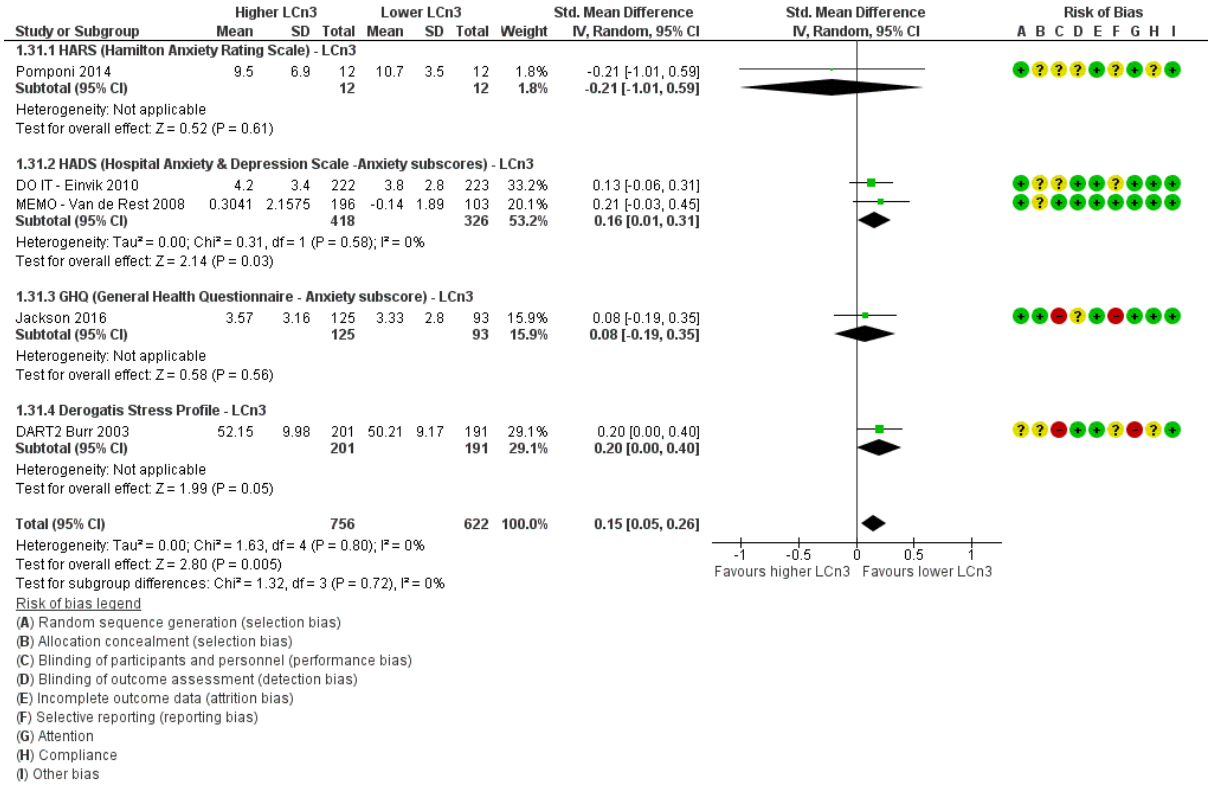

Figure 3. Meta-analysis of trials randomising to higher vs lower LCn3 intake and assessing anxiety

