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Risk assessment of eicosapentaenoic acid and docosahexaenoic acid

Opinion of the Norwegian Scientific Committee for Food and Environment

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Risk assessment of eicosapentaenoic acid and docosahexaenoic acid

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Norwegian Scientific Committee for Food and Environment (VKM)

Po 222 Skøyen

NO – 0213 Oslo

Norway

Phone: +47 21 62 28 00

Email: vkm@vkm.no

vkm.no

vkm.no/english

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Risk assessment of eicosapentaenoic acid and docosahexaenoic acid

Authors of the opinion

Members of the project group that contributed to the drafting of the opinion (in alphabetical order):

Johanna Bodin – Chair of the VKM Panel on Genetically Modified Organisms. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

Ida Henriette Caspersen – External expert. Affiliation: Norwegian Institute of Public Health

Nur Duale – Member of the VKM Panel on Genetically Modified Organisms. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

Gro Haarklou Mathisen – Project manager, the VKM secretariat. Affiliation: VKM

Camilla Svendsen – Chair of the project group and member of the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

Approval of the opinion

The opinion has been assessed and approved by the following VKM members (in alphabetical order):

Jan Alexander – Chair of the VKM Scientific Steering Committee. Affiliation: 1) VKM; 2) Retired, former Norwegian Institute of Public Health

Johanna Bodin – Chair of the VKM Panel on Genetically Modified Organisms. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

Nur Duale – Member of the VKM Panel on Genetically Modified Organisms. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

Åshild Krogdahl – Chair of the VKM Panel on Animal Feed. Affiliation: 1) VKM; 2) Norwegian University of Life Sciences

Camilla Svendsen – Member of the VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

Robin Ørnsrud – Member of the VKM Panel on Animal Feed. 1) VKM; 2) Institute of Marine Research

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

NFSA requested VKM to assess if daily intake of a food supplement containing i) 1100 mg docosahexaenoic acid (DHA), ii) 1550 mg eicosapentaenoic acid (EPA), or iii) both 1550 mg EPA and 1100 mg DHA may constitute a health risk for Norwegian children and adolescents (both sexes) from 3 to 18 years. If these daily doses may constitute a health risk, NFSA need to know which DHA and EPA doses that are safe.

DHA and EPA are omega-3 polyunsaturated fatty acids (n-3 PUFAs). Dietary sources of n-3 PUFAs includes fatty fish, human milk, and food supplements enriched with n-3 PUFAs. EPA and DHA are synthesised endogenously from α -linolenic acid in varying amounts in animal tissues. The food supplement doses included in the request from NFSA are higher than the EPA and DHA levels produced endogenously, and higher than the intake from food.

No ADME data for children and adolescents were identified, and VKM can therefore not rule out that there are important differences with regard to ADME in adults, and children and adolescents. Therefore, data from studies on adults were only used as supporting information.

Genotoxicity was assessed earlier and no concerns for genotoxicity were raised.

VKM considered that bleeding, glucose/insulin homeostasis, inflammation, lipid homeostasis, and liver effects were the most important outcomes addressed in the randomised controlled trials on children and adolescents. Note that VKM considered the data to be insufficient and that the certainty in the evidence ranged from moderate to very-low.

Conclusions and answers to the terms of reference

Daily intake of food supplement containing 1100 mg DHA

VKM concludes that a health-based guidance value or a point of departure for DHA for 3-18-year-olds cannot be established or identified. Therefore, it is not possible to conclude on the safety of the suggested daily dose of 1100 mg DHA for children and adolescents.

Daily intake of food supplement containing 1550 mg EPA

VKM concludes that a health-based guidance value or a point of departure for EPA for 3-18-year-olds cannot be established or identified. Therefore, it is not possible to conclude on the safety of the suggested daily dose of 1550 mg EPA for children and adolescents.

Daily intake of food supplements containing 1100 mg DHA and 1550 mg EPA

VKM concludes that a health-based guidance value or a point of departure for DHA+EPA for 3-18-year-olds cannot be established or identified. Therefore, it is not possible to conclude on the safety of the suggested daily doses of 1100 mg DHA and 1550 mg EPA for children and adolescents.

Key words: Adverse health effect, DHA, docosahexaenoic acid, eicosapentaenoic acid, EPA, food supplement, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food and Environment, other substances, risk assessment, VKM.

Sammendrag på norsk

På oppdrag fra Mattilsynet har Vitenskapskomiteen for mat og miljø (VKM) vurdert risiko ved daglig inntak av 1100 mg dokosaheksaensyre (DHA), 1550 mg eikosapentaensyre (EPA), og både 1550 mg EPA og 1100 mg DHA fra kosttilskudd. Risikovurderingen inkluderer barn og unge i alderen 3 til 18 år. Hvis disse dosene kan utgjøre en helserisiko, ba Mattilsynet VKM om å identifisere hva som vil være en trygg dose.

DHA og EPA er omega-3 flerumettede fettsyrer. Viktige kilder i kosten er fet fisk, morsmelk og kosttilskudd med omega-3 flerumettede fettsyrer. EPA og DHA produseres i varierende mengder i dyrevev fra α -linolensyre. Dosene som VKM har vurdert, er høyere enn mengdene av EPA og DHA som produseres i kroppen og høyere enn inntaket fra mat.

Det ble ikke funnet data på ADME (absorpsjon, distribusjon, metabolisme og eliminasjon) for barn og unge, og VKM kan derfor ikke utelukke at det er viktige forskjeller med hensyn til ADME hos voksne og barn og unge. Derfor ble data fra studier på voksne bare brukt som støttelitteratur.

Gentoksisitet, det vil si om stoffet kan skade DNA, var undersøkt tidligere, og det var ikke uttrykt bekymring for at DHA og EPA er gentoksiske.

Blødning, glukose/insulin homeostase, betennelse, lipidhomeostase og levereffekter ble vurdert å være de viktigste utfallene som var studert i de randomiserte kontrollerte studiene på barn og unge. Merk at VKM anså dataene som utilstrekkelige, og at tiltro til evidensen varierte fra moderat til veldig lav.

Konklusjon og svar på oppdraget:

Daglig inntak av kosttilskudd som inneholder 1100 mg DHA

VKM har for lite data til å kunne fastsette et utgangspunkt for å utlede en trygg dose for DHA for 3-18-åringer. Derfor er det ikke mulig å konkludere på om den foreslåtte daglige dosen på 1100 mg DHA er trygg for barn og unge.

VKM har for lite data til å kunne fastsette et utgangspunkt for å utlede en trygg dose for EPA for 3-18-åringer. Derfor er det ikke mulig å konkludere på om den foreslåtte daglige dosen på 1550 mg EPA er trygg for barn og unge.

VKM har for lite data til å kunne fastsette et utgangspunkt for å utlede en trygg dose for kombinert inntak av DHA og EPA for 3-18-åringer. Derfor er det ikke mulig å konkludere på om den foreslåtte daglige dosen på 1100 mg DHA og 1550 mg EPA er trygg for barn og unge.

Abbreviations and glossary

Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AESAN	Spanish Agency for Food Safety and Nutrition
AI	adequate intake
ALA	α -linolenic acid
bw	body weight
CRP	C-reactive protein
DHA	docosahexaenoic acid
DRV	dietary reference value
EFSA	European Food Safety Authority
EPA	eicosapentaenoic acid
FA	fatty acid
HBGV	health based guidance value
HDL	high-density lipoprotein
Hs-CRP	high-sensitivity C-reactive protein
IL	interleukin
IOM	Institute of Medicine
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LCn3	long-chain omega-3
LCPUFA	long chain polyunsaturated fatty acids
LDL	low-density lipoprotein
MOE	margin of exposure
NFSA	Norwegian Food Safety Authority
NOAEL	no observed adverse health effect
n3-PUFA	omega-3 polyunsaturated fatty acids
PL	phospholipid
POD	point of departure
PRI	population reference intake
PUFA	polyunsaturated fatty acids
RCT	randomised controlled trial
TAG	triacylglycerol
TNF- α	tumor necrosis factor alpha
UL	tolerable upper intake level
VKM	Norwegian Scientific Committee for Food and Environment
WHO	World Health Organization

Glossary

Acceptable daily intake

An estimate of the amount of a substance in food or drinking water that can be consumed daily over a lifetime without presenting an appreciable risk to health. It is usually expressed as milligrams of the substance per kilogram of body weight and applies to chemical substances such as food additives, pesticide residues and veterinary drugs (EFSA Glossary).

Absorption, distribution, metabolism and excretion

The four key processes which describe how drugs and chemicals get into the body, what happens to them while they are there, and how they are eliminated (EFSA Glossary).

Adequate intake

A dietary recommendation used when there isn't enough data to calculate an average requirement. An adequate intake is the average nutrient level consumed daily by a typical healthy population that is assumed to be adequate for the population's needs (EFSA glossary).

Adverse health effect

A change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO, 1994).

Certainty of evidence

The certainty (or quality) of evidence is the extent to which we can be confident that what the research tells us about a particular treatment effect is likely to be accurate. Concerns about factors such as bias can reduce the certainty of the evidence. Evidence may be of high certainty; moderate certainty; low certainty or very-low certainty (Cochrane glossary).

Dietary reference value

The complete set of reference values for nutrient intake comprising Population Reference Intakes (PRI), Average Requirements (AR), Adequate Intakes (AI), Lower Threshold Intakes (LTI) and Reference Intakes (RI). DRVs are typically used as a basis for reference values in food labelling and for establishing food-based dietary guidelines (EFSA glossary).

Health-based guidance value

Guidance on safe consumption of substances that takes into account current safety data, uncertainties in these data, and the likely duration of consumption (EFSA glossary).

"Other substances"

A substance other than a vitamin or mineral that have a nutritional or physiological effect (Regulation (EC) No 1925/2006 of the European Parliament and of the Council).

Point of departure

The point on a dose-response curve established from experimental data used to derive a safe level (EFSA Glossary).

Population reference intake

The intake of a nutrient that is likely to meet the needs of almost all healthy people in a population (EFSA glossary).

“Positive list”

Annex to Regulation (EC) No 1925/2006 including “other substances” and levels thereof allowed for addition to foods.

ROBIS tool

A tool for assessing the risk of bias in systematic reviews. The tool is completed in 3 phases: (1) assess relevance (optional), (2) identify concerns with the review process and (3) judge risk of bias (<https://www.bristol.ac.uk/population-health-sciences/projects/robis/robis-tool/>).

Systematic review (synonym: systematic overview):

A specific research question and the specific criteria used for selecting studies are described, a systematic literature search are performed, and a quality assessment of the selected studies is included (Cochrane Glossary, 2020).

Tolerable upper intake level

The maximum intake of substances in food, such as nutrients or contaminants, that can be consumed daily over a lifetime without adverse health effects (EFSA glossary).

Background as provided by the Norwegian Food Safety Authority

"Other substances" are substances that have a nutritional or physiological effect but are not vitamins or minerals. Examples of "other substances" include fatty acids, amino acids, coenzyme Q10 and caffeine. Excessive intake of certain "other substances" may be associated with health risks.

In the European Economic Area (EEA), the provisions on the addition of "other substances" to foods are currently only partially harmonised in Regulation (EC) No 1925/2006. This means that Member States may lay down national supplementary provisions on the aspects that are not harmonised. Any national supplementary provisions must comply, inter alia, with the general principles of EEA law on the free movement of goods, "mutual recognition" and the legal exceptions to these EEA principles.

In Norway new supplementary national provisions regarding the addition of certain "other substances" to foods including food supplements entered into force on 1 January 2020. The new national supplementary provisions are included in the Norwegian regulation "[Forskrift 26. februar 2010 nr. 247 om tilsetning av vitaminer, mineraler og visse andre stoffer til næringsmidler](#)", which also implements Regulation (EC) No 1925/2006 in Norwegian internal law.

A so-called "positive list" for the addition of certain "other substances", was introduced as an Annex to the regulation. The intention is to reduce health risks that can occur when consuming certain "other substances" in foods, including food supplements.

The new national supplementary provisions only apply to the addition of "other substances" that a) have a purity of at least 50% or are concentrated 40 times or more, and b) are not normally consumed as a food in themselves and not normally used as an ingredient in foods.

Furthermore, the supplementary national provisions do not apply to the addition of the following "other substances": a) plants or parts of plants in fresh, dried, chopped, cut or powdered form, b) extracts of plants or parts of plants exclusively made through basic aqueous extraction, possibly followed by dehydration, c) enzymes and microorganisms and d) "other substances" listed in Parts A and B of Annex III to Regulation (EC) No 1925/2006.

It is only permitted to add "other substances" that are listed in the "positive list" in Annex 3 to foods, including food supplements. Such addition to foods must be in accordance with the terms and conditions set in the "positive list", including the limits that are set for the different substances. Substances regulated by other legislations like those for novel foods, food additives, flavourings, Foods for Specific Groups, etc. is outside the scope of the national supplementary provisions.

If a food business operator wants to add different quantities or use different conditions of a substance that is included in the "positive list", the food business operator must notify the NFSA. If a food business operator wants to add new substances, not currently included in the "positive list", the food business operator must apply for authorisation to the NFSA.

When needed for the NFSA to process an application or notification, the Norwegian Scientific Committee for Food and Environment (VKM) is requested to perform a risk assessment so that new substances or higher amounts of substances listed in the "positive list" are risk assessed.

Terms of reference as provided by the Norwegian Food Safety Authority

Terms of references

The NFSA hereby asks the Norwegian Scientific Committee for Food and Environment (VKM) to examine whether the exposure to EPA (CAS registry number 10417-94-4) and DHA (CAS registry number 6217-54-5) in food supplements to children and adolescents, that is covered by the national supplementary provision, might constitute a health risk for Norwegian children and adolescents (both sexes) in the age group from 3 to 18 years.

In the report «Risk assessment of «other substances» - eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid VKM Report 2015:27» it is assessed as unlikely that the daily dose of 1290 mg DHA in food supplements will cause negative health effects in the age group 10-18 years. The NFSA needs a similar assessment of DHA given to children in the age from 3-10 years, of EPA for all ages (3-18 years), and an assessment of a total daily intake of DHA and EPA for all ages (3-18 years).

EPA and DHA in food supplements for children and adolescents

- 1) The NFSA asks the Norwegian Scientific Committee for Food and Environment (VKM) to assess if a daily intake of a food supplement containing 1100 mg DHA can constitute a health risk for Norwegian children and adolescents (both sexes) in the ages from 3 to 18 years.
- 2) The NFSA asks VKM to assess if a daily intake of a food supplement containing 1550 mg EPA can constitute a health risk for Norwegian children and adolescents (both sexes) in the ages from 3 to 18 years.
- 3) The NFSA asks VKM to assess if a daily intake of a food supplement containing both 1550 mg EPA and 1100 mg DHA can constitute a health risk for Norwegian children and adolescents (both sexes) in the ages from 3 to 18 years.
- 4) If these daily doses of EPA and DHA (the doses specified in question 1-3) are not considered as safe: Which doses can be assessed as safe?

This includes:

- Identify and characterise adverse health effects.
 - Identify and describe toxicological reference point(s).
 - Describe uncertainty related to the toxicological reference point(s).
- Estimate the exposure

- Estimate exposure for the dose(s) and age groups given above.
 - Describe uncertainty related to the exposure estimates.
- Characterise health risks associated with exposure to EPA and DHA, and describe uncertainty that may have an impact on the conclusions.
- Identify and describe main knowledge gaps that may have an impact on the conclusions.

Assessment

1 Introduction

"Other substances" are substances that have a nutritional or physiological effect but are not vitamins or minerals (Regulation (EC) No 1925/2006 of the European Parliament and of the Council). Excessive intake of certain "other substances" may be associated with health risks.

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food and Environment (VKM) to assess if daily intake of a food supplement containing i) 1100 mg docosahexaenoic acid (DHA), ii) 1550 mg eicosapentaenoic acid (EPA), or iii) both 1550 mg EPA and 1100 mg DHA can constitute a health risk for Norwegian children and adolescents (both sexes) from 3 to 18 years. If these daily doses may constitute a health risk, NFSA need to know which DHA and EPA doses that are safe.

Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) have a double bond located three carbon atoms from the methyl end of the molecule. The main n-3 PUFAs in the diet are α -linolenic acid (ALA, which is an essential fatty acid as it cannot be synthesised by the body), DHA and EPA (EFSA, 2012), and humans require a dietary source (IOM, 2005). PUFAs with ≥ 20 carbon atoms and ≥ 3 double bonds are also defined as n-3 long chain PUFAs (LCPUFAs), and includes ALA, DHA and EPA.

EPA and DHA are synthesised endogenously from ALA in varying amounts in animal tissues, especially fatty fish, but not in plant cells (IOM, 2005). A lack of α -linolenic acid in the diet can result in clinical symptoms of a deficiency (IOM, 2005). Dietary sources of n-3 PUFAs includes fatty fish, human milk, and food supplements enriched with n-3 PUFAs (EFSA, 2012). Regular foods contain combinations of EPA and DHA, food supplements or fortified foods are the only sources for isolated single EPA and DHA. Endogenous production of EPA and DHA is insignificant compared to doses used in supplementation studies (EFSA, 2012). The intakes of EPA and DHA from regular foods are in the form of triacylglycerol (TAG) and phospholipids (PLs). In food supplements DHA and EPA can be bound to TAGs and PLs, but given as single fatty acids most formulations are as ethyl esters or as free fatty acids (VKM, 2015).

According to EFSA (2012), dietary recommendations from national and international bodies for n-3 PUFAs (mostly as EPA and DHA) range from 200 mg to >600 mg/day for adults, and from 40 mg to 250 mg/day for infants older than six months and for children and adolescents. No tolerable upper intake level (UL) for EPA or DHA has been set by any authoritative body (EFSA, 2012; VKM, 2015).

Adverse effects of high intakes of n-3 PUFAs noted in previous reports/assessments includes bleeding episodes, impaired immune function, increased lipid peroxidation, and impaired lipid and glucose metabolism (IOM, 2005; VKM, 2011; EFSA, 2012; VKM, 2015).

1.1 Limitations of the present risk assessment

General for the risk assessments of other substances is that there are relatively short deadlines for the assignments, which results in limitations in literature searches.

Further limitations:

- The assessment is performed for EPA and DHA, and only for the doses in the mandate given by NFSA.
- The assessment covers the general healthy population, not groups in the population that may have a high exposure due to e.g. certain dietary habits, or population groups that may be especially vulnerable due to e.g. certain genetic variants, diseases, drug use or age/life stages.
- The age groups included are given in the mandate from the NFSA.
- Documentation of any claimed beneficial effects is not evaluated.
- Stability of EPA and DHA in a product is not addressed.
- Interaction with other components in a product is not addressed.
- Potential impurities are not addressed.

2 Substance specifications

Name and other identifiers and physical and chemical properties of DHA and EPA (Chemical Book, 2021a; Chemical Book, 2021b; PubChem, 2021a; PubChem, 2021b) are presented in Table 2-1 and 2-2.

Table 2-1. Docosahexaenoic acid; name and other identifiers, physical and chemical properties.

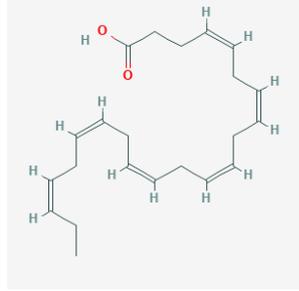
Substance name	Docosahexaenoic acid
Synonym	cis-4,7,10,13,16,19-Docosahexaenoic acid, DHA
CAS number	6217-54-5
EINECS number	612-950-9
Molecular formula	C ₂₂ H ₃₂ O ₂
Molecular weight	328.5
Structural formula	
SMILES	CCC=CCC=CCC=CCC=CCC=CCCCCCC(=O)O
Physical state	Liquid, clear colorless to light yellow oil
Boiling point (liquids)	446.7±24.0 °C (predicted)
Stability	Light and air sensitive
Density	0.943±0.06 g/cm ³ (predicted)
Vapor pressure	Not available
Partition coefficient (LogP)	8.833

Table 2-2. Eicosapentaenoic acid; name and other identifiers, physical and chemical properties.

Substance name	Eicosapentaenoic acid
Synonym	5,8,11,14,17-Eicosapentaenoic acid, icosapent, icosapentaenoic acid, timnodonic acid, EPA
CAS number	10417-94-4
EINECS number	Not available
Molecular formula	C ₂₀ H ₃₀ O ₂
Molecular weight	302.5

Structural formula	
SMILES	<chem>CCC=CCC=CCC=CCC=CCC=CCCCC(=O)O</chem>
Physical state	Liquid
Boiling point (liquids)	439°C
Stability	Light and air sensitive
Density	0.943 g/mL at 25°C
Vapor pressure	Not available
Partition coefficient (LogP)	6.1

3 Exposure

The dietary surveys Ungkost 3 (Hansen et al., 2016) and Norkost 3 (Totland et al., 2012) were used to estimate the intake of DHA and EPA from food. Ungkost 3 is a nationwide dietary survey carried out in 2015 and 2016 by the University of Oslo, the Norwegian Food Safety Authority, the Norwegian Directorate of Health and the Norwegian Institute of Public Health. The dietary assessment tool was a 4-day validated web-based food diary. The study was conducted among 4-year-olds (n= 399), 8-9-year-olds (n=636) and 12-13-year-olds (n=687). Norkost 3 is a nationwide dietary survey carried out in 2010/2011 among adults (n=1787), aged 18-70 years. Norkost 3 is based on two 24-hour recalls by telephone interviews, performed at least one month apart.

The estimated daily intake of DHA and EPA alone and combined from food for 4-year-olds, 8-9-year-olds, 12-13-year-olds, and 18-27-years olds are shown in Table 3-1, 3-2, and 3-3, respectively. There were only ten 18-year-olds in Norkost 3, so in order to get enough participants to be able to calculate the intake, all participants aged 18-27 were included. A comparison of the DHA and EPA doses included in this evaluation (1100 mg DHA and 1550 mg EPA) and the intake from food is shown in Table 3-4.

Table 3-1. Daily DHA intake from food, in mg/day.

Age groups	Median	P95	Mean	SD
4-year-olds (n=399, Ungkost 3)	148	828	229	248
8-9-year-olds (n=636, Ungkost 3)	81	834	206	303
12-13-year-olds (n=687, Ungkost 3)	84	882	226	380
18-27-year-olds (n=224, Norkost 3)	144	1294	344	477

P95: 95th percentile; SD: standard deviation.