Study	Population, country	Intervention	Comparison	Participants randomised	Trial Duration
AlphaOmega – Kromhout 2010 ALA(30, 69)	60-80 year olds with previous myocardial infarction, Netherlands	ALA-rich supplementary margarine, 2g ALA/d	ALA vs MUFA	2409 int, 2428 cont	3.3 years
AlphaOmega – Kromhout 2010 EPA & DHA(30, 69)	60-80 year olds with previous myocardial infarction, Netherlands	EPA & DHA-rich supplementary margarine, 0.24g/d EPA & 0.16g/d DHA	LCn3 vs MUFA	2404 int, 2433 cont	3.3 years
AREDS2 2014(31)	50-85 year olds at high risk of progression to advanced age-related macular degeneration, USA	EPA & DHA supplement, 0.65g/d EPA & 0.35g/d DHA	LCn3 vs nil	2157 int, 2046 cont	5 years
ASCEND 2018(59)	Patients with diabetes, without apparent vascular disease, UK	EPA & DHA supplement, 0.46g/d EPA & 0.38g/d DHA	LCn3 vs MUFA	7740 int, 7740 cont	7.4 years
Chiu 2008(32)	Older adults with Alzheimer's Disease or Mild Cognitive Impairment, Taiwan	EPA & DHA supplement, 1.08g/d EPA & 0.72g/d DHA	LCn3 vs MUFA	24 int, 22 cont	0.5 years
DART2 Burr 2003(70)	Men treated for angina, UK	Dietary fish advice or EPA supplement, 2.4g/week EPA	LCn3 vs nil	1571 int, 1543 cont	3-9 years
Derosa 2016(34)	Overweight/obese Caucasians with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), Italy	EPA & DHA supplement, 0.83g/d EPA & 1.57g/d DHA	LCn3 vs non-fat	138 int, 143 cont	1.5 years

DIPP – Tokudome 2015(35)	Patients previously polypectomised for colorectal tumours, Japan	Dietary advice to increase oily fish & ALA-rich oil & EPA & DHA supplement, unclear EPA & DHA	LCn3 vs non-fat	104 int, 101 cont	2 years
DO IT - Einvik 2010(36)	Elderly men with long standing dyslipidaemia or hypertension, Norway	EPA & DHA supplement, 0.84g/d EPA & 0.48g/d DHA	LCn3 vs n6	282 int, 281 cont	3 years
EPE-A – Sanyal 2014(37)	People with non-alcoholic steatohepatitis (NASH) & non-alcoholic fatty liver disease (NAFLD), USA	EPA supplement, 0.9g/d EPA	LCn3 vs unclear	86 int, 82 cont	1 year
EPOCH – Danthiir 2011(38)	Healthy older adults with no cognitive impairment, Australia	EPA & DHA supplement, 0.6g/d EPA & 1.72g/d DHA	LCn3 vs MUFA	195 int, 196 cont	1.5 years
Ferreira 2015(39)	Adults with Huntington's disease, 6 European countries	EPA supplement, 2.0g/d EPA	LCn3 vs unclear	147 int, 143 cont	0.5 years
Hashimoto 2016(40)	Healthy older people, Japan	EPA & DHA supplement, 0.18g/d EPA & 0.81g/d DHA	LCn3 vs MUFA	43 int, 32 cont	1 year
Jackson 2016(41)	Healthy adults with subjective memory deficit, UK	EPA & DHA supplement, 0.13g/d EPA & 0.90g/d DHA	LCn3 vs MUFA	33 int, 32 cont	0.5 years
Lee 2012(42)	People aged ≥60 years, low to middle socioeconomic status, Malaysia	EPA & DHA supplement, 0.15g/d EPA & 0.43g/d DHA	LCn3 vs n6	18 int, 18 cont	1 year

MAPT – Vellas 2017(60)	People aged ≥70 years with memory complaint, IADL limitation or slow gait speed, France & Monaco	EPA & DHA supplement, 0.23g/d EPA & 0.80g/d DHA	LCn3 vs non-fat	840 int, 840 cont	3 years
MEMO – Van de Rest 2008(61)	Independently living people aged ≥65 years, Netherlands	EPA & DHA supplement, 1.09g/d EPA & 0.85g/d DHA or 0.23g/d EPA & 0.18g/d DHA	LCn3 vs MUFA	196 int, 106 cont	0.5 years
MIDAS – Yurko-Mauro 2010(45)	Healthy older people with subjective memory complaints, USA	DHA supplement, 0.90g/d DHA	LCn3 vs n6	242 int, 243 cont	0.5 years
NEURAPRO – McGorry 2017(46)	Young people at ultra-high risk for psychotic disorders, Australia, Switzerland, Germany, China, Austria, Singapore, Netherlands	EPA & DHA supplement, 0.84g/d EPA & 0.56g/d DHA	LCn3 vs non-fat	153 int, 151 cont	0.5 years
OFAMS – Torkildsen 2012(47)	People with relapsing remitting multiple sclerosis, Norway	EPA & DHA supplement, 1.35g/d EPA & 0.85g/d DHA	LCn3 vs n6	46 int, 46 cont	0.5 years
OFFER – Pawelczyk 2015(48)	People with first episode of schizophrenia aged 16–35, Poland	EPA & DHA supplement, 1.32g/d EPA & 0.88g/d DHA	LCn3 vs MUFA	36 int, 35 cont	0.5 years
OmegaAD – Freund-Levi 2008 (49)	People with mild to moderate Alzheimer's disease, Sweden	EPA & DHA supplement, 0.60g/d EPA & 1.72g/d DHA	LCn3 vs n6	103 int, 101 cont	0.5 years
OMEGA – Rauch 2010(50)	People who have had an acute myocardial infarction, Germany	EPA & DHA supplement, 0.46g/d EPA & 0.39g/d DHA	LCn3 vs MUFA	1940 int, 1911 cont	1 year

OPAL – Dangour 2010(51)	Healthy cognitively normal adults aged 70-79, UK	EPA & DHA supplement, 0.20g/d EPA & 0.50g/d DHA	LCn3 vs MUFA	434 int, 433 cont	1 year
Palma 2015(52)	People with schizophrenia, Spain	EPA & DHA supplement, 0.84g/d EPA & 0.47g/d DHA	LCn3 vs nil	30 int, 30 cont	1 year
Pomponi 2014(53)	Adults with mild to moderate Parkinson's disease (some with depression), Italy	EPA & DHA supplement, 0.29g/d EPA & 0.80g/d DHA	LCn3 vs n6	12 int, 12 cont	0.5 years
Pratt 2009(54)	Adults with paroxysmal or persistent atrial fibrillation, USA	EPA & DHA supplement, 1.86g/d EPA & 1.5g/d DHA	LCn3 vs n6	332 int, 331 cont	0.5 years
PREDIMED – Estruch 2013(62)	Men aged 55 to 80 years and women aged 60 to 80 years, free of CVD but with diabetes or at least 3 CVD risk factors, Spain	Dietary advice and food supplement (mixed nuts), PUFA dose unclear	PUFA vs MUFA	2454 int, 2543 cont	5 years
Sinn 2012(55)	Older people with mild cognitive impairment & few comorbidities, Australia	EPA & DHA supplement, 1.67g/d EPA & 0.16g/d DHA or 0.4g/d EPA & 1.55g/d DHA	LCn3 vs n6	36 int, 18 cont	0.5 years
SUFOLOM3 – Galan 2010(56)	People with a history of MI, unstable angina or ischemic stroke, France	EPA & DHA supplement, 0.4g/d EPA & 0.2g/d DHA	LCn3 vs non-fat	1248 int, 1253 cont	4 years
Tajalizadekhoob 2011(28)	Elderly poor people with mild or moderate depression, Iran	EPA & DHA supplement, 0.18g/d EPA & 0.12g/d DHA	LCn3 vs mixed fat	33 int, 33 cont	0.5 years
THIS DIET – Tuttle 2008(57)	Survivors of recent first myocardial infarction, USA	LCn3 dietary advice, dose unclear	LCn3 vs mixed fat	51 int, 50 cont	2 years

TREND-HD 2008(58)	People with Huntington's disease, USA & Canada	EPA supplement, 0.95g/d EPA	LCn3 vs non-fat	158 int, 158 cont	0.5 years

Table 1: Brief characteristics of included studies (see Supplementary Table 1 for further details)