Table 3-2. Daily EPA intake from food, in mg/day.

Age groups	Median	P95	Mean	SD
4-year-olds (n=399, Ungkost 3)	75	523	141	162
8-9-year-olds (n=636, Ungkost 3)	40	587	130	211
12-13-year-olds (n=687, Ungkost 3)	43	612	147	272
18-27-year-olds (n=224, Norkost 3)	63	784	190	278

P95: 95th percentile; SD: standard deviation

Table 3-3. Daily DHA and EPA intake from food, in mg/day.

Age groups	Median	P95	Mean	SD
4-year-olds (n=399, Ungkost 3)	219	1382	371	408
8-9-year-olds (n=636, Ungkost 3)	120	1416	336	512
12-13-year-olds (n=687, Ungkost 3)	124	1499	373	651

Age groups	Median	P95	Mean	SD
18-27-year-olds (n=224, Norkost 3)	196	2058	533	750

P95: 95th percentile; SD: standard deviation

Table 3-4. The ratio of 1100 mg DHA and 1550 mg EPA doses and the intake from food (Ungkost 3 and Norkost 3).

Food supplement/ exposure from food	4-year-olds	8-9-year-olds	12-13-year-olds	18-27-year-olds
DHA/median	7.4	13.5	13.1	7.6
DHA/mean	4.8	5.3	4.9	3.2
DHA/P95	1.3	1.3	1.3	0.9
EPA/median	20.7	38.8	36.1	24.6
EPA/mean	11	11.9	10.5	8.2
EPA/P95	3.0	2.6	2.5	2.0
(DHA+EPA)/median	12.1	22.1	21.4	13.5
(DHA+EPA)/mean	7.1	7.9	7.1	5.0
(DHA+EPA)/P95	1.9	1.9	1.8	1.3

P95: 95th percentile; SD: standard deviation

4 Hazard identification and characterisation

The questions to be answered in the hazard identification and characterisation for oral intake of DHA and EPA from food supplements are presented in Table 4-1. An overview of the hazard identification and characterisation process is given in Figure 4-1.

Table 4-1. Hazard: Research questions.

Hazard identification	1	Is there a concern for genotoxicity?
	2	Is exposure to docosahexaenoic acid and/or eicosapentaenoic acid associated with adverse health effects?
Hazard characterisation	3	What is the dose-response relationships between exposure to docosahexaenoic acid and/or eicosapentaenoic acid and the adverse effects?
	4	Can a health-based guidance value be established or a point of departure be identified?

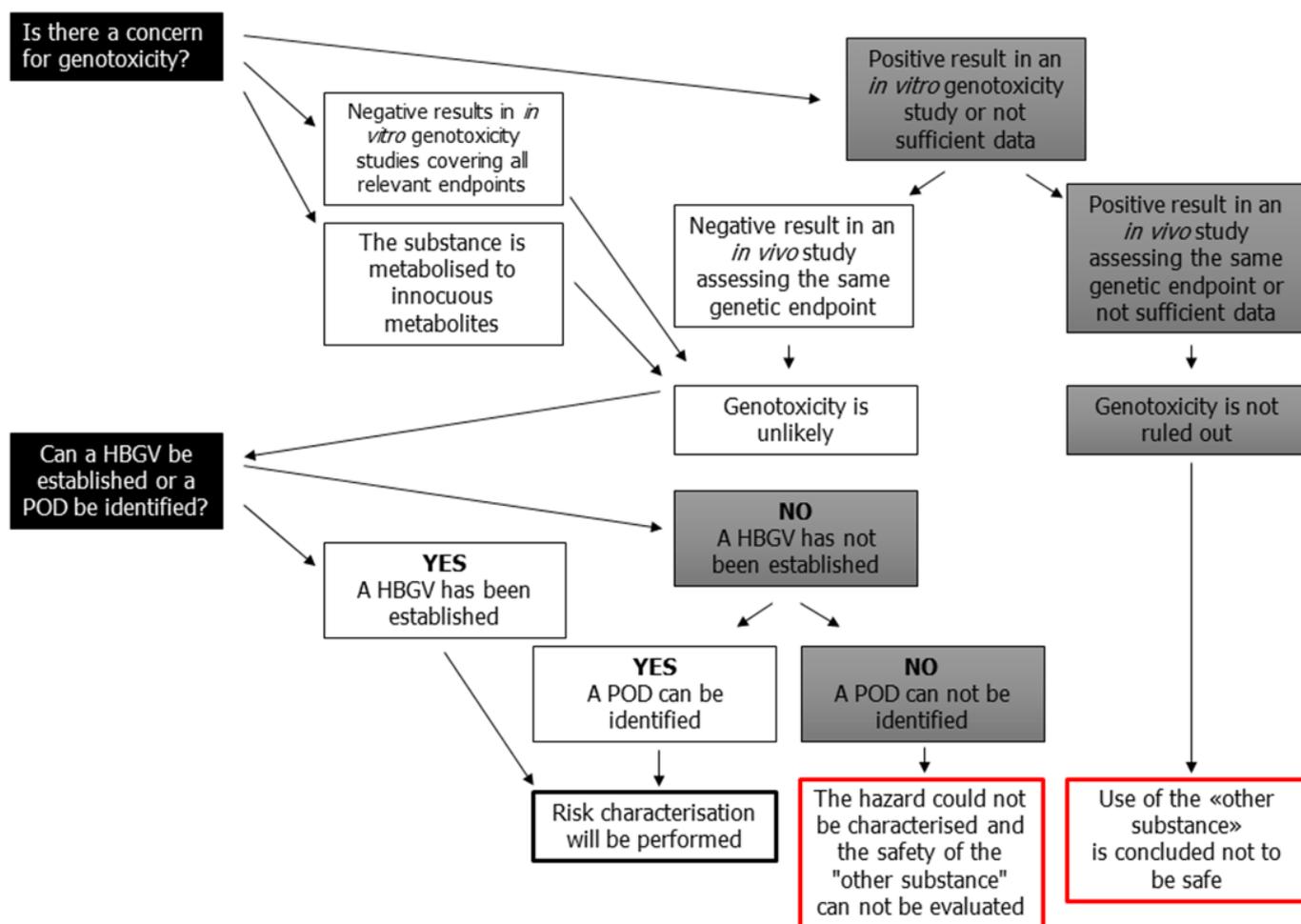


Figure 4-1. Flow chart for the hazard identification and characterisation. HBGV = health-based guidance value; POD = point of departure.

4.1 Literature

To identify relevant data of sufficient quality to answer question 1-4 (Table 4-1), websites of international relevant organisations were searched to identify reports (including risk- or safety assessments) on DHA and EPA. In addition, literature searches in electronic databases were performed.

Five reports/risk assessments including relevant data were identified from the searches of websites:

- Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acid. Institute of medicine (IOM, 2005).
- Evaluation of negative and positive health effects of n-3 fatty acids as constituents of food supplements and fortified foods (VKM, 2011).
- Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) (EFSA, 2012).

- Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the use conditions for certain substances other than vitamins, minerals and plants in food supplements (AESAN, 2012).
- Risk assessment of “other substances” – eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid (VKM, 2015).

A systematic literature search in the electronic databases MEDLINE (Ovid) and Embase (Ovid) were performed. The search strategies for systematic reviews and RCTs are reported in Section 10.1 and 10.2 (Appendix), respectively.

4.1.1 Literature search for systematic reviews

4.1.1.1 Publication selection

Literature retrieved from the searches were screened based on the eligibility criteria presented in Table 4.1.1.1-1.

Outcomes defined as “any adverse health effect” identified in the RCT were included. In addition, outcomes defined as “adverse” or as “side-effects” in the systematic reviews were included.

A publication qualified as a systematic review if 1) a specific research question and the specific criteria used for selecting studies were described, 2) a systematic literature search was performed, and 3) a quality assessment of the selected studies was included (Cochrane Glossary, 2020).

Table 4.1.1.1-1. Hazard: eligibility criteria for systematic reviews.

Population	Humans
Exposure	Docosahexaenoic acid and eicosapentaenoic acid are tested alone (not part of a mixture), or docosahexaenoic acid and eicosapentaenoic acid are tested together. Exposure route is oral
Outcome of interest	Any adverse health effect related to exposure to the substance
Language of the full-text	English, Norwegian, Swedish, Danish, German
Publication type	Systematic reviews

The literature search identified 955 publications. First, pairs of reviewers screened titles and abstracts independently, and 231 publications were included. Next, pairs of reviewers

screened the full-text articles independently, and 66 was considered to fulfil the eligibility criteria (Figure 4.1.1.3-1).

4.1.1.2 Evaluation of risk of bias

ROBIS, a tool for assessing the risk of bias (RoB) in systematic reviews, was used (Whiting et al., 2016). The reviewers calibrated themselves once to ensure similar evaluation, and the pair of reviewers independently assessed RoB. The tool includes three phases: First, the relevance is assessed, next, concerns with the review process are identified, and last, the risk of bias is judged.

Although fulfilling the eligibility criteria, 29 systematic reviews were considered as not relevant as they contained no data on adverse effects usable for our review, or because the participants were not representative for the whole population, but critically ill patient groups. An overview of the rating of relevance is available in Section 10.1.2 (Appendix). None of the eligible systematic reviews were considered to be relevant, however, 37 were considered partly relevant. Phase two and phase three of the ROBIS RoB tool was performed for the partly relevant systematic reviews (Table 4.1.1.2-1, for detailed evaluations, see Section 10.1.3). Five of the systematic reviews had low RoB, seven had unclear RoB and 25 had high RoB. Systematic reviews with high RoB were not included in the evidence synthesis due to the high concern for bias on key element(s).

Table 4.1.1.2-1. Assessment of risk of bias using the ROBIS tool.

Reference	Concerns with the review process				RoB
	Eligibility criteria	Identification and selection of studies	Data collection and study appraisal	Synthesis and findings	
Abdelhamid et al. (2020)	Low	Low	Low	Low	Low
AbuMweis et al. (2021)	Unclear	High	Unclear	Unclear	High
AbuMweis et al. (2018)	Unclear	High	Unclear	High	High
AlAmmar et al. (2019)	Unclear	High	High	High	High
Azzi et al. (2018)	Unclear	High	High	Unclear	High
Balk et al. (2016)	High	High	High	High	High
Becic and Studenik (2018)	Unclear	High	Unclear	Unclear	High
Bernstein et al. (2012)	Unclear	Unclear	High	High	High
Casula et al. (2020)	Unclear	Unclear	High	High	High
Chang et al. (2018a)	Unclear	Low	High	Unclear	High

Reference	Concerns with the review process				RoB
	Eligibility criteria	Identification and selection of studies	Data collection and study appraisal	Synthesis and findings	
Chen et al. (2015)	Unclear	Unclear	Unclear	High	High
Chua et al. (2012)	Unclear	Low	Low	Unclear	Unclear
Craddock et al. (2017)	Low	Unclear	High	High	High
Downie et al. (2019)	Low	Low	Low	Low	Low
Fogacci et al. (2020)	Unclear	Low	Low	High	High
Fu et al. (2015)	Unclear	Unclear	Unclear	Unclear	Unclear
Goh et al. (2021)	Unclear	High	Low	High	High
Guo et al. (2019)	High	High	Unclear	High	High
Hu et al. (2018)	High	High	High	High	High
Innes and Calder (2018)	High	High	High	High	High
Irving et al. (2006)	Low	Low	Low	Unclear	Unclear
Kar et al. (2016)	Unclear	Unclear	Unclear	High	High
Kwak et al. (2012)	Unclear	High	High	High	High
Lehner et al. (2021)	Unclear	Unclear	High	Unclear	High
Lopez-Huertas (2012)	Unclear	Unclear	High	High	High
Mocellin et al. (2016)	Unclear	Unclear	Low	Unclear	Unclear
Newberry et al. (2016)	Low	Unclear	Unclear	Low	Unclear
Ostadrahimi et al. (2016)	Unclear	High	Low	High	High
Quin et al. (2016)	Unclear	Low	Unclear	High	High
Ren et al. (2021)	Low	Unclear	Low	Low	Low
Sadeghi et al. (2017)	Unclear	High	High	High	High
Sarmento Vasconcelos et al. (2016)	Low	Low	Low	Unclear	Unclear
Su et al. (2021)	Low	Unclear	Low	Low	Low
Villani et al. (2013)	Unclear	High	Low	High	High
Wang et al. (2006)	Unclear	Unclear	Unclear	Unclear	High
Watson and Stackhouse (2020)	Low	Low	Low	High	Low
Xin et al. (2013)	Unclear	Unclear	Low	Unclear	Unclear

4.1.1.3 Summary of the literature search for systematic reviews

Systematic literature searches for systematic reviews addressing adverse effects related to DHA and EPA were performed in two electronic databases. An overview of study selection, the evaluation of risk of bias, and finally the number of included systematic reviews, is shown in Figure 4.1.1.3-1.

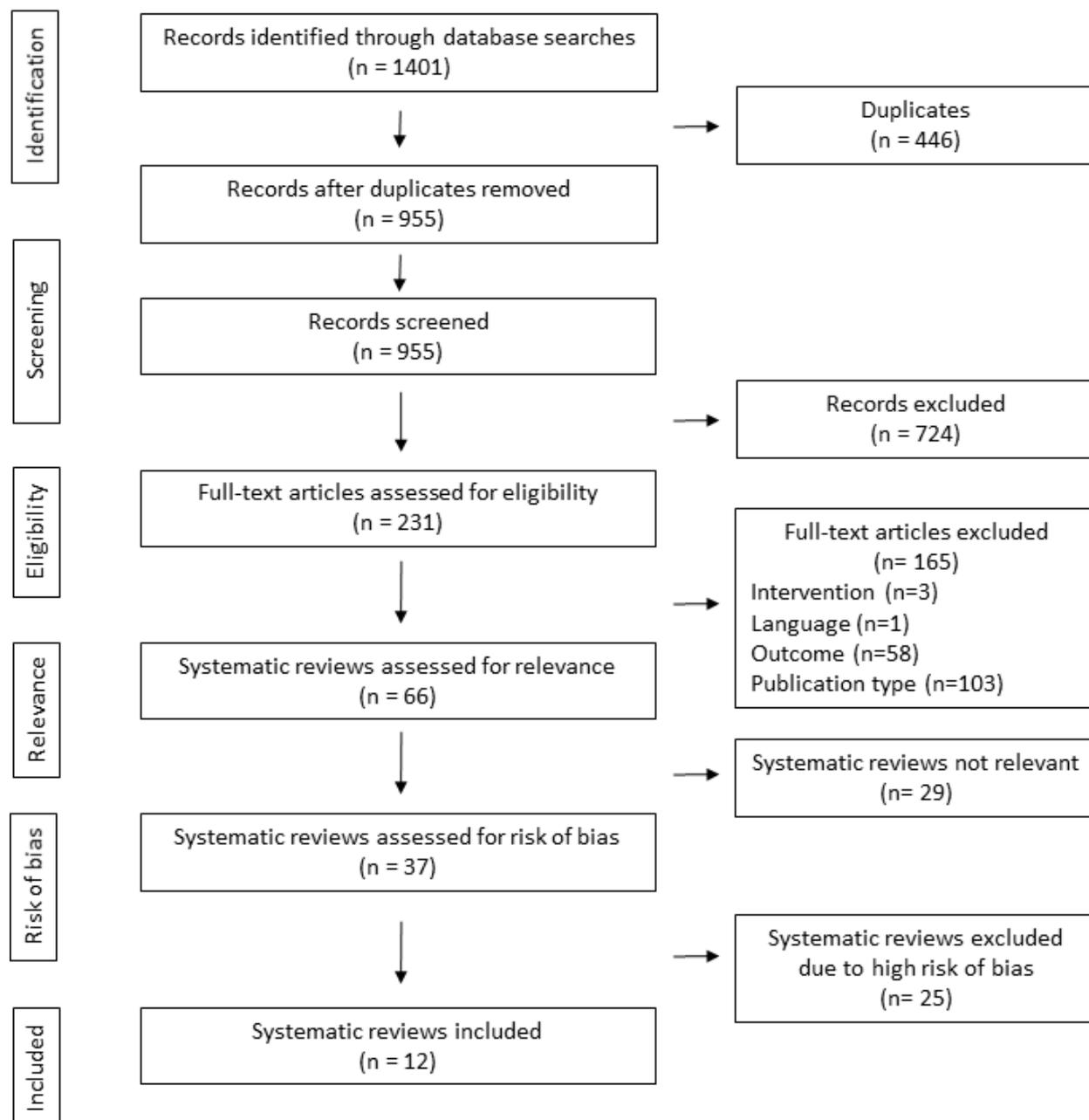


Figure 4.1.1.3-1. Flowchart for the selection of systematic reviews of sufficient quality addressing adverse effects related to DHA and EPA (modified from Moher et al. (2009)).

4.1.2 Literature search for RCTs

Literature searches in MEDLINE (Ovid) and Embase (Ovid) was performed. The search strategy is reported in Section 10.2 (Appendix).

4.1.2.1 Publication selection

Literature retrieved from the searches were screened based on the eligibility criteria presented in Table 4.1.2.1-1.

Table 4.1.2.1-1. Hazard: eligibility criteria for human studies.

Population	Children and adolescents, 0-18 years.
Exposure	Docosahexaenoic acid and eicosapentaenoic acid are tested alone (not part of a mixture), or docosahexaenoic acid and eicosapentaenoic acid are tested together. Exposure route is oral
Outcome of interest	Any adverse health effect related to exposure to the substance
Language of the full-text	English
Publication type	Randomised controlled trials

The literature search identified 1100 publications. First, pairs of reviewers screened titles and abstracts independently, and 60 publications were included. Next, pairs of reviewers screened the full-text articles independently, and 19 were included. In addition, reference lists of the included risk assessments were screened and one RCT fulfilling the eligibility criteria was identified.

4.1.2.2 Evaluation of risk of bias

Risk of bias (RoB) was evaluated using the OHAT (Office of Health Assessment and Translation) tool (OHAT, 2015; OHAT 2019). This tool includes questions considering aspects relevant for RoB evaluation of human randomised controlled trials.

The RoB questions addressing key elements such as exposure assessment and outcome assessment, were defined as key questions. The rating of all questions, key and non-key, was integrated to classify the studies into tiers to characterise the overall RoB as shown in Table 4.1.2.2-1. Tier 1 represents low RoB, tier 3 represents high RoB. Tier 2 studies did not

meet the criteria for tier 1 or 3. For RCTs, we defined questions 1, 2, 3, 5 and 6 as key questions, whereas questions 4, 7 and 8 were defined as non-key questions (Table 4.1.2.2-2; *=key questions). The key questions address the elements selection bias (randomisation and allocation to study groups), performance bias (identical experimental conditions across study groups and blinding of personnel and participants), and detection bias (confidence in the exposure characterisation and the outcome assessment). The non-key questions address the elements attrition/exclusion bias, selective reporting bias, and other sources of bias.

The response options and symbols (in parentheses) used for the rating are i) definitely low risk of bias (+++); ii) probably low risk of bias (+); iii) probably high risk of bias/not reported (NR) (-); and iv) definitely high risk of bias (--).

Table 4.1.2.2-1. Classification of studies into tiers according to overall RoB.

Tier	1	2	3
Criteria for classification	All key questions are scored +/++ AND No more than one non-key question is scored - AND No non-key question is scored - -	All combinations not falling under tier 1 or 3	Any key or non-key question is scored - - OR More than one key question is scored -

Two reviewers independently assessed RoB. The detailed evaluation for each RoB question is included in the appendix (Section 11.2). The RoB scores are shown in Tables 4.1.2.2-2 to 4.1.2.2-14. The detailed evaluations are available in Section 10.2.2 (Appendix). The overall classification in tiers were as follows:

- Allergic skin reactions: one RCT tier 3, two RCTs tier 2.
- Bleeding: two RCTs tier 3, one RCT tier 2.
- Gastrointestinal effects: four RCTs tier 3, three RCTs tier 2, one RCT tier 1.
- Glucose and insulin: two RCTs tier 3, two RCTs tier 2, two RCTs tier 1.
- Headache: two RCTs tier 3, two RCTs tier 2.
- Inflammatory markers: two RCTs tier 3, five RCTs tier 2, three RCTs tier 1.
- Joint, lumbar and muscle pain: one RCT tier 3, two RCTs tier 2.
- Kidney effects: one RCT tier 3.
- Lipid profile: two RCTs tier 3, three RCTs tier 2, one RCT tier 1.
- Liver effects: two RCTs tier 3, one RCT tier 2, one RCT tier 1.
- Other side effects: seven RCTs tier 3, three RCTs tier 2.
- Oxidative stress: One RCT tier 2.
- Skin problems: two RCTs tier 3, two RCTs tier 2.

Table 4.1.2.2-2. Allergic skin reactions: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Cornu et al. (2018)	++	++	++	+	+	-	+	-	2
Mazahery et al. (2019)	++	++	++	-	+	-	++	-	2
Van der Wurff et al. (2019)	++	++	++	-	-	-	+	-	3

Table 4.1.2.2-3. Bleeding: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Hanssen et al. (2016)	++	++	+	+	-	-	--	+	3
Hughbanks-Wheaton et al. (2014)	++	-	-	-	+	-	++	++	3
Mazahery et al. (2019)	++	++	++	-	+	-	++	-	2

Table 4.1.2.2-4. Gastrointestinal effects: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Cornu et al. (2018)	++	++	++	+	+	-	+	-	2
de Ferranti et al. (2014) (3 months)	++	++	++	-	+	-	-	-	2
de Ferranti et al. (2014) (6 months)	++	++	++	--	+	-	-	-	3
Hughbanks-Wheaton et al. (2014)	++	-	-	-	+	-	++	++	3
Mazahery et al. (2019)	++	++	++	-	+	-	++	-	2
Milte et al. (2015)	++	-	+	+	+	-	++	-	3
Montgomery et al. (2018)	++	++	++	+	+	++	++	+	1
Van der Wurff et al. (2019)	++	++	++	-	-	-	+	-	3

Table 4.1.2.2-5. Glucose and insulin: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
de Ferranti et al. (2014) (3 months)	++	++	++	-	+	-	-	-	2
de Ferranti et al. (2014) (6 months)	++	++	++	--	+	-	-	-	3
Hughbanks-Wheaton et al. (2014)	++	-	-	-	+	-	++	++	3
Janczyk et al. (2015)	++	++	+	+	+	-	++	+	2
Pacifico et al. (2015)	++	+	++	+	+	++	+	+	1
Rodriguez et al. (2019)	++	++	++	-	+	++	+	+	1

Table 4.1.2.2-6. Headache: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Cornu et al. (2018)	++	++	++	+	+	-	+	-	2
Mazahery et al. (2019)	++	++	++	-	+	-	++	-	2
Van der Wurff et al. (2019)	++	++	++	-	-	-	+	-	3
Voigt et al. (2014)	++	-	++	-	+	++	-	-	3

Table 4.1.2.2-7. Inflammatory markers: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Chang et al. (2019)	++	+	+	-	-	++	++	+	2
Chase et al. (2015)	+	-	+	-	++	++	++	+	2
de Ferranti et al. (2014) (3 months)	++	++	++	-	+	++	-	-	2
de Ferranti et al. (2014) (6 months)	++	++	++	--	+	++	-	-	3
Engler et al. (2004)	+	+	++	-	+	++	++	-	2
Hanssen et al. (2016)	++	++	+	+	-	-	--	+	3
Janczyk et al. (2015)	++	++	+	+	+	-	++	+	2
Pacifico et al. (2015)	++	+	++	+	+	++	+	+	1
Rodriguez et al. (2018)	++	++	++	++	++	++	++	+	1
Smuts et al. (2015)	++	++	++	++	+	++	++	+	1

Table 4.1.2.2-8. Joint, lumbar and muscle pain: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Cornu et al. (2018)	++	++	++	+	+	-	+	-	2
de Ferranti et al. (2014) (3 months)	++	++	++	-	+	-	-	-	2
de Ferranti et al. (2014) (6 months)	++	++	++	--	+	-	-	-	3

Table 4.1.2.2-9. Kidney effects: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Hughbanks-Wheaton et al. (2014)	++	-	-	-	+	-	++	++	3

Table 4.1.2.2-10. Lipid profile: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
de Ferranti et al. (2014) (3 months)	++	++	++	-	+	-	-	-	2
de Ferranti et al. 2014 (6 months)	++	++	++	--	+	-	-	-	3

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Engler et al. (2004)	+	+	++	-	+	++	++	-	2
Hughbanks-Wheaton et al. (2014)	++	-	-	-	+	-	++	++	3
Janczyk et al. (2015)	++	++	+	+	+	-	++	+	2
Verduci et al. (2014)	++	++	++	++	+	++	+	+	1

Table 4.1.2.2-11. Liver effects: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Hanssen et al. (2016)	++	++	+	+	-	-	--	+	3
Hughbanks-Wheaton et al. (2014)	++	-	-	-	+	-	++	++	3
Janczyk et al. (2015)	++	++	+	+	+	-	++	+	2
Pacifico et al. (2015)	++	+	++	+	+	++	+	+	1

Table 4.1.2.2-12. Other side effects: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Crippa et al. (2019)	++	++	++	-	+	-	-	-	2
Hanssen et al. (2016)	++	++	+	+	-	-	--	+	3
Mazahery et al. (2019)	++	++	++	-	+	-	++	-	2
Manos et al. (2019)	++	++	++	-	-	-	++	+	3
Meguid et al. (2016)	-	-	-	+	-	-	-	-	3
Pacifico et al. (2015)	++	+	++	+	+	-	-	-	2
Rodriguez et al. (2018)	++	++	++	++	+	-	--	-	3
Rodriguez et al. (2019)	++	++	++	-	+	-	--	-	3
Verduci et al. (2014)	++	++	++	-	+	-	+	-	3

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Voigt et al. (2014)	++	-	++	-	+	++	-	-	3

Table 4.1.2.2-13. Oxidative stress: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Engler et al. (2004)	+	+	++	-	+	++	++	-	2

Table 4.1.2.2-14. Skin problem: evaluation of RoB.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Cornu et al. (2018)	++	++	++	+	+	-	+	-	2
de Ferranti et al. (2014) (3 months)	++	++	++	-	+	-	-	-	2
de Ferranti et al. (2014) (6 months)	++	++	++	--	+	-	-	-	3
Van der Wurff et al. (2019)	++	++	++	-	-	-	+	-	3

4.1.2.3 Summary of literature search RCTs

Systematic literature searches for RCTs addressing adverse effects related to DHA and EPA were performed in two electronic databases. An overview of study selection, the evaluation of risk of bias, and finally the number of included RCTs, is shown in Figure 4.1.2.3-1.

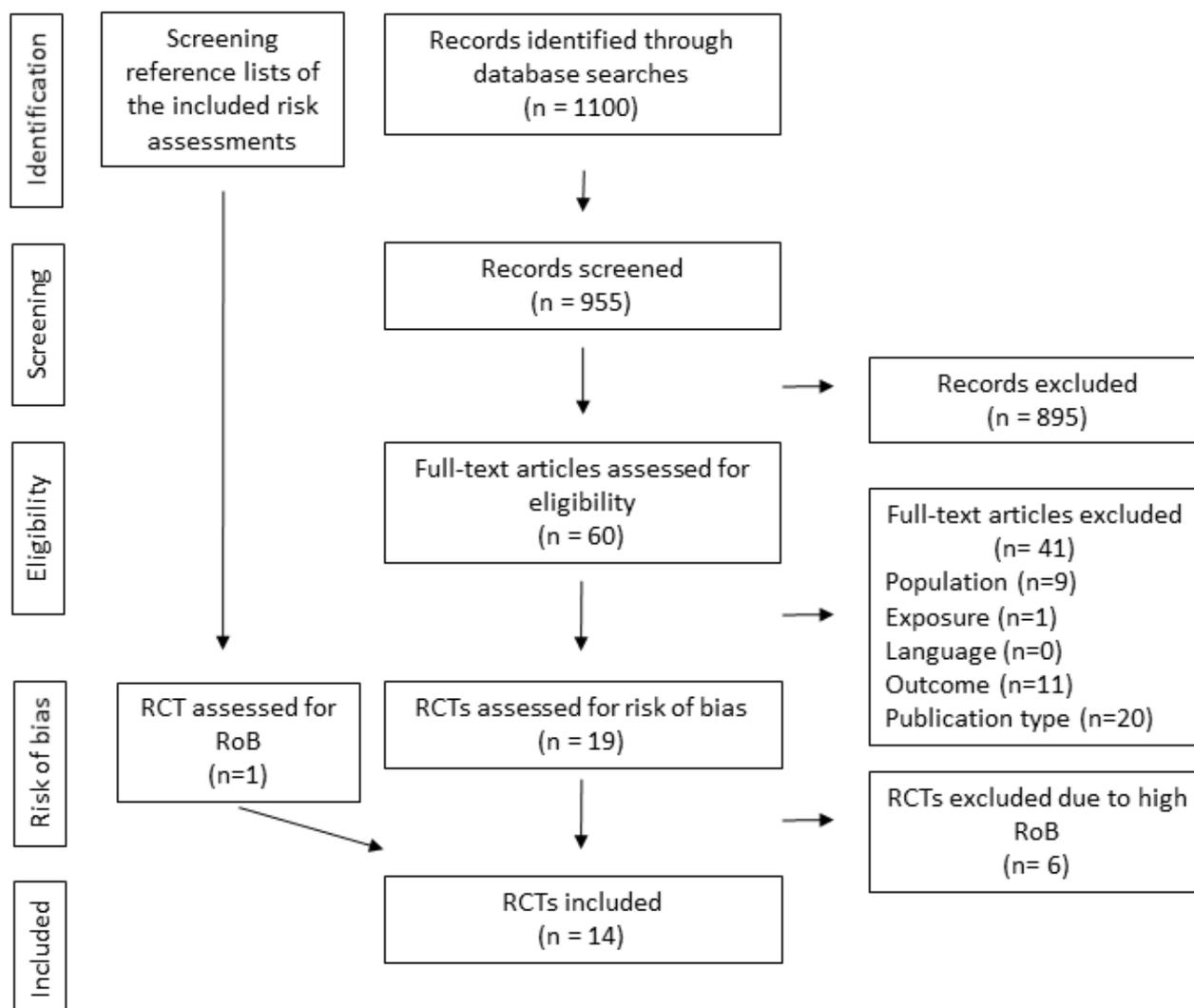


Figure 4.1.2.3-1. Flowchart for the selection of RCTs of sufficient quality addressing adverse effects related to DHA and EPA (modified from Moher et al. (2009)).

4.2 Absorption, distribution, metabolism and elimination (ADME)

According to the protocol for the risk assessment of "other substances", the following ADME questions should be addressed and answered:

- What is the ADME of DHA and EPA in humans?
- Is DHA and EPA metabolised to innocuous metabolites?
- Is DHA and EPA endogenous to humans? If yes, is the dose given in the mandate from NFSA resulting in body levels within the range normally metabolised and eliminated?

No data ADME data on n-3 PUFAs in children and adolescents were identified in the included literature. A literature search for ADME data was not performed due to the limited time available.

ADME data for adults:

According to IOM (2005), n-3 PUFAs are almost completely absorbed and either oxidised to carbon dioxide and water, incorporated into tissue lipids, or utilised in eicosanoid synthesis. EPA and DHA are incorporated into cell membranes, and thus may impact cellular metabolism, signal transduction, and regulation of gene expression. EPA can be transformed to eicosanoids, including prostaglandins, prostacyclin and leukotrienes, which participate in the regulation of blood pressure, renal function, blood coagulation, inflammatory and immunological reactions and other functions in tissues (VKM, 2015). DHA is metabolised to F4-neuroprostanes and endocannabinoids (VKM, 2015). Other metabolites of DHA and EPA includes resolvins, poxytrins, neuroprotectins and maresins, which are thought to be involved in resolution of inflammatory responses (VKM, 2015). High dietary intakes of EPA and DHA result in decreased tissue concentrations of arachidonic acid, increased concentrations of EPA and DHA, and changes in the balance of eicosanoids synthesized from the n-6 and n-3 fatty acids (IOM, 2005).

EPA and DHA are synthesised endogenously from ALA in varying amounts in animal tissues. The endogenous production of EPA and DHA from ALA in humans may be negligible compared to the food and food supplement doses used in the studies considered for the assessment of the safety of n-3 LCPUFAs (EFSA, 2012). The DHA and EPA doses in food supplements assessed in the present risk assessment are higher than the levels produced endogenously, and also higher than the intake from food (shown in Table 3-1, 3-2, and 3-3).

4.3 Genotoxic potential

See Section 4.4.2-5. Genotoxicity has been assessed earlier and no concerns for genotoxicity were raised (VKM 2015).

4.4 Adverse health effects

4.4.1 Summary of hazard data from the included reports

4.4.1.1 IOM (2005)

From the hazard identification summary: *“While there is evidence to suggest that high intakes of n-3 polyunsaturated fatty acids, particularly EPA and DHA, may impair immune response and result in excessively prolonged bleeding times, it is not possible to establish a UL. Studies on immune function were done in vitro and it is difficult, if not impossible, to know how well these artificial conditions simulate human immune cell response in vivo. Data on EPA and DHA intakes and bleeding times are mixed and a dose–response effect was not observed. EPA and DHA are available as dietary supplements, and until more information is available on the adverse effects of EPA and DHA, these supplements should be taken with caution.”*

4.4.1.2 VKM (2011)

From the summary: *“The following negative health effects have been identified in studies with EPA and DHA; bleeding tendency, lipid peroxidation, impaired inflammation and other immune functions, impaired lipid and glucose metabolism and gastrointestinal disturbances.*

An increased bleeding time has been found after intake of 6.9 g/day EPA and DHA in coronary heart disease patients on anti-coagulant medication. However, no negative health effects regarding bleeding complication in connection with EPA and DHA supplementations have been reported.

A limited number of studies have reported data on lipid peroxidation following n-3 fatty acid supplementation. Most of these did not show any increase in lipid peroxidation biomarkers. One large study with myocardial infarction patients taking 3.5 g EPA and DHA per day as ethyl ester showed increased thiobarbituric acid reactive substances (TBARS) in plasma. The relationship between in vivo lipid peroxidation and TBARS is uncertain. Moreover, none of the oxidative stress biomarkers are presently defined as risk factors of disease. The clinical relevance of lipid peroxidation is therefore unclear.

In several studies biomarkers of systemic inflammation in healthy subjects and different patient groups supplemented with n-3 fatty acids have been measured. No increase in C-reactive protein (CRP) after intake of marine n-3 fatty acids has been observed. EPA and DHA at doses of 5 g/day have been shown to activate endothelial cells (increased sVCAM-1 and sE-selectin) among individuals at high risk of cardiovascular diseases and in patients with coronary heart disease. Although low-grade systemic inflammation plays an important role in the pathology of some diseases, such as cardiovascular disease and type 2 diabetes, the clinical relevance of an increase of low-grade systemic inflammation is still uncertain.

The findings in the reviewed literature indicates no effects on glucose control in subjects with type 2 diabetes of supplementation with EPA and DHA in doses ranging from 0.8 to 4.8 g/day (mean: 2.4 g/day). A minor increase in LDL-cholesterol (1-3%) in subjects with type 2 diabetes has been reported in meta-analyses following supplementation with EPA and DHA in doses ranging from 0.8 to 4.8 g/day (mean: 2.4 g/day). No dose response relationship has been reported. However, the clinical relevance in subjects with type 2 diabetes of this minor increase in LDL-cholesterol is unclear because of a concomitant reduction in serum triacylglycerol and unchanged apolipoprotein B in the same subjects. No change in LDL cholesterol was reported in the large coronary heart intervention trials including both subjects with and without type 2 diabetes. Since the effect on LDL-cholesterol is minor, and of uncertain clinical significance, the Scientific Steering Committee will put less emphasis on this effect.

Negative health effects regarding gastrointestinal function, including abdominal cramps, flatulence, eructation, vomiting and diarrhea, have been reported, but seem to be associated with intake of an oily substance and not ascribed specifically to EPA and/or DHA. Based on the reviewed literature, it is not possible to identify clear adverse effects from EPA and/or DHA, which can be used for setting tolerable upper intake levels.”

4.4.1.3 EFSA (2012)

From the summary: “Adverse effects, which have been described in humans in association with high intakes of EPA and DHA, include bleeding episodes, impaired immune function, increased lipid peroxidation, and impaired lipid and glucose metabolism. However, no tolerable upper intake level (UL) for EPA, DHA or DPA has been set by any authoritative body.

Previous assessments on the safety of n-LCPUFAs referred to mixtures of EPA and DHA (DPA was not explicitly mentioned), and were primarily based on a large number of human studies. The Panel considers that the evaluation of the safety of n-3 LCPUFA intakes should be based on the human studies available.

The majority of human intervention studies which have investigated the effects of n-3 LCPUFAs on different health outcomes have used fish oils containing known amounts of EPA and DHA and generally unknown (but relatively low) amounts of DPA; EPA and DHA in combination as ethyl esters; or more rarely mostly EPA or mostly DHA. Very few studies are available using krill oil as a source of EPA and DHA, and no studies have been conducted with sources containing mainly DPA, or with DPA alone.

Long-term human intervention studies which have investigated the effects of supplemental intakes of EPA and DHA, either alone or in combination, at doses up to about 1 g/day on a variety of health outcomes (e.g., cardiovascular, neurological, immunological), have generally reported no adverse effects in relation to the consumption of EPA or DHA at these doses.

Long-term supplemental intakes of EPA and DHA combined up to about 5 g/day do not increase the risk of spontaneous bleeding episodes or bleeding complications even in subjects at high risk of bleeding (e.g. taking acetylsalicylic acid or anti-coagulants).

Supplemental intakes of EPA and DHA combined at doses up to 5 g/day consumed for up to 12 weeks do not significantly affect glucose homeostasis in healthy or diabetic subjects, nor do they induce changes in immune functions which might raise concern in relation to the risk of infections or inappropriate activation of inflammatory responses. The data available are insufficient to conclude on whether the same doses administered mostly as EPA or mostly as DHA would have different effects on these outcomes.

Supplemental intakes of EPA and DHA consumed either alone or in combination at doses up to about 5 g/day for up to 16 weeks do not induce changes in lipid peroxidation which might raise concern in relation to cardiovascular disease (CVD) risk as long as the oxidative stability of these n-3 LCPUFA is guaranteed.

Supplemental intakes of EPA and DHA combined of 2-6 g/day, and supplemental intakes of mostly DHA of 2-4 g/day, increase blood concentrations of LDL cholesterol by about 3 %. Such increase is accompanied by a decrease in triglycerides with no changes in total (or non-HDL) cholesterol concentrations. Supplemental intakes of mostly EPA at doses up to 4 g/day have no significant effect on LDL-cholesterol concentrations. The Panel considers that the small increase in LDL-cholesterol concentrations associated with combined EPA and DHA supplementation or with DHA supplementation alone at the doses mentioned above may not have an adverse effect on CVD risk.

The Panel concludes that the available data are not sufficient to establish a tolerable upper intake level for n-3 LCPUFA (DHA, EPA, and DPA, individually or combined) for any population group.

At observed intake levels, consumption of n-3 LCPUFA has not been associated with adverse effects in healthy children or adults.

The Panel considers that supplemental intakes of EPA and DHA combined at doses up to 5 g/day, and supplemental intakes of EPA alone up to 1.8 g/day, do not raise safety concerns for the adult population. Limited data are available on the effects of long-term supplementation with these n-3 LCPUFAs at higher doses. The Panel also notes that observed intakes of EPA and DHA from food and food supplements in European populations are generally below these amounts. Dietary recommendations for EPA and DHA based on CVD risk considerations for European adults are between 250 and 500 mg/day. There are no specific recommendations for EPA.

The Panel also considers that supplemental intakes of DHA alone up to about 1 g/day do not raise safety concerns for the general population. Limited data are available on the effects of long-term supplementation with DHA alone at higher doses. The Panel notes that specific

dietary recommendations for DHA for European adults and children are well below this amount.”

4.4.1.4 AESAN (2012)

The safety assessment of EPA and DHA in the AESAN report was based on the EFSA (2012) report.

4.4.1.5 VKM (2015)

From the summary: “The major concerns with high intake of EPA and DHA have been increased bleeding time, adverse effects related to immune function, lipid peroxidation and glucose homeostasis. EFSA concluded in 2012 that long-term supplemental intakes of 5 g/day of the n-3 LCPUFA do not raise safety concerns for adults with regard to an increased risk of spontaneous bleeding episodes or bleeding complications, or affect glucose homeostasis, immune function or lipid peroxidation. In 2011, VKM concluded that an intake n-3 LCPUFA up to 6.9 g/day was not associated with increased risk of any serious adverse events.

Some adverse health effects related to gastrointestinal function, including abdominal cramps, flatulence, eructation, vomiting and diarrhea have been reported, but seem to be associated with intake of an oily substance and not related specifically to EPA, DPA and/or DHA.

EPA

In the report from 2012, EFSA concluded that 1.8 g/day of supplemental EPA does not raise safety concerns in adults. None of the included studies from our literature searches limited to 2012 and onwards have investigated bleeding complications. The dosages of EPA in the three included studies in this report range from 1.8 to 3.8 g/day for 12 weeks. The main endpoints in the studies included lipid peroxidation, inflammation biomarkers of cardiovascular diseases and no serious adverse events were found related to the main endpoints. In general, adverse events were described as gastrointestinal discomforts and not related to dosage.

Studies of longer duration are necessary before an intake above 1.8 g of EPA can be considered safe.

The Norwegian Scientific Committee for Food Safety (VKM) concludes that the specified doses of 1500, 1750, 1825 mg/day of EPA in food supplements are unlikely to cause adverse health effects in adults (≥18 years).

In 2012, EFSA did not make conclusions for children or adolescents for EPA. No new studies with EPA supplementation have been identified in children or adolescents after 2012, and therefore no risk assessment can be made for children (≥10 years) or adolescents.

DHA

EFSA concluded that 1 g/day of supplemental DHA does not raise safety concerns for the general population (including children and adolescents). The dosages of DHA in the included trials in this report range from 1.0 to 3.6 g/day and the duration from five weeks to four years. Six out of seven studies have used dosages from 1 to 2 g DHA/day. The last study included up to 3.6 g DHA/day for four years and the age spanned from 7 to 31 years. The main endpoints in all studies included lipid peroxidation, inflammation, cognitive performance, blood pressure and biomarkers of cardiovascular diseases and no serious adverse events were found related to the main endpoints. In general, adverse events were described as gastrointestinal discomforts and not related to dosage. VKM therefore considers that the specified daily doses of DHA that moderately exceed 1 g per day (1050 and 1290 mg/day) are unlikely to cause adverse health effects in the general population including children ≥ 10 years and adolescents.

VKM concludes that the specified doses of 1050 and 1290 mg/day of DHA in food supplements are unlikely to cause adverse health effects in the general population including children (≥ 10 years), adolescents and adults (≥ 18 years)."

The VKM risk assessment (2015), also describes the safety assessment of a registered drug (Omacore) which is a mixture of EPA and DHA in the form of ethyl esters (the pharmacology review: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-654_Omacor_Pharmr.pdf):

"Chronic toxicity

In 1 year toxicity studies, 2000 mg/kg bw/day and 1000 mg/kg bw/day produced clinical signs such as fur staining in rats and dogs, respectively. In rats, the main target organ of toxicity was the liver, and in the dogs the main target organs were adrenals, kidneys and testes. The NOAEL in rats (both sexes) was identified to be 600 mg/kg bw/day. This dose corresponds to about 8 g/day in humans with bw of 60 kg, when the comparison is made on the basis of body surface area. Such a comparison takes into account that the metabolism and surface/volume ratio is progressively higher in smaller animals. The NOAEL (adrenal, kidney and testis) in male dogs was reported to be 50 mg/kg bw/day, which corresponds to 1.6 g/day in humans, based on body surface area comparison. In female dogs the NOAEL (adrenals) was reported to be 300 mg/kg bw/day, corresponding to 8 g/day in humans, based on body surface area.

Dermal symptoms were consistently observed in several studies in rats, mice and dogs and were mainly and more severely observed in males.

Carcinogenicity

In a 2-year rat carcinogenicity study with oral gavage doses of 100, 600, 2000 mg/kg bw/day, males were treated with n-3 fatty acid as ethyl esters for 101 weeks and females for

89 weeks without an increased incidence of tumors. The highest dose corresponds to 20 g/day in humans (5-fold the human dose for a standard body weight of 60 kg), when a comparison due to body surface area is done. Standard lifetime carcinogenicity bioassays were not conducted in mice.

Genotoxicity

N-3 fatty acid as ethyl esters were not mutagenic or clastogenic with or without metabolic activation in the bacterial mutagenesis (Ames) test with Salmonella typhimurium and Escherichia coli or in the chromosomal aberration assay in Chinese hamster V79 lung cells or human lymphocytes. N-3-acid ethyl esters were negative in the in vivo mouse micronucleus assay.

Reproductive and developmental toxicity

In a rat fertility study with oral gavage doses of 100, 600, 2000 mg/kg bw/day, males were treated for 10 weeks prior to mating and females were treated for 2 weeks prior to and throughout mating, gestation and lactation. No adverse effect on fertility was observed even at the highest dose 2000 mg/kg bw/day, which corresponds to 20 g/day in humans (5-fold the human dose for standard body weight of 60 kg), when a comparison due to body surface area is done. In rabbits, the maternal NOAEL for reproductive toxicity was 375 mg/kg bw/day and embryo-fetal NOAEL was 750 mg/kg bw/day, representing 8-16 g/day in humans based on body surface area comparison. Studies in pregnant rats have reported conflicting results. In one study, no adverse reproductive effects were observed at doses up to 6000 mg/kg bw/day. Two studies reported embryotoxicity and/or decreased survival to postnatal day 4 (40% reduction) at doses resulting in exposures 7 times the recommended human dose of 4 g/day based on a body surface area comparison.

Adverse effects in humans

Controlled safety data are available from 226 patients treated with 4 g/day of EPA and DHA as ethyl ester (Medical Reviews part of the drug approval package for the registered drug http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-654_Omacor.cfm). A total of 665 patients received EPA and DHA therapy (any dose) in studies for which case report forms were available. More patients treated with EPA and DHA as ethyl ester experienced an adverse effect compared to placebo (35% vs 28%). However, the reported adverse effects were mild and only 8 patients on drug treatment discontinued as a result of adverse effects. The incidence of serious adverse effects was similar between EPA and DHA-group (3.1%) and placebo (2.6%). The most common adverse effects in the treatment group were eructation (4.9%) followed by infection (4.4%), flu syndrome (3.5%), and diarrhea (3.5%). Adverse effects in the digestive system such as eructation, dyspepsia, nausea and diarrhea accounted for 15% of the reported effects. The differences in incidences of adverse effects were not statistically significant between the treatment and the placebo group. The only side effect that occurred at a significantly higher rate in the 4 g/day EPA and DHA group in

comparison with placebo was taste perversion (primarily "fishy taste"), with an incidence of 2.7% in the treatment group versus none in the placebo group (p=0.015)."

4.4.2 Systematic reviews: study characteristics

An overview of study characteristics for the systematic reviews with low and unclear risk of bias for the potential adverse outcomes addressed are presented in Tables 4.4.2-1 to 4.4.2-17. Detailed data extraction forms are available in Section 10.1.4 (Appendix). Publications classified as high RoB are not included in the evidence synthesis.

Table 4.4.2-1. Data on age-related macular degeneration. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	1	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).	-	-	x	Low

Table 4.4.2-2. Data on liver and biliary tract. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Irving et al. (2006)	To assess the effects of essential fatty acid supplementation of antipsychotic treatment for schizophrenia-like illnesses.	1	This is an update of previous systematic reviews, and the search was performed in 2006.	People with schizophrenia or similar chronic mental illnesses, diagnosed by any criteria, irrespective of gender, age or race. Participants: both sex, >18 years.	-	X	-	Unclear

Table 4.4.2-3. Data on bleeding. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	11	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).	-	x	x	Low
Xin et al. (2013)	To investigate the possible role of fish oil supplementation for the prevention of postoperative atrial fibrillation.	5	The final literature search was performed on November 25th, 2012. There was no restriction on the start date of the search.	Patients undergoing cardiac surgery. Mean age for patients in the included studies reporting on bleeding were >60 years.	-	-	x	Unclear

Table 4.4.2-4. Data on gastrointestinal effects. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	33	Searches were performed in 2019. As this was an update, date limits to the terms from	Adults (18 years or older, men and women) at any risk of cardiovascular disease	x	x	x	Low

			the original search strategies were applied.	(with or without existing cardiovascular disease).				
Downie et al. (2019)	Assess the effects of omega-3 and omega-6 polyunsaturated fatty acid supplements on dry eye signs and symptoms, and to document any potential treatment-related adverse events.	3	The searches were performed February 2018. No restrictions were applied to the year of publication.	Trials in which participants received the diagnosis of dry eye.	-	-	x	Low
Irving et al. (2006)	To assess the effects of essential fatty acid supplementation of antipsychotic treatment for schizophrenia-like illnesses.	2	This is an update of previous systematic reviews, and the search was performed in 2006.	People with schizophrenia or similar chronic mental illnesses, diagnosed by any criteria, irrespective of gender, age or race. Participants: both sex, >18 years.	-	X	-	Unclear
Newberry et al. (2016)	To update a prior systematic review on the effects of n-3 PUFAs on maternal and child health and to assess the evidence for their effects on, and associations with, additional outcomes.	9	January 1, 2000 to August 24, 2015.	Pregnant or breastfeeding women	-	-	x	Unclear
		13		Healthy term infants or preterm infants	-	-	x	Unclear
Sarmiento et al. (2016)	To assess the effectiveness and tolerability of EPA and DHA in the control of seizures in people with refractory epilepsy.	2	Up to November 2015.	All individuals (adults and children) with a diagnosis of drug-resistant epilepsy, irrespective of their	-	-	x	Unclear

				seizure type or epilepsy syndrome.				
Watson and Stackhouse (2020)	To determine whether there is evidence that omega-3 polyunsaturated fatty acid supplementation reduces morbidity and mortality. To identify any adverse events associated with omega-3 polyunsaturated fatty acid supplementation in cystic fibrosis.	1	Searches were not restricted by year. The search was performed 1 April 2020	Children and adults with cystic fibrosis. Details on age and sex for the included studies are not reported.	-	-	x	Low

Table 4.4.2-5. Data on haemorrhagic stroke. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	10	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).	-	x	x	Low

Table 4.4.2-6. Data on headache or worsening migraine. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	4	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).	-	-	x	Low

Table 4.4.2-7. Data on inflammatory markers.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Mocellin et al. (2016)	To evaluate the effects of n-3 PUFA on inflammatory mediators in colorectal cancer patients.	7	Not reported	Adults over 18 years of age and, only affected by malignant colorectal neoplasm	-	-	x	Unclear
Su et al. (2021)	To investigate the effect of omega-3 PUFA supplements on outcomes in lower extremity arterial disease patients.	4	Not reported	Patients with lower extremity arterial disease	-	x	x	Low

Table 4.4.2-8. Data on joint, lumbar and muscle pain. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	3	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).	-	x	x	Low
Irving et al. (2006)	To assess the effects of essential fatty acid supplementation of antipsychotic treatment for schizophrenia-like illnesses.	1	This is an update of previous systematic reviews, and the search was performed in 2006.	People with schizophrenia or similar chronic mental illnesses, diagnosed by any criteria, irrespective of gender, age or race. Participants: both sex, >18 years.	-	X	-	Unclear

Table 4.4.2-9. Data on lipid profile. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	30 (total cholesterol) 30 (HDL) 25 (LDL)	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).	x	x	x	Low

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Su et al. (2021)	To investigate the effect of omega-3 PUFA supplements on outcomes in lower extremity arterial disease patients.	9	Not reported	Patients with lower extremity arterial disease	x	x	x	Low

Table 4.4.2-10. Data on metabolic and nutritional effects (e.g. body weight). X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Irving et al. (2006)	To assess the effects of essential fatty acid supplementation of antipsychotic treatment for schizophrenia-like illnesses.	1	This is an update of previous systematic reviews, and the search was performed in 2006.	People with schizophrenia or similar chronic mental illnesses, diagnosed by any criteria, irrespective of gender, age or race. Participants: both sex, >18 years.	-	X)	-	Unclear

Table 4.4.2-11. Data on movement disorder. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Irving et al. (2006)	To assess the effects of essential fatty acid supplementation of antipsychotic treatment for schizophrenia-like illnesses.	1	This is an update of previous systematic reviews, and the search was performed in 2006.	People with schizophrenia or similar chronic mental illnesses, diagnosed by any criteria, irrespective of gender, age or race. Participants: both sex, >18 years.	-	X	-	Unclear

Table 4.4.2-12. Data on other side effects. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Sarmiento et al. (2016)	To assess the effectiveness and tolerability of omega-3 polyunsaturated fatty acids (EPA and DHA) in the control of seizures in people with refractory epilepsy.	1	Up to November 2015.	All individuals (adults and children) with a diagnosis of drug-resistant epilepsy.	-	-	x	Unclear
Irving et al. (2006)	To assess the effects of essential fatty acid supplementation of antipsychotic treatment for schizophrenia-like illnesses.	1	This is an update of previous systematic reviews, and the search was performed in 2006.	People with schizophrenia or similar chronic mental illnesses, diagnosed by any criteria, irrespective of gender, age or race. Participants: both sex, >18 years.	-	X	-	Unclear

Table 4.4.2-13. Data on preterm birth and birth weight. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participants	DHA alone	EPA alone	DHA+EPA	RoB
Newberry et al. (2016)	To update a prior systematic review on the effects of omega-3 fatty acids on maternal and child health and to assess the evidence for their effects on, and associations with, additional outcomes.	Preterm birth: 16 RCTs, 2 observational studies	January 1, 2000 to August 24, 2015.	Healthy pregnant women	x	-	x	Unclear
		Birth weight: 37 RCTs, 19 observational studies	January 1, 2000 to August 24, 2015.	Healthy pregnant women	-	-	x	Unclear
Ren et al. (2021)	To evaluate whether levels of EPA, DHA, and trans fatty acids during pregnancy influenced offspring weight development.	19	Up to 2019	Healthy pregnant women and children	x	x	x	Low

Table 4.4.2-14. Data on prostate cancer. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participant characteristics (health condition/age/gender)	DHA alone	EPA alone	DHA+EPA	RoB
Chua et al. (2012)	To give a systematic review through quality assessment of all available literatures regarding the association of omega fatty acids and prostate cancer.	5	Up to June 2011	Males	-	-	x	Unclear

Reference	Aim	Trials on this outcome (n)	Literature search period	Participant characteristics (health condition/age/gender)	DHA alone	EPA alone	DHA+EPA	RoB
Fu et al. (2015)	To estimate the trend and quantify the association for both dietary intakes and blood concentration of individual n-3 PUFAs with prostate cancer risk based on prospective studies only.	7	Up to February 2014	Males (age 27-80 years old).	-	-	x	Unclear

Table 4.4.2-15. Data on pulmonary embolus or deep vein thrombosis (DVT). X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participant characteristics (health condition/age/gender)	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	5	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).	x	x	x	Low

Table 4.4.2-16. Data on reflux. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participant characteristics (health condition/age/gender)	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	3	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).	-	x	x	Low

Table 4.4.2-17. Data on skin problem effects. X: studies included; -: no studies included.

Reference	Aim	Trials on this outcome (n)	Literature search period	Participant characteristics (health condition/age/gender)	DHA alone	EPA alone	DHA+EPA	RoB
Abdelhamid et al. (2020)	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.	9	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).	-	-	x	Low

For the outcomes where only one systematic review was identified, all included trials from that systematic review were included in the evidence synthesis. For each outcome, where there was data from more than one systematic review, a judgement of which of the systematic reviews

that should be included in the evidence synthesis was done. The judgement was based on a combination of the result of our RoB evaluation, publication year/literature search period, and overlapping of included studies.

Bleeding have been assessed by two systematic reviews and there is no overlapping of included studies. However, Xin et al. 2013, only assessed major bleeding after surgery and these data will only be used as supporting information.

Six systematic reviews have assessed effects of EPA and/or DHA on gastrointestinal effects. The participants in the included studies in these six systematic reviews have different underlying diseases or conditions and there is no overlap between studies. All six are included in the evidence synthesis.

Two systematic reviews have assessed effects of joint, lumbar and muscle pain. No overlap between studies included in the two reviews and both will therefore be included in the evidence synthesis.

Effects of EPA and DHA alone or in combination on lipids have been assessed by two systematic reviews which have no overlap of included studies. Both reviews are therefore included in the evidence synthesis.

General side effects following exposure EPA and/or DHA have been assessed in two systematic reviews and there is no overlap between studies. Both reviews are included in the evidence synthesis.

4.4.3 RCTs: study characteristics

As publications classified as tier 3 (high RoB) are not included in the evidence synthesis due to the high concern for bias on key element(s), only study characteristics for the eligible publications classified as tier 1 (low RoB) or tier 2 (moderate RoB) are reported. A brief overview is given in Table 4.4.3-1, whereas detailed descriptions and data extraction forms are available in Section 10.2.3 (Appendix).

Table 4.4.3-1. RCTs addressing possible adverse effects.

Reference	Aim	Participant characteristics, n, age	Dose and duration	Possible adverse endpoints addressed/ results	RoB (tier)
Chang et al. (2019)	Compare the effect of a high dose of EPA (1.2 g/ day) with placebo on cognitive function, and the inflammatory biomarker high-sensitivity CRP.	Youth with Attention Deficit Hyperactivity Disorder. n = 92 youth, age 6–18-years-old.	1.2 g/day EPA or placebo (1.2 g/day soybean oil). 12 weeks.	Inflammatory markers: no effect on hs-CRP.	Inflammatory markers: 2.
Chase et al. (2015)	Investigate the effect of DHA supplementation on stimulated inflammatory cytokine production in white blood cells.	Infants with a high genetic risk for type 1 diabetes (T1D). 41 infants.	Group A mothers were randomized to receive DHA (800 mg/d) or corn/soy oil (800 mg/d) in the last trimester of pregnancy and continued on this same dose after delivery if breast-feeding. Formula fed infants received formula with 10.2 mg DHA/ounce (treatment) or 3.4 mg DHA/ounce (control). Formula-fed infants and infants of breast-feeding mothers in Group B (57 infants) were randomized in the first five postnatal months to receive similar dosages of DHA or corn/soy oil as their counterparts in Group A. 36 months.	Inflammatory markers, measured at 6, 12, 18, 24, 30 and 36 months: No effect on IL-6 and IL-10. There were no statistically significant reductions in production of the inflammatory cytokines, IL-1 β , TNF α , or IL-12p40 at any of the six time points measured. The inflammatory marker, high-sensitivity C-reactive protein (hs-CRP), was significantly lower in breast-fed DHA-treated infants compared to all formula-fed infants at the age of 12 months.	Inflammatory markers: 2.

Reference	Aim	Participant characteristics, n, age	Dose and duration	Possible adverse endpoints addressed/ results	RoB (tier)
Cornu et al. (2018)	Investigate the effect of omega-3 supplements on ADHD.	Children with established diagnosis of ADHD. N=148, aged 6–15 years.	Children aged 6–8 years, EPA 336 mg and DHA 84 mg; for children aged 9–11 years, EPA 504 mg and DHA 126 mg, and for children aged 12–15 years EPA 672 mg and DHA 168 mg. Study duration was 3 months.	Eleven (14.9%) children in the DHA–EPA group experienced 13 adverse events, 2 of which were judged to be related to the study treatment by the study authors: hip pain, fatigue, headache, fever and cough (n = 2), dermatitis and allergic skin reactions. (n = 2), abdominal pain, diarrhoea (n = 3), and depression. Eight children (10.7%) in the placebo group experienced 10 adverse events: fatigue, influenza (n = 2), abdominal pain, dermatitis, swollen eyes, vomiting, and diarrhoea (n = 3). Two patients in the DHA–EPA group and none in the placebo group experienced a severe adverse event (hospitalisation for worsening ADHD symptoms).	Gastrointestinal effects: 2. Headache: 2. Joint, lumbar and muscle pain: 2. Skin problems, including allergic reactions: 4.
Crippa et al. (2019)	Investigate the efficacy of DHA dietary	Drug-naïve children with attention-	500 mg DHA per day. 6 months.	Other side effects: no instances of either major or small adverse events were reported.	Other side effects: 2.

Reference	Aim	Participant characteristics, n, age	Dose and duration	Possible adverse endpoints addressed/ results	RoB (tier)
	supplementation on behavior and cognition in school-aged, drug-naïve children with attention-deficit/hyperactivity disorder.	deficit/hyperactivity disorder. N=50, aged 7-14 years.			
de Ferranti et al. (2014)	Evaluate the effect of omega-3 fatty acid supplements on triglycerides in hypertriglyceridemic adolescents.	Hypertriglyceridemic adolescents. N=25, aged 10-19 years.	~3360 mg DHA + EPA per day vs placebo.	<ul style="list-style-type: none"> • Inflammatory markers: no effect on CRP • Joint, lumbar and muscle pain: no effect • Skin problem: no effect • Gastrointestinal effects; no effect • Lipid profile; LDL levels increased significantly in the omega-3 FA group by 14 ± 6 mg/dL ($p=0.02$) at 3 months but not at 6 months (7 ± 8 mg/dl; $p=0.37$) and were unchanged in the placebo group at both timepoints. Again, when the change from baseline to 3 months or 6 months was compared between groups, there was no 	Gastrointestinal effects: 2. Glucose/insulin: 2. Inflammatory markers: 2. Joint, lumbar and muscle pain: 2. Lipid profile: 2. Skin problem: 2.

Reference	Aim	Participant characteristics, n, age	Dose and duration	Possible adverse endpoints addressed/ results	RoB (tier)
				<p>significant difference in LDL change. VLDL cholesterol decreased significantly in the treatment group at 6 months (p=0.04). No significant changes were seen in any of the other lipid parameters within or between groups at any other time points.</p> <ul style="list-style-type: none"> Fasting glucose and insulin: no effect. 	
Engler et al. (2004)	To determine whether supplementation with DHA affects endothelial function in children with familial combined hyperlipidemia.	<p>Children with familial combined hyperlipidemia.</p> <p>N=20, aged 9-19 years.</p>	<p>DHA 1.2 g/day.</p> <p>6 weeks.</p>	<p>Inflammatory markers: No effect on CRP.</p> <p>Lipid profile: DHA supplementation was associated with increased levels of total cholesterol, LDL- and HDL cholesterol concentrations.</p> <p>Oxidative stress: No effect on biomarkers for oxidative stress (F2 isoprostanes or 8-OH-2-dG).</p>	<p>Inflammatory markers: 2.</p> <p>Lipid profile: 2.</p> <p>Oxidative stress: 2.</p>
Janczyk et al. (2015)	Assess the efficacy of omega-3 LC-PUFAs in children	Children with nonalcoholic fatty liver disease.	<p>Dose:</p> <ul style="list-style-type: none"> Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day 	<ul style="list-style-type: none"> Inflammatory markers: no effect Lipid profile: no effect 	Glucose/insulin: 2.

Reference	Aim	Participant characteristics, n, age	Dose and duration	Possible adverse endpoints addressed/ results	RoB (tier)
	with nonalcoholic fatty liver disease.	64 completed the trial. Median age was 13 years.	- Body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day - Body weight >60 kg: 800 mg DHA and 532.5 mg EPA per day. Study duration was 24 weeks.	- Fasting glucose and insulin: no effect - Liver effects: no differences in intervention and placebo in effect on ALT. AST levels was significantly lower in the intervention group.	Inflammatory markers: 2. Lipid profile: 2. Liver effects: 2.
Mazahery et al. (2019)	Evaluate the efficacy of vitamin D (VID), omega-3 long chain polyunsaturated fatty acids (omega-3 LCPUFA, OM), or both (VIDOM) on core symptoms of autism spectrum disorder.	Children with autism spectrum disorder. N=73, aged 2.5 to 8 years.	722 mg DHA. Study duration = 12 months.	Events reported over the study period by the OM and the placebo group: Headache: 2 (OM); 0 (placebo). Allergic skin reactions: 5 (OM); placebo (0). Gastrointestinal effects: 10 (OM); 8 (placebo). Nose-bleeding: 1 (OM); 0 (placebo). Other side effects: 5 (OM); 3 (placebo).	Allergic skin reactions: 2. Bleeding: 2. Gastrointestinal effects: 2. Headache: 2. Other side effects: 2.

Reference	Aim	Participant characteristics, n, age	Dose and duration	Possible adverse endpoints addressed/ results	RoB (tier)
Montgomery et al. (2018)	Replication of the 2012 DOLAB 1 study findings that a dietary supplementation with the long-chain omega-3 DHA had beneficial effects on the reading, working memory, and behavior of healthy school children.	Healthy school children. N=185 (intervention) and 187 (placebo), aged 7 to 9 years.	600 mg/day DHA, placebo: taste/color matched corn/soybean oil. Study duration: 16 weeks.	Gastrointestinal effects: no effect. Headache: no effect.	Gastrointestinal effects: 1.
Pacifico et al. (2015)	Investigate whether treatment with DHA improves hepatic fat and other fat depots, and their associated CVD risk factors.	Children with biopsy-proven nonalcoholic fatty liver disease. 58 participants randomized, 51 (25 DHA, 26 placebo) completed the study. Age at study start was 10.8 (2.8) years for placebo and 11.0 (2.6) years for intervention.	DHA: 250 mg/day. 6 months.	<ul style="list-style-type: none"> Inflammatory markers: hs-CRP, no effect. Glucose: no effect. Insulin: significantly reduced fasting insulin. Lipid profile: No effect on HDL or total cholesterol. Liver effects: ALT changes – p=0.06. No side effects were reported. 	Glucose/insulin: 1. Inflammatory markers: 1. Liver effects: 1. Other side effects: 2.

Reference	Aim	Participant characteristics, n, age	Dose and duration	Possible adverse endpoints addressed/ results	RoB (tier)
Rodriguez et al. (2018)	Analyze the influence of n-3 long chain-PUFA intake on gene expression and blood inflammatory markers.	Boys with Duchenne Muscular Dystrophy. 36 participants, between 3 and 18 years (inclusion criteria).	450 mg EPA + 2250 mg DHA + 200 mg of additional omega 3 fatty acids per day. 6 months.	Inflammatory markers: Omega-3 LC-PUFA intake decreased the serum IL-1 β (-59.5%; p -0.011) and IL-6 (-54.8%; p -0.041), and increased the serum IL-10 (99.9%, p<0.005), in relation to those with placebo treatment.	Inflammatory markers: 1.
Rodriguez et al. (2019)	Analyze the impact of n-3 LCPUFA consumption on lean mass, fat mass, hyperinsulinemia, and insulin resistance (IR).	Children with Duchenne Muscular Dystrophy. 28 participants completed the trial. Age: 8.4 \pm 2.3 years (placebo) and 6.96 \pm 2.5 years (intervention).	450 mg EPA + 2250 mg DHA + 200 mg of additional omega 3 fatty acids per day. 6 months.	Fasting insulin, percentage of boys with hyperinsulinemia, and IR were similar between the placebo and n-3 LCPUFA groups during the 6 months of supplementation. The percentage of boys with IR was significantly (p -0.045) lower at month 6 of supplementation in the n-3 LCPUFA group than in the placebo group.	Glucose/insulin: 1.
Smuts et al. (2015)	Investigate the effects of Fe and DHA + EPA supplementation, alone or in combination, on	Healthy Fe-deficient school children. 98 participants, 6-11 years.	420 mg DHA + 80 mg EPA. 8.5 months.	Inflammatory markers: no effects on CRP (baseline: 0.57 (0.00-10.30); completed study: 0.70 (0.00-7.32).	Inflammatory markers: 1.

Reference	Aim	Participant characteristics, n, age	Dose and duration	Possible adverse endpoints addressed/ results	RoB (tier)
	physical activity during school days and on teacher-rated behaviour.				
Verduci et al. (2014)	Explore the effect size of different dietary long chain polyunsaturated supplementations on blood lipid profile.	Children with primary hyperlipidemia. 36 participants, 8-13 years.	After an 8-week stabilization period on the Step I diet, participants were randomized to additionally receive for a 16-week period one capsule (500 mg) daily of DHA alone or a DHA plus EPA mixture (45.6% DHA; 41.6% EPA) or wheat germ oil (control).	Lipid profile; An effect size (as percentage change from baseline) of +8%, -12% and -16% for high-density lipoprotein cholesterol (HDL-C), total cholesterol/HDL-C ratio and triglycerides was observed in children supplemented with DHA, compared to +2%, -8% and -12%, respectively, in children supplemented with DHA plus EPA.	Lipid profile: 1.

4.4.4 Evidence synthesis

Twenty possible adverse outcomes are included in the evidence synthesis (Section 4.4.4.1 to 4.4.4.20). VKM considered bleeding, glucose/insulin homeostasis, inflammation, lipid homeostasis, and liver effects to be the most important outcomes. For the systematic reviews, the certainty in the evidence was evaluated by the systematic review authors. For the RCTs, VKM assessed the certainty in the evidence according to OHAT (2019). Two persons independently evaluated the certainty in evidence for each outcome. A short description of the method

is available in Section 10.2.4 (Appendix).

Table 4.4.4-1. Summary of findings for the different lines of evidence for each outcome.

Included literature	Participants (n and age for children and adolescents)	Intervention	Duration	Outcome	VKM conclusions on dose, effect and certainty in the evidence
Age-related macular degeneration					
1 systematic review Abdelhamid et al. (2020)	Adults	350 mg DHA + 650 mg EPA	5 years.	RR 0.96 (95% CI 0.90 to 1.02); 1 trial, 4,203 participants.	Daily doses of 650 mg EPA and 350 mg DHA for five years did not affect the progress of age-related macular degeneration in adults. Since there is only data on adults and certainty in evidence have not been assessed, the findings will only be used as supporting information. VKM considers that this outcome is of minor relevance for the children and adolescents.
Bleeding					
2 systematic reviews Abdelhamid et al. (2020); Xin et al. (2013) *data from the key	Adults	EPA+DHA: 0.84 - 3 g/day. EPA: 1.8 - 3.99 g/day.	EPA+DHA: 1-6 years. EPA: about 5 years.	No (small).	There were no data on children or adolescents. There is insufficient evidence to conclude whether exposure to EPA and DHA or EPA alone constitute a risk of spontaneous bleeding or the size of the effect in adults. VKM considers the outcome to be of relevance for children and adolescents.

study reported					
1 RCT Mazahery et al. (2019)	2.5 to 8-year-olds, n=73.	DHA: 722 mg/day.	12 months.	Intervention: 1 event (nose-bleeding); 0 placebo.	No studies on DHA+EPA were included. There is very-low certainty evidence that 722 mg of DHA for 12 months causes no or very little increased risk for nose-bleed for children aged 2.5 to 8 years. No studies on EPA alone were included.
2 risk assessments (EFSA, 2012; VKM, 2015)					The findings on bleeding from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.
Fasting glucose and insulin, and/or insulin resistance					
4 RCTs de Ferranti et al. (2014); Janczyk et al. (2015); Pacifico et al. (2015); Rodriguez et al. (2019)	N=25, aged 10-19 years. N=64, median age 13 years. N=58, aged 10.8 (2.8) years (placebo) and 11.0 (2.6) years (intervention).	DHA+EPA: 3360 mg/day. DHA+EPA: Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day; body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day; body weight >60 kg: 800 mg	3 months. 24 weeks. 6 months. 6 months.	No effect on fasting glucose and insulin. No effect on fasting glucose and insulin. No effect on fasting glucose. Significantly reduced fasting insulin. Fasting insulin, percentage of boys with hyperinsulinemia, and insulin resistance were similar between the placebo and n-3 LCPUFA groups during the 6 months of supplementation. The percentage of boys with IR was significantly (p -0.045) lower at month 6 of supplementation in	There is moderate certainty evidence that doses up to 450 mg EPA and 2250 mg DHA combined for up to six months do not cause negative effects on glucose and insulin homeostasis in children and adolescents aged 6-18 years. There is low certainty evidence that doses of 250 mg DHA for six months do not cause negative effects on glucose and insulin homeostasis in children and adolescents aged 8-13 years. No studies on EPA alone were included.

	N=28, aged 8.4±2.3 years (placebo) and 6.96±2.5 years (intervention).	DHA and 532.5 mg EPA per day. DHA: 250 mg/day. DHA+EPA: 2250+45 mg/day.		the n-3 LCPUFA group than in the placebo group.	
Gastrointestinal effects					
6 systematic reviews Abdelhamid et al. (2020); Downie et al. (2019); Irving et al. (2006); Newberry et al (2016); Sarmiento et al. (2016); Watson and Stackhouse (2020)	Adults	DHA+EPA: up to 5.5 g/day. DHA: 1 g/day. EPA: up to 4 g/day.	Up to 5.5 years. 18 months. Up to 5 years.	Yes/minor. Serious gastrointestinal events: RR 1.34, 95% CI 0.64 to 2.80 (3 trials). Increased abdominal pain or discomfort: RR 1.05, 95% CI 0.91 to 1.20 (9 trials). Diarrhoea: RR 1.02, 95% CI 0.87 to 1.19 (13 trials). RR 2.04 CI 0.91 to 4.54 (2 trials). OR 0.6, 95% CI 0.05 to 6.79 (1 trial). Nausea: RR 1.20, 95% CI 0.96 to 1.49 (8 trials). Any gastrointestinal side effect: RR 1.10, 95% CI 0.97 to 1.26 33 trials). RR 0.78, 95% CI 0.32 to 1.89 (2 trials).	There is insufficient evidence to conclude whether EPA and DHA in combination or alone increases the risk for gastrointestinal effects. VKM considers that potential gastrointestinal effects following exposure to EPA and DHA in combination or alone are minor and the symptoms are mild.

4 RCTs Cornu et al. (2018); de Ferranti et al. (2014); Mazahery et al. (2019); Montgomery et al. (2018)	N=148, aged 6-15 years.	EPA+DHA: 336+84 mg/day for 6-8-year-olds; 504+126 mg/day for 9-11-year-olds; 672+168 for 12-15-year-olds.	3 months.	Similar effects reported for intervention and control.	There is low certainty evidence that doses up to 1800 mg EPA and 1500 mg DHA for three months do not significantly cause gastrointestinal effects different from that of placebo. There is low certainty evidence that doses up to 722 mg DHA for 12 months do not significantly cause gastrointestinal effects different from that of placebo. No studies on EPA alone were included.
	N=25, aged 10-19 years.		3 months.	No effects reported.	
	N=73, aged 2.5-8 years.	DHA+EPA: 3360 mg/day.	12 months.	Intervention: 10 events; placebo: 8 events.	
	N= 372, aged 7-9 years.	DHA: 722 mg/day. DHA: 600 mg/day.	16 weeks.	No effects reported.	
Glucose and lipid homeostasis					
2 risk assessments (EFSA, 2012; VKM 2015)					The findings on glucose and lipid homeostasis from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.
Haemorrhagic stroke					
1 systematic review Abdelhamid et al. (2020)	Adults	DHA+EPA: up to 3.4 g/day. EPA: Up to 4 g/day.	Up to 5 years.	Yes: RR 1.23, 95% CI 0.93 to 1.64; I ² =0, 10 trials, n=70,695.	There were no data on children and adolescents. It is not possible to conclude the effects of EPA and DHA or EPA alone on haemorrhagic stroke in adults since all studies irrespective of intervention are summarised together. There were no studies with exposure

					to DHA alone. VKM considers this outcome to be of relevance for children.
Headache and worsening migraine					
1 systematic review Abdelhamid et al. (2020)	Adults	EPA+DHA: up to 3 g/day.	1 year.	No: RR 0.85, 95% CI 0.51 to 1.40; I ² = 0%; 4 trials, 1526 participants, 60 events.	There were no data on children or adolescents. Data on adults suggested that 2.7 g EPA and 0.8 g DHA for 12 months did not cause headache or worsening of migraine. However, due to lack of ADME data and lack of certainty in evidence assessment, the findings will only be used as supporting information. VKM considers the outcome to be of relevance for children and adolescents.
2 RCTs Cornu et al. (2018); Mazahery et al. (2019)	N=148, 6-15 years. N=73, 2.5-8 years.	EPA+DHA: 336+84 mg/day for 6-8-year-olds; 504+126 mg/day for 9-11-year-olds; 672+168 for 12-15-year-olds. DHA: 722 mg/day.	3 months. 12 months.	One participant in the intervention group reported headache, no reported headache in the control group. Two events in the intervention group, none in the placebo group.	There is very-low certainty evidence that daily doses of 336 mg EPA and 84 mg DHA for three months do not or only to a small extent increase the risk of headache in children aged 6-8 years. There is very-low certainty evidence that a daily dose 722 mg DHA for 12 months do not or only to a small extent increase the risk of headache in children aged 2.5-8 years. No studies on EPA alone were included.
Inflammatory markers					
2 systematic reviews Mocellin et al. (2016);	Adults	DHA+EPA: up to 4.4 g/day. EPA: 1.8 g/day.	Up to 3 months. 7 weeks.	No effect on CRP: 4 trials; n=166; Effect sizes (95% CI): -0.0164 (-0.3215; 0.2887), p= 0.9162.	VKM considers the body of evidence sufficient to conclude on EPA and DHA combined exposure, but insufficient for EPA alone due to no possibility to separate the effects from those of EPA and DHA alone and due to low number

Su et al. (2021)					of studies. There were no studies on exposure to DHA alone. Data indicate that exposure to EPA and DHA combined do not cause inflammation in adults. However, as we lack ADME data and no assessment of certainty in evidence were performed, the data will only be used as supporting information.
8 RCTs Chang et al. (2019); Chase et al. (2015); de Ferranti et al. (2014); Engler et al. (2004); Janczyk et al. (2015); Pacifico et al. (2015); Rodriguez et al. (2018); Smuts et al. (2015)	N=92, 6-18 years. N=41, infants. N=25, 10-19 years. N=20, 9-19 years. N=64, median age was 13 years. N=58, aged 10.8 (2.8) years (placebo) and 11.0 (2.6) years (intervention).	EPA: 1.2 g/day. During last trimester, formula fed. DHA+EPA: 3360 mg/day. DHA: 1.2 g/day. Dose: Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day; body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day; body weight >60 kg: 800 mg	12 weeks. 5 postnatal months. 3 months. 6 weeks. 24 weeks. 6 months. 6 months. 8.5 months.	No effect on hs-CRP. Hs-CRP was significantly lower in breast-fed DHA-treated infants compared to all formula-fed infants at the age of 12 months. No effects on CRP. No effects reported. No effect on hs-CRP. Omega-3 LC-PUFA intake decreased the serum IL-1 β (-59.5%; p -0.011) and IL-6 (-54.8%; p -0.041), and increased the serum IL-10 (99.9%, p<0.005), in relation to those with placebo treatment. No effects on CRP (baseline: 0.57 (0.00-10.30); completed study: 0.70 (0.00-7.32).	There is very-low certainty evidence that a daily dose of 1.2 g EPA for 12 weeks do not cause inflammation in children aged 6-18 years. There is moderate certainty evidence that a daily dose of 1.2 g DHA for six weeks and 250 mg DHA for six months do not cause inflammation in infants <5 months and children aged 8-18 years. There is moderate certainty evidence that daily doses of 1800 mg EPA + 1500 mg DHA for three months and doses up to 450 EPA + 2250 DHA for six months do not cause inflammation in children aged 3-18 years.

	N=36, 3-18 years. N=98, 6-11 years.	DHA and 532.5 mg EPA per day. DHA: 250 mg/day. DHA+EPA: 2250 + 450 mg/day. DHA+EPA: 420 + 80 mg/day.			
2 risk assessments (EFSA, 2012; VKM, 2015)					The findings on inflammatory markers from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.
Joint, lumbar and muscle pain					
2 systematic reviews Abdelhamid et al. (2020); Irving et al. (2006)	Adults	DHA+EPA: 1+2 g/day. EPA: up to 4 g/day.	12 months. Up to 5 years.	Little or no effect. One systematic review reported RR 0.95, 95% CI 0.74 to 1.23; I ² =61.51% > 27,000 participants. The other reported that no differences were apparent for any dose of E-EPA supplementation compared with placebo.	There were no data on children and adolescents. There is insufficient evidence to conclude whether exposure the combination of EPA and DHA constitute a risk of joint, lumbar and muscle pain. Data on adults suggested that doses of EPA up to 3.99 g for up to five years do not cause joint, lumbar or muscle pain. However, due to lack of ADME data and lack of certainty in evidence assessment, the findings will only be used as supporting information. VKM considers the outcome to be of relevance for children and adolescents.

<p>2 RCTs</p> <p>Cornu et al. (2018); de Ferranti et al. (2014)</p>	<p>N=148, 6-15 years.</p> <p>N=25, 10-19 years.</p>	<p>EPA+DHA: 336+84 mg/day for 6-8-year-olds; 504+126 mg/day for 9-11-year-olds; 672+168 for 12-15-year-olds.</p> <p>DHA+EPA: 3360 mg/day.</p>	<p>3 months.</p> <p>3 months.</p>	<p>One participant in the intervention group reported hip pain, no such effects reported in the control group.</p> <p>No effects reported on back pain, muscle or joint ache.</p>	<p>There is low certainty evidence that daily doses up to 1800 mg EPA and 1500 mg DHA for three months do not cause or to a minor extent cause joint, lumbar or muscle pain in children aged 6-18 years.</p> <p>No studies on DHA alone were included.</p> <p>No studies on EPA alone were included.</p>
Blood lipid profile					
<p>2 systematic reviews</p> <p>Abdelhamid et al. (2020); Su et al. (2021)</p>	<p>Adults</p>	<p>DHA+EPA: up to 6 g/day.</p> <p>EPA: up to 4 g/day.</p> <p>DHA: 1.2 g/day.</p>	<p>Up to 7.4 years.</p>	<p>Systematic review 1: GRADE assessment suggests high certainty evidence that LCn3intake makes little or no difference to serum total cholesterol. Mean difference (random, 95% CI): -0.01, 95% CI -0.05 to 0.03; I² = 14.34%; 30 trials, 35,000> participants.</p> <p>GRADE assessment suggests high certainty evidence that LCn3intake has little or no effect on HDL cholesterol. Mean difference (random, 95% CI): 0.03, 95% CI -0.01 to 0.05; I² = 50.41%; 30 trials, 40,000> participants.</p> <p>GRADE assessment suggests high certainty evidence that LCn3 intake makes little or no difference to LDL</p>	<p>It is not possible to conclude on the effects of EPA and DHA or EPA alone on lipid metabolism in adults since all studies irrespective of intervention are summarised together.</p>

				<p>cholesterol. Mean difference (random, 95% CI): 0.01, 95% CI -0.01 to 0.03; I² = 0%; 25 trials, 40,000> participants.</p> <p>Systematic review 2: No significant association was found for n-3 PUFAs and HDL or LDL. The grade certainty was moderate.</p>	
<p>4 RCTs</p> <p>de Ferranti et al. (2014); Engler et al. (2004); Janczyk et al. (2015); Verduci et al. (2014)</p>	<p>N=25, 10-19 years.</p> <p>N=20, 9-19 years.</p> <p>N=64, mean age was 13 years.</p> <p>N=36, 8-13 years.</p>	<p>DHA+EPA: 3360 mg/day.</p> <p>DHA: 1.2 g/day.</p> <p>Dose: Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day; body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day; body weight >60 kg: 800 mg DHA and 532.5 mg EPA per day. Study duration was 24 weeks.</p>	<p>3 months.</p> <p>6 weeks.</p> <p>24 weeks.</p> <p>16 weeks.</p>	<p>LDL levels increased significantly in the omega-3 FA group by 14 ± 6 mg/dL (p=0.02) at 3 months but not at 6 months (7 ± 8 mg/dl; p=0.37) and were unchanged in the placebo group at both timepoints.</p> <p>DHA supplementation was associated with increased levels of total cholesterol, LDL- and HDL cholesterol concentrations.</p> <p>No effects were reported.</p> <p>An effect size (as percentage change from baseline) of +8%, -12% and -16% for high-density lipoprotein cholesterol (HDL-C), total cholesterol/HDL-C ratio and triglycerides was observed in children supplemented with DHA, compared to +2%, -8% and -12%,</p>	<p>There is very-low certainty evidence that daily doses up to 1800 mg EPA and 1500 mg DHA for three months causes a small increase in LDL in children and adolescents aged 8-18 years.</p> <p>There is low certainty evidence that a daily dose of 1.2 g DHA for six weeks and 500 mg DHA for 16 weeks causes a small increase in LDL in children and adolescents aged 8-18 years.</p> <p>No studies on EPA alone were included.</p>

		DHA: 500 mg/day or DHA+EPA: 500 mg/day.		respectively, in children supplemented with DHA plus EPA.	
1 risk assessment (EFSA, 2012)					The findings on lipid profile from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.
Liver and biliary tract effects					
1 systematic review Irving et al. (2006)	Adults	E-EPA: up to 4 g/day.	12 weeks.	No differences were apparent for any dose of E-EPA supplementation compared with placebo.	There were no data on children and adolescents. There is insufficient evidence to conclude whether EPA alone increases the risk for liver and biliary effects. No studies on EPA+DHA or DHA alone were included. VKM considers this outcome of relevance for children and adolescents.
2 RCTs Janczyk et al. (2015); Pacifico et al. (2015)	N=64, mean age was 13 years. N=58, mean age about 11 years.	Dose: Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day; body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day; body weight >60 kg: 800 mg DHA and 532.5 mg EPA per day.	24 weeks. 6 months.	No differences in intervention and placebo in effect on ALT. ALT changes – p=0.06.	There is very-low certainty evidence that daily doses of DHA 267-800 mg and 177.5-532.5 mg EPA for 24 weeks do not cause adverse liver effects in children and adolescents (median age of 13 years). There is low certainty evidence that a daily dose of 250 mg DHA for six months do not cause adverse liver effects in children aged 8-13 years. No studies on EPA alone were included.

		DHA: 250 mg/day.			
Metabolic and nutritional effects (e.g. weight gain)					
1 systematic review Irving et al. (2006)	Adults	E-EPA: up to 4 g/day.	12 weeks.	No differences were apparent for any dose of E-EPA supplementation compared with placebo.	There were not data on children and adolescents. There is insufficient evidence to conclude whether exposure to E-EPA constitute a risk for metabolic effects in adults. No studies on DHA+EPA or DHA alone were included. It is unclear what the study authors have measured except for weight gain, and therefore the findings are of limited value.
Movement disorders					
1 systematic review Irving et al. (2006)	Adults	E-EPA: 500 mg/day.	16 weeks.	No	There were no data on children and adolescents. There is insufficient evidence to conclude whether exposure to E-EPA constitute a risk for movement disorders in adults. No studies on DHA+EPA or DHA alone were included. VKM considers this outcome as relevant for children and adolescents.
Other side effects					
2 systematic reviews Sarmiento et al. (2016); Irving et al. (2006)	Adults	DHA+EPA: 0.7+1.0 g/day. E-EPA: up to 4 g/day.	12 weeks. 12 weeks.	Systematic review 1: There were a few reports of other adverse events, all in one study. In the PUFA group there was one case of sleepiness, one participant complained of fatigue and breathlessness and one had recurrence of depression and paranoia. In the placebo group there were two cases of	It is not possible to conclude for adults regarding other side effects, as the data are too scarce.

				<p>sleepiness and one case of aggression and fatigue.</p> <p>Systematic review 2: Psychosexual difficulties, infections: No statistically significant differences were found, however it appears as though there is an effect. Based on one short-term study only.</p>	
3 RCTs	<p>N=73, 2.5-8 years.</p> <p>N=58, mean age about 11 years.</p>	<p>DHA: 722 mg/day.</p> <p>DHA: 250 mg/day.</p>	<p>12 months.</p> <p>6 months.</p>	<p>Five events were reported in the intervention group, 3 in the placebo group.</p> <p>No other side effects reported.</p>	<p>No studies on DHA+EPA alone were included.</p> <p>No studies on EPA alone were included.</p> <p>There is low certainty evidence that daily doses of DHA up to 722 mg for up to 12 months causes little or no side effects for children aged 2.5-14 years.</p>
Oxidative stress/lipid peroxidation					
1 RCT	N=20, 9-19 years.	DHA: 1.2 g/day.	6 months.	No effect on biomarkers for oxidative stress (F2 isoprostanes or 8-OH-2-dG).	<p>No studies on DHA+EPA were included.</p> <p>There is very-low certainty evidence that a daily dose of 1.2 g DHA for six weeks do not cause oxidative stress in children and adolescents aged 9-18 years.</p> <p>No studies on EPA alone were included.</p>
2 risk assessments (EFSA, 2012); VKM, 2015)					The findings on lipid peroxidation from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.

Preterm birth					
2 systematic reviews Newberry et al. (2016); Ren et al. (2021)	Pregnant and infants	<p>Pregnant women: Supplementation and/or diet type EPA, DHA, ALA.</p> <p>Children: route of delivery: via umbilical cord & placenta, mother's milk, child's supplementation (e.g., formula) and/or diet, and with vs without mother's intake via diet and/or supplementation during pregnancy and/or breastfeeding.</p> <p>Most of the studies used both DHA and EPA as supplements.</p>	<p>During pregnancy vs post-delivery.</p> <p>During pregnancy.</p>	<p>Gestational length and risk for preterm birth: Prenatal DHA or DHA-enriched fish oil supplementation had a small positive effect on length of gestation (moderate SoE), but no effect on risk for preterm birth (low SoE)/ Prenatal EPA plus DHA-containing fish oil supplementation has no effect on length of gestation (low SoE). Supplementation with DHA, or EPA plus DHA-, or DHA-enriched fish oil does not decrease risk for preterm birth (low SoE). Birth weight and risk for low birth weight: Changes in maternal n-3 FA biomarkers were significantly associated with birth weight./ Prenatal algal DHA or DHA-enriched fish oil supplementation had a positive effect on birth weight among healthy term infants (moderate SoE), but prenatal DHA supplementation had no effect on risk for low birth weight (low SoE). Prenatal EPA plus DHA or alpha-linolenic acid (ALA) supplementation had no effect on birth weight (low SoE). Fetal, Infant, and Child Exposures and Outcomes, adverse events: Prenatal and infant supplementation with n-3 FA</p>	<p>There were no data on EPA alone. Data from RCTs indicated no effect on preterm birth for both intake of DHA alone and EPA+DHA. The certainty of the evidence was low.</p>

		Seven studies used DHA as the single supplement. The majority of the trials used fish oil.		<p>or fortification of food with n-3 FA did not result in any serious or nonserious adverse events (moderate SoE); with the exception of an increased risk for mild gastrointestinal symptoms.</p> <p>The meta-analysis showed significant overall higher birth weight in the n-3 LCPUFA-supplemented compared to the control groups. The overall quality of the meta-analysis results for birth weight was rated as moderate quality.</p>	
Prostate cancer					
2 systematic reviews					
Chua et al. (2012); Fu et al. (2015)	-	-	-	-	No conclusion as outcome is to be of minor relevance for children and adolescents.
Pulmonary embolus or deep vein thrombosis					
1 systematic review	Adults	<p>DHA+EPA: up to 3.5 g/day.</p> <p>DHA: about 1 g/day.</p> <p>EPA: 0.5 g/day.</p>	Up to 3 years.	<p>Pulmonary embolus or DVT (RR 1.15, 95% CI 0.44 to 2.98; I² =0%; 5 trials, > 3000 participants, 20 events). Assessed as being very-low certainty evidence (GRADE), so the effect of LCn3 on pulmonary embolus or DVT is unclear.</p>	There is insufficient evidence to conclude whether EPA and DHA in combination or alone increases may have an effect on pulmonary embolus.
Reflux					

1 systematic review Abdelhamid et al. (2020)	Adults	DHA+EPA: up to 3 g/day. EPA: 4 g/day.	Up to 4.9 years	Reflux: there were limited data (RR 1.23, 95% CI 0.79 to 1.91; I ² =32%; 3 trials, > 8000 participants, 282 events).	There is insufficient evidence to conclude whether EPA and DHA in combination or alone increases may have an effect on reflux.
Skin problems					
1 systematic review Abdelhamid et al. (2020)	Adults	DHA+EPA: up to 3.36 g/day. EPA: 1.8 g/day.	Up to 7.4 years	Skin problems, including itching or rashes: these were not affected by LCn3 in a meta-analysis with high heterogeneity (RR1.11, 95% CI 0.52 to 2.37; I ² = 68%; 9 trials, > 36,000 participants, 293 events).	There is low certainty evidence that EPA+DHA and EPA alone does not cause skin problems.
3 RCTs Cornu et al. (2018); de Ferranti et al. (2014); Mazahery et al. (2019)	N=148, 6-15 years. N=25, 10-19 years. N=73, 2.5-8 years.	EPA+DHA: 336+84 mg/day for 6-8-year-olds; 504+126 mg/day for 9-11-year-olds; 672+168 for 12-15-year-olds. DHA+EPA: 3360 mg/day. DHA: 722 mg/day.	3 months. 3 months. 12 months.	One participant in the intervention group and one in the control group reported dermatitis as an effect. No effects reported. Five events reported by the participants in the intervention group, none in the placebo group.	There is very-low certainty evidence that daily doses of 1800 mg EPA + 1500 mg DHA for three months do not cause skin problems in children aged 6-18 years. There is very-low certainty evidence that a daily dose 722 mg DHA for 12 months do not or only to a small extent increase the risk of headache in children aged 2.5-8 years. No studies on EPA alone were included.

4.4.4.1 Age-related macular degeneration

One systematic review addressing age-related macular degeneration was included (Table 4.4.4.1-1).

Table 4.4.4.1-1. Systematic review addressing age-related macular degeneration.

Reference	Study/ intervention	Results
Abdelhamid et al. (2020)	AREDS2 2014: 5 years. RCT with parallel design. People aged 50-85 years at high risk of progression to advanced age-related macular degeneration. N: 2147 intervention (1068 DHA/EPA, 1079 DHA/EPA + lutein/zeaxanthin), 2056 control (1012 placebo, 1044 lutein/zeaxanthin). Intervention: 350 mg/day DHA plus 650 mg/day EPA added to the standard AREDS supplement of Vitamin C (500 mg/day), Vitamin E (440 IU/day), beta-carotene (15 mg/day), zinc oxide (80 mg/day) and cupric oxide (2 mg/day). Dose: 1 g/day EPA + DHA. Control: standard AREDS supplement. RoB: low.	Progression to advanced age-related macular degeneration (RR 0.96, 95% CI 0.90 to 1.02; 1 trial, 4,203 participants, 2049 events).
<p>VKM concludes: Daily doses of 650 mg EPA and 350 mg DHA for five years did not affect the progress of age-related macular degeneration in adults. Since there is only data on adults and certainty in evidence have not been assessed, the findings will only be used as supporting information. VKM considers that this outcome is of minor relevance for the children and adolescents.</p> <p>Summary: Data from one RCT with parallel design showed no effect following exposure to EPA+DHA for 60 months on the progression of age-related macular degeneration. The participants included were adults >50 years at high risk for the disease and can therefore be considered as a sensitive group for this outcome. Additionally, the long-term exposure, high number of participants and low risk of bias suggest that the finding is credible. However, certainty in evidence have not been assessed.</p>		

4.4.4.2 Bleeding

Systematic reviews, RCTs and risk assessments addressing bleeding were included (Table 4.4.4.2-1, 4.4.4.2-2 and 4.4.4.2-3).

Table 4.4.4.2-1. Systematic reviews addressing bleeding.

Reference	Study/ intervention	Results, certainty in the evidence
Abdelhamid et al. (2020)	<p><u>Studies with combined EPA and DHA exposure:</u></p> <p>Dream Asbell 2018. 12 months. RCT with parallel design. N: 349 intervention randomised, 186 control randomised. Intervention: omega-3 supplements (2000 mg EPA + 1000 mg DHA/day as 5 gel caps). Dose: 3.0 g/day LCn3. Control: olive oil supplements (5 gel caps). RoB: low.</p> <p>Afford 2013. 12 months. RCT with parallel design. N: 165 intervention, 172 control (Analysed, intervention: 153 control: 163). 4 × 1 g enteric-coated fish oil capsules/day (1.6 g/day EPA + 0.8 g/day DHA). Dose: 2.4 g/day EPA + DHA. Control: 4 × 1 g matching placebo capsules, 4 g/day safflower oil. RoB: moderate or high.</p> <p>EPIC-1 2008. 52 weeks. RCT with parallel design. N: 188 intervention, 186 control. Intervention: 2 × 2 1 g gelatin capsules omega-3 free FAs (2.2 g EPA, 0.8 g DHA). Dose: 3 g/day EPA + DHA. Control: 4 x1 g capsules medium-chain triglyceride oil. RoB: moderate or high.</p>	<p>Bleeding (RR 1.12, 95% CI 0.91 to 1.37; I² = 44%; 11 trials, > 80,000 participants, 1324 events). Assessed as being very-low certainty evidence, so the effect of LCn3 on bleeding is unclear.</p>

	<p>EPIC-2 2008. 58 weeks. RCT with parallel design. N: intervention, 189, control 190 (187 intervention, 188 control analysed). Intervention: 2 × 2 1 g gelatin capsules omega-3 free FAs providing total dose ~2.2 g/day EPA, 0.8 g/day DHA. Dose: ~3.0 g/day EPA + DHA. Control: 2 × 2 1 g capsules medium-chain triglyceride oil. RoB: moderate or high.</p> <p>Fostar 2016. 24 months. RCT with parallel design. N: 101 intervention, 101 control. Intervention: 1-3 × a day drink of fruit juice mixed with day total = 15 mL of fish oil supplement (18% EPA, 12% DHA, 4.5 g/day total omega-3). Dose: 4.5 g/day EPA + DHA. Control: liquid oral oil 15 mL Sunola oil/day (which contains fish oil 2 mL plus 13 mL canola oil) (total omega-3 fat: ≥ 0.45 g EPA + DHA from 15 mL). RoB: low.</p> <p>ORIGIN 2012. 72 months. RCT, 2x2 factorial. N: 6319 intervention, 6292 control (analysed, intervention: 6281 control: 6255). People at high risk of cardiovascular events with impaired fasting glucose, impaired glucose tolerance or diabetes. Intervention: 1 gelatin capsule/day Omacor containing at least 900 mg ethyl esters of n-3 fats (465 mg EPA + 375 mg DHA). Dose: 0.84 g/day EPA + DHA. Control: 1 × 1 g gelatin capsule/day olive oil. RoB: low.</p> <p>Risk and prevention 2013. 60 months. RCT with parallel design. N: 6244 intervention, 6269 control (analysed, intervention: 6239 control: 6266). Intervention: 1 g/day n-3 capsules polyunsaturated FA ethyl esters (EPA and DHA content 850-882 mg with an average ratio of 1.0:1.2). Dose: ~0.87 g/day</p>	
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	<p>EPA + DHA. Control: 1 g/day olive oil capsules. Rob: moderate or high.</p> <p>SHOT 1996. 1 year. RCT with parallel design. N: 317 intervention, 293 control. Intervention: 4 fish-oil concentrate soft gelatin capsules/day containing 51% EPA and 32% DHA ethyl esters and 3.7 mg vitamin E as an antioxidant. Dose: 3.3 g/day EPA + DHA. Control: no treatment. RoB: moderate or high.</p> <p>VITAL 2019. 5.3 years. RCT with parallel design 2x2. N: 12,933 intervention, 12,938 control (analysed intervention 12,933, control 12,938). Intervention: Arm 1: omega-3, 1 capsule/day, Omacor fish oil, EPA + DHA 840 mg/day: 465 mg EPA; 375 mg DHA provided in calendar packs and placebo D3. Arm 3: omega-3 as in Arm 1 and vitamin D3 (1/d, 2000 IU). Control: Arm 2: placebo omega-3 and vitamin D3 (1/d, 2,000IU). Arm 4: placebo omega-3 and placebo D3. Dose: 840 mg/day LCn3, or 0.38% E. RoB: low.</p> <p><u>Studies with EPA exposure:</u></p> <p>JELIS 2007. Maximum 5 years, mean 4.7 years. RCT with parallel design. N: intervention, 9326, control 9319 (analysed intervention 9326, control 9319). Intervention: 3 × 2 × 300 mg capsules/day EPA ethyl ester (total dose of 1.8 g/day EPA), after meals. Dose: 1.8 g/day EPA. Control: nothing (though all in both groups received "appropriate" dietary advice).</p>	
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	<p>RoB: moderate or high.</p> <p>REDUCE-IT 2019. 4.9 years (median). RCT with parallel design. N: intervention 4089 randomised, control 4090 randomised (analysed, intervention: 4083 control: 4077). Intervention: EPA ethyl ester derived from fish oil (AMR101 4 g/d, Amarin), 3.99 g/day EPA plus 8 mg/day vitamin E (2 capsules twice/day). Control: 3.73 g/day light liquid paraffin oil in 4 capsules (2 capsules twice a day). RoB: moderate or high.</p>	
Xin et al. (2013)	<p>Calo 2005. At least 5 days before surgery until discharge. N=160. Intervention: EPA: 0.58 g/day; DHA 1.16 g/day. Control: no treatment.</p> <p>Heidarsdottir 2010. 6 days (median) before surgery until discharge or 14 days after surgery. N=168. Intervention: EPA: 1.24 g/day; DHA 1.0 g/day. Control: olive oil.</p> <p>Farquharson 2011. 21 days before surgery until discharge or 6 days after surgery. N= 194. Intervention: EPA: 2.7 g/day; DHA 1.9 g/day. Control: sunola.</p> <p>Sandesara 2012. 2.5 days (median) before surgery until 14 days after surgery. N= 2430. Intervention: EPA: 1.07 g/day; DHA 0.86 g/day. Control: corn oil.</p> <p>Mozaffarian 2012. 2–5 days before surgery until discharge or 10 days after surgery. N= 1516. Intervention: EPA: 1.03 g/day; DHA 0.83 g/day. Control: Olive oil.</p>	<p>Major bleeding was defined as bleeding more than 3 L through chest tube drain, needing transfusion, or needing reexploration or reoperation. By pooling these studies (5 in total), no significant influence of fish oil on the incidence of major bleeding ($I^2= 39%$, fixed-effect model: RR = 0.82, 95% CI 0.59 to 1.55, p = 0.26; random-effect model: RR = 0.83, 95% CI 0.51 to 1.35, p = 0.45) were found.</p>

VKM concludes: There were no data on children or adolescents. There is insufficient evidence to conclude whether exposure to EPA and DHA or EPA alone constitute a risk of spontaneous bleeding or the size of the effect in adults. VKM considers the outcome to be of relevance for children and adolescents.

Summary: Two systematic reviews have summarised data on spontaneous bleeding following exposure to EPA and DHA. Abdelhamid et al. 2020 have summarized data on spontaneous bleeding following exposure to EPA+DHA and EPA alone in adults with increased risk for CVD. No trials with exposure to DHA alone were identified. Eleven trials which combined include more than 80 000 participants were identified (nine with EPA+DHA exposure and two with EPA alone). The doses tested varied from 0.84 g EPA+DHA to 3 g EPA+DHA and 1.8 to 3.99 g of EPA.

No significant effect of exposure to DHA and EPA and EPA alone on spontaneous bleeding was observed, however, the evidence was judged as very-low certainty evidence. Seven of the eleven trials had moderate to high risk of bias. The certainty in evidence were also downgraded due to the large confidence intervals. The authors noted that the 95% confidence intervals do not exclude large and important benefits or harms. It is stated that the effect size changed direction from harmful to protective, when only trials with low RoB were included in the analysis. VKM notes that the inconsistencies in direction of effect cannot be explained by study size, dose or duration of intervention. VKM considers that the findings are of limited value, as all studies, independent of intervention, were summarised together. Therefore, it is not possible assess the effect of EPA and DHA in combination or EPA alone, separately.

In Xin et al. 2013 the effect of EPA and DHA in combination on major bleeding (>3L) after surgery has been assessed. No significant effect was identified; however, the confidence interval is large.

Table 4.4.4.2-2. RCTs addressing bleeding in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results
Mazahery et al. (2019)	Children with autism spectrum disorder. N=73, aged 2.5 to 8 years. Dose: 722 mg DHA. Study duration = 12 months.	Tier 2	Nose-bleeding: 1 (OM); 0 (placebo)
Overall evaluation of certainty in the evidence on bleeding			
	Elements triggering downgrading	Elements triggering upgrading	Overall rating

Initial rating	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
DHA	Serious	Not evaluated	Not serious	Serious	No	No	Not evaluated	Very-low
1 RCT, initial rating ++++		Serious						
VKM concludes:								
<ul style="list-style-type: none"> • No studies on DHA+EPA were included. • There is very-low certainty evidence that 722 mg of DHA for 12 months causes no or very little increased risk for nose-bleed for children aged 2.5 to 8 years. • No studies on EPA alone were included. 								

Table 4.4.4.2-3. Risk assessments addressing bleeding.

Reference	Direct quotation from summary / conclusion in the risk assessment
EFSA, 2012	<p><i>"The Panel considers that supplemental intakes of EPA and DHA combined of up to about 5 g/day for up to two years and up to about 7 g/day for up to six months, do not increase the risk of spontaneous bleeding episodes or bleeding complications, even in subjects at high risk of bleeding (e.g. taking acetylsalicylic acid or anti-coagulants). The Panel notes that the data available are insufficient to conclude on whether the same doses administered mostly as EPA or mostly as DHA would have different effects on this outcome. The Panel considers that intakes of EPA alone at doses up to 1.8g/day for two years do not increase the risk of bleeding complications.</i></p> <p><i>The Panel notes that supplemental intakes of EPA and DHA combined of up to about 6 g/day do not enhance the effects of anti-platelet or antithrombotic medications on bleeding time, and that the changes in bleeding times within the normal range which have been observed in</i></p>

	<p><i>some intervention studies are not considered to be adverse as they were not associated with an increased risk of clinical complications (e.g. spontaneous bleeding).</i></p> <p><i>The Panel notes that the changes on platelet function which are observed at supplemental intakes of EPA and DHA (either alone or in combination) up to about 4 g/day are not considered to be adverse as they are not associated with an increased risk of clinical complications (e.g. spontaneous bleeding)."</i></p>
VKM, 2015	<p><i>"Increased tendency to bleed from the nose and urinary tract, and increased mortality from haemorrhagic stroke have been reported in observational studies of Greenlandic Eskimos (mean intakes of n-3 LCPUFAs about 6.5 g/day) as well as prolonged bleeding time and reduced platelet aggregation (Dyerberg and Bang, 1979). These studies were uncontrolled for factors other than the intake of n-3 LCPUFA.</i></p> <p><i>Bleeding complications were studied in an open label human intervention study (Yokoyama et al., 2007) cited in VKM (2011) and EFSA (2012), which investigated the effects of 1.8g/day of EPA as ethyl esters consumed for five years in combination with statins (n=9326) vs. statins alone (n=9319) in hypercholesterolemic, high fish consumers on primary and secondary prevention of coronary heart disease. In this study adverse outcomes were assessed and published in a separate paper (Tanaka et al., 2008) cited in EFSA 2012. Bleeding (cerebral and fundal bleedings, epistaxis, and subcutaneous bleeding combined) was more frequently reported in the EPA group than in controls. EFSA (2012) noted that nose-or subcutaneous bleeding was self-reported, and that self-reported side effects are subject to high reporting bias in open label studies. EFSA further noted that no statistically significant differences in the total incidence of stroke, or in the incidence of cerebral or subarachnoid haemorrhage, were observed between groups. EFSA considered that an intake of EPA alone at doses up to 1.8 g/day for five years does not increase the risk of bleeding complications. The aim of a review article from 2014 was to look at the effects of n-3 fatty acids on bleeding complications in a variety of clinical settings and in combinations with different anti-platelet drugs or anticoagulant therapies (Wachira et al., 2014). It was stated that although platelets are affected by n-3 fatty acids supplementation, impact on bleeding time and bleeding complications is minor. New insights indicate that fish oil can somewhat lower platelet arachidonic acid levels and possibly slightly diminish the cyclooxygenase (COX)-derived signalling cascade. Besides COX, n-3 fatty acids are substrates for several enzymes that produce active metabolites which can affect platelets function.</i></p> <p><i>Neither in the present nor in the EFSA opinion, were any studies with single DPA or DHA supplementation investigating bleeding complications or bleeding time identified."</i></p>
<p>VKM concludes: The findings on bleeding from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.</p>	

Summary: Risk for bleeding complications following EPA and DHA exposure have been assessed in previous risk assessments. In EFSA 2012, it was concluded that supplemental intakes of EPA and DHA combined of up to about 5 g/day for up to two years and up to about 7 g/day for up to six months, do not increase the risk of spontaneous bleeding episodes or bleeding complications, even in subjects at high risk of bleeding (e.g. taking acetylsalicylic acid or anti-coagulants). EFSA considered that an intake of EPA alone at doses up to 1.8 g/day for five years does not increase the risk of bleeding complications. VKM notes that no systematic approach has been used to assess all relevant data on the subject and also no quality assessment has been performed on the studies which of the conclusion have been based on.

4.4.4.3 Fasting glucose and insulin, and insulin resistance

Four RCTs addressing fasting glucose and insulin and/or insulin resistance were included (Table 4.4.4.3-1).

Table 4.4.4.3.-1. RCTs addressing fasting glucose and insulin, and/or insulin resistance in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results
de Ferranti et al. (2014)	Hypertriglyceridemic adolescents. N=25, aged 10-19 years. Dose: ~3360 mg DHA + EPA per day vs placebo. 3 months.	Tier 2	No effect on fasting glucose and insulin.
Janczyk et al. (2015)	Children with nonalcoholic fatty liver disease. 64 completed the trial. Dose: Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day; body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day; body weight >60 kg: 800 mg DHA and 532.5 mg EPA per day. Study duration was 24 weeks. Median age was 13 years.	Tier 2	No effect on fasting glucose and insulin.
Pacifico et al. (2015)	Children with biopsy-proven nonalcoholic fatty liver disease. 58 participants randomized, 51 (25 DHA, 26 placebo) completed the study. Age at study start was 10.8 (2.8) years for placebo and 11.0 (2.6) years for	Tier 1	No effect on fasting glucose. Significantly reduced fasting insulin.

	intervention. DHA dose: 250 mg/day. Study duration was 6 months.							
Rodriguez et al. (2019)	Children with with Duchenne Muscular Dystrophy. 28 participants completed the trial. Age: 8.4±2.3 years (placebo) and 6.96±2.5 years (intervention). Dose: 450 mg EPA + 2250 mg DHA + 200 mg of additional omega 3 fatty acids per day. Study duration was 6 months.			Tier 1	Fasting insulin, percentage of boys with hyperinsulinemia, and insulin resistance were similar between the placebo and n-3 LCPUFA groups during the 6 months of supplementation. The percentage of boys with IR was significantly (p -0.045) lower at month 6 of supplementation in the n-3 LCPUFA group than in the placebo group.			
Overall evaluation of certainty in the evidence on fasting glucose and insulin, and/or insulin resistance								
Initial rating	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose-response relationship	Consistency	
DHA+EPA 3 RCTs initial rating ++++	Serious	Not serious	Not serious	Serious	No	No	Yes	Moderate
DHA 1 RCTs initial rating ++++	Not serious	Not evaluated Serious	Not serious	Serious	No	No	Not evaluated	Low
VKM concludes:								
<ul style="list-style-type: none"> • There is moderate certainty evidence that doses up to 450 mg EPA and 2250 mg DHA combined for up to six months do not cause negative effects on glucose and insulin homeostasis in children and adolescents aged 6-18 years. • There is low certainty evidence that doses of 250 mg DHA for six months do not cause negative effects on glucose and insulin homeostasis in children and adolescents aged 8-13 years • No studies on EPA alone were included. 								

4.4.4.4 Gastrointestinal effects

Systematic reviews and RCTs addressing gastrointestinal effects were included (Table 4.4.4.4-1 and 4.4.4.4-2).

Table 4.4.4.4-1. Systematic reviews addressing gastrointestinal effects.

Reference	Study/ intervention	Results, certainty in the evidence
Abdelhamid et al. (2020)	<p><u>Studies with combined EPA and DHA exposure:</u></p> <p>Afford 2013. 12 months. RCT with parallel design. N: 165 intervention, 172 control (Analysed, intervention: 153 control: 163). 4 × 1 g enteric-coated fish oil capsules/day (1.6 g/day EPA + 0.8 g/day DHA). Dose: 2.4 g/day EPA + DHA. Control: 4 × 1 g matching placebo capsules, 4 g/day safflower oil. RoB: moderate or high.</p> <p>AlphaOmega - EPA+DHA 2010. 40 months. RCT. N: 1192 EPA/DHA intervention, 1236 control. Adults with previous myocardial infarction. Intervention: 20 g/day enriched margarine incorporating 400 mg EPA-DHA (240 mg EPA and 160 mg DHA). Dose: average achieved 376 mg/day EPA + DHA. Control: 20 g/day margarine. No additional n-3 PUFAs.</p>	<p>Serious gastrointestinal events (RR 1.34, 95% CI 0.64 to 2.80; I²=22%; 3 trials, 774 participants, 49 events).</p> <p>Increased abdominal pain or discomfort: data suggest an association with higher LCn3 (RR 1.05, 95% CI 0.91 to 1.20; I² =16%; 9 trials, > 41,000 participants, 10,040 events).</p> <p>Diarrhoea: the data suggested an increased risk with increased LCn3 (RR 1.02, 95% CI 0.87 to 1.19; I² = 49%; 13 trials, > 37,000 participants, 12,303 events).</p> <p>Nausea: risk increased with LCn3 (RR 1.20, 95% CI 0.96 to 1.49; I²= 54%; 8 trials, > 35,000 participants, 7639 events).</p> <p>Any gastrointestinal side effect: risk appeared to increase with LCn3, albeit with very high heterogeneity (RR 1.10, 95% CI 0.97 to 1.26; I² = 74%; 33 trials, > 895,000 participants, 6651events).</p>

	<p>Identical margarine (oleic acid) placebo. RoB: low.</p> <p>ASCEND 2018. 7.4 years. RCT, parallel design. N: 7740 intervention, 7740 control. Intervention: 840 mg/day EPA+DHA (460 mg/day EPA plus 380 mg/day DHA) as 1 capsule daily.</p> <ul style="list-style-type: none"> • Arm 1: omega-3 (1 g/d: 0.41 g EPA, 0.34 g DHA) and placebo tablets for aspirin. • Arm 3: omega-3 (1 g/d) and aspirin (100 mg/d) Control: 1 capsule/day of olive oil. • Arm 2: aspirin (100 mg/d) and olive oil placebo capsule. • Arm 4: olive oil placebo and placebo tablets for aspirin. RoB: moderate or high. <p>Dream Asbell 2018. 12 months. RCT with parallel design. N: 349 intervention randomised, 186 control randomised. Adults with dry eye. Intervention: omega-3 supplements (2000 mg EPA + 1000 mg DHA/day as 5 gel caps). Dose: 3.0 g/day LCn3. Control: olive oil supplements (5 gel caps). RoB: low.</p> <p>AREDS2 2014: 5 years. RCT with parallel design. People aged 50-85 years at high risk of progression to advanced age-related macular degeneration.</p>	
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	<p>N: 2147 intervention (1068 DHA/EPA, 1079 DHA/EPA + lutein/zeaxanthin), 2056 control (1012 placebo, 1044 lutein/zeaxanthin). Intervention 350 mg/day DHA plus 650 mg/day EPA added to the standard AREDS supplement of Vitamin C (500 mg/day), Vitamin E (440 IU/day), beta-carotene (15 mg/d), zinc oxide (80 mg/day) and cupric oxide (2 mg/day). Dose: 1 g/day EPA + DHA. Control: standard AREDS supplement. RoB: low.</p> <p>Energise 2018. 12 months. RCT. N: 148 intervention randomised (122 fish oil only, 26 fish oil plus losartan), 141 control randomised (102 placebo only, 39 placebo plus losartan). People with self-reported walking or stair-climbing difficulty Intervention: Arm 1: omega-3 fish oil (1.4 g/day for 6 months, discontinued if AF or intolerance at 6 months, increased to 2.8 g/day if IL-6 remained high at 6 months). Arm 4: omega-3 plus losartan Dose: 1.2 g/day LCn3 (0.8 g/day EPA plus 0.4 g/day DHA), increasing to 2.4 g/day (1.6 g/day EPA plus 0.8 g/day DHA) in some from 6-12 months. Control: Arm 2: losartan 25 mg/d. Arm 3: placebo corn oil (for omega-3) plus placebo cellulose (for losartan). Arm 5: placebo corn oil (for omega-3). Arm 6: placebo cellulose (for losartan). RoB: moderate or high.</p> <p>EPE-A 2014. 12 months. RCT with parallel design. N: 86 intervention-high, 82 intervention low, 75</p>	
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	<p>control. People with nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. RCT with parallel design. Comparison 1: high EPA vs low EPA (unclear what replaced EPA) Comparison 2: EPA vs unclear (placebo contents not reported). Intervention-high: EPA-E 2.7 g/d, 3 × EPA-E 300 mg capsules. Dose: 2.7 g/day EPA + DHA Intervention-low: EPA-E 1.8 g/d, 2 × EPA-E 300 mg capsules + 1 placebo capsule Dose: 1.8 g/day EPA + DHA. Control: 3 × placebo capsules. The pills were identical with respect to size, colour and smell. RoB: moderate or high.</p> <p>EPIC-1 2008. 52 weeks. RCT with parallel design N: 188 intervention, 186 control. Adults with quiescent Crohn's disease. Intervention: 2 × 2 1 g gelatin capsules omega-3 free FAs (2.2 g EPA, 0.8 g DHA). Dose: 3 g/day EPA + DHA. Control: 4 x1 g capsules medium-chain triglyceride oil. RoB: moderate or high.</p> <p>EPIC-2 2008. 58 weeks. RCT with parallel design N: intervention, 189, control 190 (187 intervention, 188 control analysed). Adults with a confirmed diagnosis of Crohn's Disease. Intervention: 2 × 2 1 g gelatin capsules omega-3 free FAs providing total dose ~2.2 g/day EPA, 0.8 g/day DHA. Dose: ~3.0 g/day EPA + DHA. Control: 2 × 2 1 g capsules medium-chain triglyceride</p>	
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	<p>oil. RoB: moderate or high.</p> <p>FORWARD 2013. 12 months. RCT, parallel design. N: 289 intervention, 297 control. People with paroxysmal atrial fibrillation. Intervention: 1 capsule/day containing 1 g of n-3 PUFA (provided 850 mg-882 mg EPA/DHA). Dose: 0.85 g/day EPA + DHA. Control: identical placebo capsule containing olive oil. RoB: low.</p> <p>Fostar 2016. 24 months. RCT with parallel design. N: 101 intervention, 101 control. Adults with knee osteoarthritis. Intervention: 1-3 × a day drink of fruit juice mixed with day total = 15 mL of fish oil supplement (18% EPA, 12% DHA, 4.5 g/day total omega-3). Dose: 4.5 g/day EPA + DHA. Control: liquid oral oil 15 mL Sunola oil/day (which contains fish oil 2 mL plus 13 mL canola oil) (total omega-3 fat: ≥ 0.45 g EPA + DHA from 15 mL). RoB: low.</p> <p>GISSI-HF 2008, 3.9 years. RCT with parallel design. N: 3494 intervention, 3481 control. People with chronic heart failure. Intervention: 1 capsule/day of 1f n-3 mainly EPA and DHA as ethyl esters in the average ratio of 1:1.2. Dose approximately 0.866 g/day EPA+DHA. Control: 1g/day matching olive oil placebo capsule. RoB: moderate or high.</p>	
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	<p>Kumar 2012. 12 months. RCT with parallel design. N: 92 intervention, 90 control. People with persistent atrial fibrillation on warfarin. Intervention: 6 capsules/day of a fish oil preparation containing a total dose of 1.02 g of EPA and 0.72 g DHA. Participants in the omega-3 group were asked to continue fish oils till a maximum of 1 year or till return of persistent AF. Dose: 1.7 g/day EPA + DHA. Control: no supplements. Participants were advised not to take any fish oil supplements. RoB: moderate or high.</p> <p>Kumar 2013. 12 months. RCT with parallel design. N: 39 intervention, 39 control randomised (18 intervention vs 39 control at 12 months). People >60 years with sinoatrial node disease and dual chamber pacemakers. Intervention: a triglyceride preparation containing a total of 6 g/day of omega-3 polyunsaturated FAs of which 1.8 g/day were n-3 (1.02 g EPA and 0.72 g DHA). Dose: 1.8 g/day EPA + DHA. Control: no supplements. RoB: moderate or high.</p> <p>Lorenz-Meyer 1996. 12 months. N: 70 intervention, 63 control. People with Crohn's disease in remission. Intervention: 2 × 3 1 g gelatin capsules/day of ethylester fish oil concentrate (3.3 g/day EPA + 1.8 g/day DHA). Dose: 5.1 g/day EPA + DHA.</p>	
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	<p>Control: 2 × 3 1 g gelatin capsules/day of corn oil. RoB: low.</p> <p>MAPT 2017. 36 months. RCT with parallel design, 4 arms. N: 840 intervention (arms 1 and 3), 840 (control arms 2 and 4) randomised. Intervention Arm 1: omega-3 (V0137 CA 800 mg/day DHA; 225 mg/day EPA in soft capsules). Dose for arms 1 and 3: 1.025 g/day EPA + DHA. Arm 3: omega-3 (800 mg/day DHA; 225 mg/day EPA) plus multi-domain intervention (nutrition, physical exercise, cognitive stimulation, social activities). Control: Arm 2: placebo capsules containing flavoured paraffin oil. Arm 4: placebo capsules plus multi-domain intervention (nutrition, physical exercise, cognitive stimulation, social activities). RoB: low.</p> <p>Proudman 2015. 12 months. RCT with parallel design. N: 87 intervention, 53 control (analysed, intervention: 75 control: 47). People with RA <12 months duration, disease-modifying antirheumatic drug-naive. Intervention: 10 mL/day fish oil concentrate providing 5.5 g/day (3.2 EPA + 2.3 DHA). Dose: 5.5 g/day EPA + DHA. Control: 10 mL/day Sunola oil:capelin oil (2:1) providing 0.21 g EPA + 0.19 g DHA/day as TAG (0.40 g/day EPA + DHA). RoB: low.</p>	
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	<p>OPAL 2010. 12 months. N: 434 intervention, 433 control (analysed 376 intervention, 372 control). Healthy adults. Intervention: 2 × 650 mg capsule/day (total daily dose of 200 mg EPA and 500 mg DHA). Dose: 0.7 g/day EPA + DHA. Control: 2 × 650 mg olive oil capsule identical to intervention. RoB: low.</p> <p>ORIGIN 2012. 72 months. RCT, 2x2 factorial. N: 6319 intervention, 6292 control (analysed, intervention: 6281 control: 6255). People at high risk of cardiovascular events with impaired fasting glucose, impaired glucose tolerance or diabetes. Intervention: 1 gelatin capsule/day Omacor containing at least 900 mg ethyl esters of n-3 fats (465 mg EPA + 375 mg DHA). Dose: 0.84 g/day EPA + DHA. Control: 1 × 1 g gelatin capsule/day olive oil. RoB: low.</p> <p>ORL 2013. 12 months. N: 171 intervention (4 g TAK), 165 control (2 g TAK). Adults with hypertriglyceridaemia. Intervention: Total dose of 1.86 g/day EPA + 1.5 g/day DHA. Dose: ~3.4 g/day EPA + DHA (difference of +1.7 g/day from control arm). Control: 1 capsule/day containing 2 g of TAK-085 (1 g of FA in TAK-085 capsules contains approximately 465 mg of EPA-E plus 375 mg of DHA-E). Total dose of 0.93 g/day EPA + 0.75 g/day DHA. Dose: 1.7 g/day</p>	
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	<p>EPA + DHA. RoB: moderate or high.</p> <p>Raitt 2005. 24 months. N: 100 intervention, 100 control. People with implantable cardioverter defibrillators and recent sustained ventricular tachycardia and ventricular fibrillation. Intervention: 1.8 g/day fish oil capsules (including ethyl esters of EPA and DHA, 0.76 g/ d EPA, 0.54 g/day DHA). Dose: 1.3 g/day EPA + DHA. Control: 1.8 g/day olive oil capsules (73% oleic acid). RoB: moderate or high.</p> <p>Risk and prevention 2013. 60 months. RCT with parallel design N: 6244 intervention, 6269 control (analysed, intervention: 6239 control: 6266). Intervention: 1 g/day n-3 capsules polyunsaturated FA ethyl esters (EPA and DHA content 850-882 mg with an average ratio of 1.0:1.2). Dose: ~0.87 g/day EPA + DHA. Control: 1 g/day olive oil capsules. Rob: moderate or high.</p> <p>Rossing 1996. 12 months. N: 18 intervention, 18 control (analysed, 17 intervention, 15 control). Adults with insulin-dependent diabetes mellitus. Intervention: cod-liver oil emulsion. EPA 2 g, DHA 2.6 g, total PUFA 4.6 g/day. Dose: 4.6 g/day EPA + DHA. Control: olive oil emulsion. RoB: moderate or high.</p>	
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	<p>Sandhu 2016. 24 months. RCT with parallel design. N: 54 + 53 intervention, 53 + 53 control. Healthy postmenopausal women. Intervention: group 4, Lovaza 4 g/day. Lovaza is the FDA-approved n-3 FA formulation containing 465 mg of EPA + 375 mg of DHA per gram, total dose; 1860 mg/day EPA, 1500 mg/day DHA. Group 5 as group 4 plus 30 mg raloxifene/day. Dose: 3.36 g/day EPA + DHA. Control: group 1, no treatment; group 3, 30 mg raloxifene/day. RoB: moderate or high.</p> <p>SCIMO 1999. 2 years. RCT with parallel design. N: 112 intervention, 111 control (analysed 82 intervention, 80 control). People with angiographically proven coronary artery disease. Intervention: concentrated fish oil capsules, 6 x 1 g capsules/day for first 3 months, 3 x 1 g/day for rest of trial (4 g/day EPA +DHA + DPA + ALA for first 3 months, then 2 g/d). Dose: ~2 g/day LCn3. Control: capsules containing fat that replicated the fat composition of the average European diet, 6/day for first 3 months, 3/day for rest of trial, opaque soft gelatin capsules identical to fish capsules in identical screw-top containers. RoB: low.</p> <p>Shinto 2014. 12 months. N: 13 intervention, 13 control. People with probable Alzheimer dementia diagnosis Intervention: 3 x 1 g capsules/day of fish oils (975 mg EPA, 675 mg DHA/d). Dose: 1.65 g/day EPA + DHA.</p>	
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	<p>Control: 3 × 1 g capsules/day soybean oil (which contains 5% fish oil). RoB: moderate or high.</p> <p>SHOT 1996. 1 year. RCT with parallel design. N: 317 intervention, 293 control. Intervention: 4 fish-oil concentrate soft gelatin capsules/day containing 51% EPA and 32% DHA ethyl esters and 3.7 mg vitamin E as an antioxidant. Dose: 3.3 g/day EPA + DHA. Control: no treatment. RoB: moderate or high.</p> <p>SOFA 2006. 12 months. RCT with parallel design. N: 273 intervention, 273 control (273 intervention, 273 control analysed). People with previous ventricular arrhythmias and implanted cardioverter defibrillator. Intervention: 2 g/day (4 capsules) purified fish oil. 961 mg n-3 PUFAS (464 mg EPA + 335 mg DHA and 162 mg other n-3 PUFAs) daily. 3000 ppm vitamin E. Dose: 0.8 g/day EPA + DHA. Control: 2 g/day high-oleic acid sunflower oil. 3000 ppm vitamin E. RoB: low.</p> <p>Tande 2016. 12 months. RCT with parallel design. N: 64 intervention, 63 control (50 intervention, 50 control analysed). Healthy males and females. Intervention: 2 × 500 mg Calanus oil capsules twice daily to provide a daily dose of 2 g. The Calanus oil contained approximately 85% wax ester with a sum of</p>	
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	<p>neutral lipids > 90%. Dose: 2 g/day EPA + DHA. Control: identical capsules of olive oil. Compositional analysis indicated that the FA content of the olive oil was primarily oleic acid (76.9%), palmitic acid (10.2%), and linoleic acid (7.7%). RoB: moderate or high.</p> <p><u>Studies with DHA exposure:</u></p> <p>ADCS 2010. 18 months. N: 238 intervention, 164 control. Individuals with mild to moderate Alzheimer's disease. Intervention: 2 × 1 g algal-derived DHA capsules/day, each capsule contain 45%-55% of (950 mg soft-gel capsules, which contain approximately 510 mg DHA). Dose: +DHA 1.02 g/day. Control: 2 × 1 g placebo capsules/day (made up of corn or soy oil). RoB: low.</p> <p><u>Studies with EPA exposure:</u></p> <p>Puri 2015. 12 months. N: 67 intervention, 68 control (analysed, intervention: 39 control: 44). People with Huntington's disease. Intervention: 2 × 2 × 500 mg capsules/d, total dose of 2 g/day ethyl-EPA (code name LAX-101, purity 95%). Dose: 1.9 g/day EPA. Control: 2 × 2 × 500 mg capsules/day liquid paraffin. RoB: low.</p> <p>REDUCE-IT 2019. 4.9 years (median).</p>	
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	<p>RCT with parallel design N: intervention 4089 randomised, control 4090 randomised (analysed, intervention: 4083 control: 4077). People (45 years+) with hypertriglyceridaemia, and with cardiovascular disease or with diabetes mellitus and another risk factor, and on statin. Intervention: EPA ethyl ester derived from fish oil (4 g/day), 3.99 g/day EPA plus 8 mg/day vitamin E (2 capsules twice/day). Control: 3.73 g/day light liquid paraffin oil in 4 capsules (2 capsules twice a day). RoB: moderate or high.</p> <p>JELIS 2007. 5 years. RCT with parallel design. N: intervention, 9326, control 9319 (analysed intervention 9326, control 9319). People with hypercholesterolaemia. Intervention: 3 × 2 × 300 mg capsules/day EPA ethyl ester (total dose of 1.8 g/day EPA), after meals. Dose: 1.8 g/day EPA. Control: nothing (though all in both groups received "appropriate" dietary advice). RoB: moderate or high.</p>	
Downie et al. (2019)	<p><u>Studies with EPA + DHA exposure:</u> Asbell 2018. <i>Included as Dream Asbell 2018 in Abdelhamid et al., 2020.</i></p> <p>Bhargava 2016a. 6 months. RCT, parallel design. 130 participants in total, 65 participants in each treatment groups. Participants with the diagnosis of</p>	<p>The most common side effect was temporary gastrointestinal problems.</p> <p>Of the nine studies that investigated long-chain omega-3 fatty acid supplementation relative to placebo and reported adverse events, six trials reported that participants in the omega-3 group experienced gastrointestinal disorders (including diarrhoea) and one trial reported that participants in the comparator group experienced similar effects. Three of these studies provided sufficient data for a meta-analysis related to</p>

	<p>dry eye. Age (mean \pm SD, range): 47.7 \pm 3.8 (range 24 to 68) years in the omega-3 treatment group; 48.9 \pm 4.5 (range 21 to 70) years in the control group. Daily dose of 720 mg EPA and 480 mg DHA. Placebo: Oral capsules containing olive oil (dose not reported). RoB: low in five of seven domains (unclear for blinding of outcome assessors and selective reporting).</p> <p>Brignole-Baudouin 2011. 3 months. RCT, parallel design. 121 participants in total, 56 in the intervention group, 63 in the control group, all with the symptom of dry eye. Mean age was about 60 years. Intervention: oral capsule containing fish oil (omega-3 fatty acids, average of 285 mg including EPA 142.5 mg and DHA 95 mg) and omega-6 average of 5 mg. Control: placebo capsule containing medium-chain triglycerides, 3 capsules daily (daily dose: 575 mg/d). RoB: low for four domains, high for incomplete outcome data, and unclear for selective reporting and other bias.</p> <p>Deinema 2017. 90 days. RCT, parallel design. 60 participants in total, 20 in each of the 3 intervention groups. Participants with the diagnosis of dry eye. Daily dose of 1000 mg EPA and 500 mg DHA from fish</p>	<p>gastrointestinal adverse effects. The statistical heterogeneity ($I^2 = 76\%$) was substantial. Gastrointestinal disorders were reported in between 5% and 19% of participants in the omega-3-treated group and between 0% and 24% of participants in the placebo group.</p> <p>The certainty of the body of evidence (according to the GRADE system) was downgraded by two levels to low, as results were inconsistent among studies and few events accounted for the wide confidence intervals reported (imprecision).</p>
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	<p>oil capsules (triglycerideomega-3 PUFAs). Daily dose of 945 mg EPA and 510 mg DHA from krill oil capsules (phospholipidomega-3 PUFAs). Placebo: Daily dose of 1500 mg olive oil. RoB: low in six of seven domains (high for incomplete outcome data).</p> <p>NCT01107964. 45 days. Randomised, parallel design. 27 participants in total, all with typical symptoms of dry eye. Mean age was > 50 years. Intervention: omega-3-acid ethyl esters, 1 g capsule, 4 times daily. Control: placebo (corn oil) capsules, 1 g capsule, 4 times daily. RoB: low for one domain, unclear for five domains, and high for incomplete outcome data.</p> <p>Oleñik 2013. 3 months. RCT, parallel design. 60 participants in total, all with the diagnosis of meibomian gland dysfunction. Mean age was > 50 years. Intervention: oral capsule containing EPA 42.5 mg and DHA 350 mg and 30 mg docosapentaenoic acid 3 times daily, 1 capsule at a time. Daily dose of: 127.5 mg EPA, 1050 mg DHA, and 90 mg docosapentaenoic acid. Control group: placebo capsule (500 mg) containing sunflower oil (dose not reported), 3 times daily, 1</p>	
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	<p>capsule at a time. RoB: low for two domains and unclear for three domains. High for incomplete outcome data and other bias.</p>	
Irving et al. (2006)	<p><u>Studies with EPA exposure:</u></p> <p>Fenton 2001. 16 weeks. RCT. 90 participants in total, only data from 87 participants. People with schizophrenia or similar chronic mental illnesses. Ethyl-EPA, 500 mg/day + vitamin. Mineral oil placebo + vitamin E. RoB: low for three domains. Unclear for adequate sequence generation, allocation concealment and other bias.</p> <p>Peet 2002: 12 weeks. RCT. 122 participants in total, only 115 participants analysed. People with schizophrenia or similar chronic mental illnesses. Ethyl-eicosapentenoic acid, 1 g/day, n=32. Ethyl-EPA, 2 g/day, n=32. Ethyl-EPA, 4 g/day, n=27. Placebo, n=31. Risk of bias assessment: Low risk of selection bias and observer bias. Unclear risk for attrition. High risk for selective reporting and source of funding. RoB: low for three domains. Uncertain for incomplete</p>	<p>Comparison of specific doses of E-EPA versus placebo: Most adverse effect data was derived from Peet 2002 (n=122, short term), and all events were rare, and results were inconclusive. Any doses of E-EPA, when compared with placebo, were not associated with frequent adverse effects.</p> <p>Data from two studies, the short-term Peet 2002 and medium-term Fenton 2001 do not show that people receiving less than 1 g/day of E-EPA are more likely to experience diarrhoea than those on placebo (n=150, 2 RCTs, RR 2.04 CI 0.91 to 4.54). However, these data are heterogeneous, and the results of the medium-term Fenton 2001 do suggest that there may be a link with the use of low dose E-EPA supplementation and diarrhoea (n=87, 1 RCT, RR 17.39 CI 1.03 to 292).</p>

	outcome data, high for selective reporting and other bias.	
Newberry et al. (2016)	<p><u>Studies with EPA + DHA exposure:</u></p> <p>9 RCTs addressed gastrointestinal effects (maternal), the strength of evidence was moderate. Supplementation with n-3 FA in the form of fish oil.</p> <p>13 RCTs addressed gastrointestinal effects (infants), the strength of evidence was moderate. Supplementation with n-3 FA in the form of fish oil alone or added to infant formula.</p>	Increased risk for mild gastrointestinal symptoms (maternal effect and infant effect). Moderate strength of evidence. These RCTs were determined to be too heterogeneous to permit pooling.
Sarmiento et al. (2016)	<p><u>Studies with EPA + DHA exposure:</u></p> <p>Bromfield 2008. 12 weeks. 27 participants. RCT. Adults with a diagnosis of drug-resistant epilepsy. PUFA group: one capsule (1.1 g) taken twice a day, total dose 2.2 g PUFAs daily (EPA plus DHA in a 3:2 ratio). 1.32 g EPA + 0.88 g DHA. Placebo: identical capsules containing a mineral oil. RoB: low for three domains, unclear for random sequence generation, allocation concealment, and blinding of outcome assessor.</p> <p>Yuen 2005. 12 weeks. 58 participants. RCT. Adults with a diagnosis of drug-resistant epilepsy. PUFA group: 1000 mg fish oil capsules, total daily dose 1.7 g omega-3 PUFAs (1 g EPA and 0.7 g DHA). Each</p>	<p>Two studies analysed gastrointestinal effects and there were no significant differences between PUFA versus placebo (RR 0.78, 95% CI 0.32 to 1.89) (IS = 8%). The certainty of the evidence (GRADE) was low.</p> <p>The most frequent side effects were nausea and diarrhoea (details of the severity of these symptoms were not given).</p>

	<p>capsule contained 171 mg EPA and 112 mg DHA, and < 100 IU vitamin A and < 40 IU vitamin D).</p> <p>Placebo: matching capsules containing mixed oils (palm olein 70%, rapeseed oil 15%, sunflower oil 15%).</p> <p>RoB: low.</p>	
Watson and Stackhouse (2020)	<p>Henderson 1994. 6 weeks. N=25.</p> <p>RCT, parallel design.</p> <p>Children and adults diagnosed with cystic fibrosis, and gender and age-matched people without CF.</p> <p>Intervention group: 8 x 1g capsules fish oil (4 capsules twice daily) containing 3.19 g EPA and 2.21 g DHA.</p> <p>Control: olive oil placebo capsules flavoured to obtain a slight fish taste.</p> <p>RoB: low for four domains, unclear for allocation concealment and selective reporting.</p>	<p>Adverse events: diarrhoea. Follow-up: 6 weeks. 1 study reported drop out due to diarrhoea. 2 out of 7 participants in the fish oil group dropped out and 2 out of 5 participants in the placebo group. There was no significant difference between the groups, OR 0.6 (CI 0.05 to 6.79). The certainty of the evidence (GRADE) was very-low.</p> <p>Other adverse events included stomach pains (5/35 participants) but the intervention arm wasn't specified.</p>
<p>VKM concludes: There is insufficient evidence to conclude whether EPA and DHA in combination or alone increases the risk for gastrointestinal effects. VKM considers that potential gastrointestinal effects following exposure to EPA and DHA in combination or alone are small and the symptoms are mild.</p> <p>Summary: VKM identified five systematic reviews with low or unclear risk of bias that assessed gastrointestinal effects following exposure to EPA and DHA in combination or alone. Abdelhamid 2020 also sorted the effects into serious and non-serious gastrointestinal effects, whereas severity was not addressed in the other reviews. For increased abdominal pain or discomfort, diarrhoea and nausea there seemed to be a slight increase in risk with LCn3 exposure. Also, combined for all these symptoms (any gastrointestinal side effect) there was an increased risk with LCn3 exposure, although the heterogeneity was very high. For serious gastrointestinal events the heterogeneity was very high, the confidence intervals were large, and the number of participants were low. The authors did not do a GRADE assessment. However, VKM notes that there are inconsistencies across studies and that the findings are of limited value, as all studies, independent of intervention, were summarised together. Therefore, it is not possible to assess the effect of EPA and DHA in combination or EPA alone, separately.</p> <p>Irving et al. 2006 summarised gastrointestinal effects in adults following exposure to EPA (500 mg to 4 g) alone for 12-16 weeks. The findings were inconsistent. Watson and Stackhouse 2020 included one RCT with both children and adults receiving daily doses of 3.19 g EPA and 2.21 g DHA for six weeks.</p>		

Gastrointestinal effects were similar in the intervention and placebo group. However, the number of participants were only 25 and the authors judged the certainty of the evidence to be very-low. Sarmiento 2016 included two RCT with adults receiving daily doses up to 1.32 g EPA and 0.88 g DHA for 12 weeks. No significant differences between intervention and placebo were observed. The authors judged the evidence to be of low certainty. Newberry 2016 identified an increased risk for mild gastrointestinal effects in infants supplemented with n-3 PUFAs. The authors judged the evidence to be of moderate certainty.

Table 4.4.4.4-2. RCTs addressing gastrointestinal effects in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results
Cornu et al. (2018)	Children with established diagnosis of ADHD. N=148. Children aged 6–8 years, EPA 336 mg and DHA 84 mg; for children aged 9–11 years, EPA 504 mg and DHA 126 mg, and for children aged 12–15 years EPA 672 mg and DHA 168 mg. Study duration was 3 months.	Tier 2	Eleven (14.9%) children in the DHA–EPA group experienced 13 adverse events, 2 of which were judged to be related to the study treatment by the study authors: hip pain, fatigue, headache, fever and cough (n = 2), dermatitis and allergic skin reactions (n = 4), abdominal pain, diarrhoea (n = 3), and depression. Eight children (10.7%) in the placebo group experienced 10 adverse events: fatigue, influenza (n = 2), abdominal pain, dermatitis, swollen eyes, vomiting, and diarrhoea (n = 3).
de Ferranti et al. (2014)	Hypertriglyceridemic adolescents. N=25, aged 10-19 years. Dose: ~3360 mg DHA + EPA per day vs placebo. 3 months.	Tier 2	No gastrointestinal effects reported.
Mazahery et al. (2019)	Children with autism spectrum disorder. N=73, aged 2.5 to 8 years. Dose: 722 mg DHA. Study duration = 12 months.	Tier 2	Ten events reported by participants in the intervention group, 8 in the placebo group.
Montgomery et al. (2018)	Healthy school children. N=185 (intervention) and 187 (placebo), aged 7 to 9 years. 600 mg/day DHA, placebo: taste/color matched corn/soybean oil. Study duration: 16 weeks.	Tier 1	No gastrointestinal effects reported.
Overall evaluation of certainty in the evidence on gastrointestinal effects			

Initial rating	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
DHA+EPA 2 RCTs initial rating +++++	Serious	Not serious	Not serious	Serious	No	No	Not evaluated	Low
DHA 2 RCTs, initial rating +++++	Serious	Not serious	Not serious	Serious	No	No	Not evaluated	Low

VKM concludes:

- There is low certainty evidence that doses up to 1800 mg EPA and 1500 mg DHA for three months do not significantly cause gastrointestinal effects different from that of placebo.
- There is low certainty evidence that doses up to 722 mg DHA for 12 months do not significantly cause gastrointestinal effects different from that of placebo.
- No studies on EPA alone were included.

4.4.4.5 Glucose and lipid homeostasis

Risk assessments addressing glucose and lipid homeostasis were included (Table 4.4.4.5-1).

Table 4.4.4.5-1. Risk assessments addressing glucose and lipid homeostasis.

Reference	Direct quotation from summary / conclusion in the risk assessment
EFSA, 2012	<p><i>"The Panel notes that human intervention studies which have controlled for fat intake generally do not show a differential effect of vegetable oils and supplemental fish oil at doses up to 5g/day of EPA and DHA consumed for 12 weeks on blood glucose control in diabetic subjects, or on insulin sensitivity in healthy or diabetic subjects.</i></p> <p><i>The Panel considers that supplemental intakes of EPA and DHA combined of up to 5 g/day consumed for up to 12 weeks do not significantly affect glucose homeostasis in healthy or diabetic subjects. The Panel notes that the data available are insufficient to conclude on whether the same doses administered mostly as EPA or mostly as DHA would have different effects on this outcome."</i></p>
VKM, 2015	<p><i>"From intervention studies, mostly uncontrolled, adverse effects on lipid-and glucose metabolism from supplemental intake of n-3 LCPUFA (≥ 10 g/day) have been described. EFSA concluded that an intake of EPA and DHA combined up to 5g/day consumed for 12 weeks does not significantly affect glucose homeostasis in healthy or diabetic subjects, but that scientific data is not available to conclude whether the same doses administered as EPA or DHA alone would have a different effect. Several human studies have addressed the effects of supplementation with n-3 LCPUFA on blood LDL-cholesterol concentrations. EFSA (2012) concluded that 2-6 g/day of supplemental EPA and DHA combined or 2-4g/day of mostly DHA, increases the blood concentration of LDL-cholesterol by about 3% and that such an increase is accompanied by a decrease in TAG with no changes in HDL-cholesterol concentrations. Supplementation of mostly EPA had no such effect on cholesterol concentrations. The small increase in LDL-cholesterol by EPA+DHA supplementation or DHA supplementation alone was not considered as an adverse effect by the EFSA Panel."</i></p> <p>VKM (2015) reported that none of the studies included reported on adverse effects related to glucose or lipid homeostasis.</p>
<p>VKM concludes: The findings on glucose and lipid homeostasis from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.</p>	

4.4.4.6 Haemorrhagic stroke

One systematic review addressing haemorrhagic stroke were included (Table 4.4.4.6-1).

Table 4.4.4.6-1. Systematic review addressing haemorrhagic stroke.

Reference	Study/ intervention	Results, certainty in the evidence
Abdelhamid et al. (2020)	<p><u>Studies with EPA and DHA exposure:</u></p> <p>AREDS2 2014: 5 years. RCT with parallel design. People aged 50-85 years at high risk of progression to advanced age-related macular degeneration. N: 2147 intervention (1068 DHA/EPA, 1079 DHA/EPA + lutein/zeaxanthin), 2056 control (1012 placebo, 1044 lutein/zeaxanthin). Intervention 350 mg/day DHA plus 650 mg/day EPA added to the standard AREDS supplement of Vitamin C (500 mg/day), Vitamin E (440 IU/day), beta-carotene (15 mg/d), zinc oxide (80 mg/day) and cupric oxide (2 mg/day). Dose: +1 g/day EPA + DHA. Control: standard AREDS supplement. RoB: low.</p> <p>GISSI-HF 2008, 3.9 years. RCT with parallel design. N: 3494 intervention, 3481 control. People with chronic heart failure. Intervention: 1 capsule/day of 1f n-3 mainly EPA and DHA as ethyl esters in the average ratio of 1:1.2. Dose approximately 0.866 g/day EPA+DHA. Control: 1g/day matching olive oil placebo capsule. RoB: moderate or high.</p> <p>MAPT 2017. 36 months. RCT with parallel design, 4 arms. N: 840 intervention (arms 1 and 3), 840 (control arms 2 and 4) randomised. Intervention: Arm 1: omega-3 (V0137 CA 800 mg/day DHA; 225 mg/day EPA in soft capsules). Dose for arms 1 and 3: 1.025 g/day EPA + DHA. Arm 3: omega-3 (V0137 CA 800 mg/day DHA; 225 mg/day EPA in soT capsules) plus multi-domain intervention (nutrition, physical exercise, cognitive stimulation, social activities). Control: Arm 2: placebo capsules containing flavoured paraffin oil.</p>	<p>10 trials, n=70,695.</p> <p>RR 1.23, 95% CI 0.93 to 1.64; I² =0.</p>

	<p>Arm 4: placebo capsules plus multi-domain intervention (nutrition, physical exercise, cognitive stimulation, social activities). RoB: low.</p> <p>NAT2 2013. 36 months. N: 150 intervention, 150 control. People with early age-related macular degeneration. Intervention: 3 daily fish oil capsules containing 1110 total n-3 FAs (EPA: 270 mg/day DHA: 840 mg/d) and vit E: 6 mg/day. Dose: 1.1 g/day EPA + DHA. Control: 3 × 602 mg/day olive oil capsules containing 0.2 g total PUFA and vitamin E: 0.09 g/day. RoB: low.</p> <p>ORL 2013. 12 months. N: 171 intervention (4 g TAK), 165 control (2 g TAK). Adults with hypertriglyceridaemia. Intervention: 1 × 2/day capsule each containing 2 g of TAK-085 (1 g of FA in TAK-085 capsules contains approximately 465 mg of EPA-E plus 375 mg of DHA-E). Total dose of 1.86 g/day EPA + 1.5 g/day DHA. Dose: ~3.4 g/day EPA + DHA (difference of +1.7 g/day from control arm). Control: 1 capsule/day containing 2 g of TAK-085 (1 g of FA in TAK-085 capsules contains approximately 465 mg of EPA-E plus 375 mg of DHA-E). Total dose of 0.93 g/day EPA + 0.75 g/day DHA. Dose: 1.7 g/day EPA + DHA. RoB: moderate or high.</p> <p>VITAL 2019. 5.3 years. RCT with parallel design 2x2. N: 12,933 intervention, 12,938 control (analysed intervention 12,933, control 12,938). Intervention: Arm 1: omega-3, 1 capsule/d, Omacor fish oil, EPA + DHA 840 mg/d: 465 mg EPA; 375 mg DHA provided in calendar packs and placebo D3. Arm 3: omega-3 as in Arm 1 and vitamin D3 (1/d, 2000 IU). Control: Arm 2: placebo omega-3 and vitamin D3 (1/d, 2,000IU). Arm 4: placebo omega-3 and placebo D3. Dose: 840 mg/day LCn3, or 0.38% E. RoB: low.</p> <p>SU.FOL.OM3 2010. 4 years. RCT with parallel design.</p>	
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N=1248 control, 1253 intervention. People with a history of MI, unstable angina or ischaemic stroke.

Intervention: 2 gelatin capsules omega-3 (400 mg/day EPA and 200 mg/day DHA).

Control: 2 gelatin capsules/day placebo (liquid paraffin with fish flavour).

RoB: low.

Studies with EPA exposure:

JELIS 2007. 5 years.

RCT with parallel design.

N: intervention, 9326, control 9319 (analysed intervention 9326, control 9319). People with hypercholesterolaemia.

Intervention: 3 × 2 × 300 mg capsules/day EPA ethyl ester (total dose of 1.8 g/day EPA), after meals. Dose: 1.8 g/day EPA.

Control: nothing (though all in both groups received "appropriate" dietary advice).

RoB: moderate or high.

DART 1989. 2 years.

RCT, parallel design.

N: 1015 intervention, 1018 control. Men recovering from myocardial infarction.

Intervention: advised to eat at least 2 weekly portions of 200-400 g fatty fish (mackerel, herring, kipper, pilchard, sardine, salmon, trout). If this was not possible, given MaxEPA capsules, 3/day (0.5 g EPA/d). 191/883 participants were taking MaxEPA at 2 years. Advice was reinforced 3-monthly. Dose: aimed for 0.5 g/day EPA.

Control: no such dietary advice or capsules.

RoB: moderate or high.

REDUCE-IT 2019. 4.9 years (median).

RCT with parallel design.

N: intervention 4089 randomised, control 4090 randomised (analysed, intervention: 4083 control: 4077). People (45 years+) with hypertriglyceridaemia, and with cardiovascular disease or with diabetes mellitus and another risk factor, and on statin.

Intervention: EPA ethyl ester derived from fish oil (AMR101 4 g/d, Amarin), 3.99 g/day EPA plus 8 mg/day vitamin E (2 capsules twice/day).

	Control: 3.73 g/day light liquid paraffin oil in 4 capsules (2 capsules twice a day). RoB: moderate or high.	
<p>VKM concludes: There were not data on children and adolescents. It is not possible to conclude on the effects of EPA and DHA or EPA alone on haemorrhagic stroke in adults since all studies irrespective of intervention are summarised together. There were no studies with exposure to DHA alone. VKM considers this outcome to be of relevance for children.</p> <p>Summary: The conclusions on haemorrhagic stroke are based on Abdelhamid et al., 2020, which is a systematic review with low RoB. Data from ten RCTs on haemorrhagic stroke following exposure to EPA+DHA (seven trials) and EPA alone (three trial) were summarised together by Abdelhamid et al., 2020. No trial with exposure to DHA alone were identified.</p> <p>For EPA+DHA, the doses ranged from 0.84 to 3.4 g/day. For EPA alone, the doses ranged from 0.5 to 3.99 g/day. The participants mean age were > 36 years, and the exposure period ranged from 1.0 to 5 years. The total number of participants were 70,695.</p>		

4.4.4.7 Headache or worsening migraine

Systematic reviews and RCTs addressing headache and worsening migraine were included (Table 4.4.4.7-1 and 4.4.4.7-2).

Table 4.4.4.7-1. Systematic reviews addressing headache or worsening migraine.

Reference	Study/ intervention	Results, certainty in the evidence
Abdelhamid et al. (2020)	<p><u>Studies with combined EPA and DHA exposure:</u></p> <p>Dream Asbell 2018. 12 months. RCT with parallel design. N: 349 intervention randomised, 186 control randomised. Adults with dry eye. Intervention: omega-3 supplements (2000 mg EPA + 1000 mg DHA/day as 5 gel caps). Dose: 3.0 g/day LCn3. Control: olive oil supplements (5 gel caps). RoB: low.</p>	Headache or worsening migraine: there were limited data on this outcome (RR 0.85, 95% CI 0.51 to 1.40; I ² = 0%; 4 trials, 1526 participants, 60 events).

	<p>EPE-A 2014. 12 months. RCT with parallel design. N: 86 intervention-high, 82 intervention low, 75 control. People with nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. RCT with parallel design. Comparison 1: high EPA vs low EPA (unclear what replaced EPA) Comparison 2: EPA vs unclear (placebo contents not reported). Intervention-high: EPA-E 2.7 g/d, 3 × EPA-E 300 mg capsules. Dose: 2.7 g/day EPA + DHA Intervention-low: EPA-E 1.8 g/d, 2 × EPA-E 300 mg capsules + 1 placebo capsule Dose: 1.8 g/day EPA + DHA. Control: 3 × placebo capsules. The pills were identical with respect to size, colour and smell. RoB: moderate or high.</p> <p>EPIC-1 2008. 52 weeks. RCT with parallel design N: 188 intervention, 186 control. Adults with quiescent Crohn's disease. Intervention: 2 × 2 1 g gelatin capsules omega-3 free FAs (2.2 g EPA, 0.8 g DHA). Dose: 3 g/day EPA + DHA. Control: 4 x1 g capsules medium-chain triglyceride oil. RoB: moderate or high.</p> <p>EPIC-2 2008. 58 weeks. RCT with parallel design N: intervention, 189, control 190 (187 intervention, 188 control analysed). Adults with a confirmed diagnosis of Crohn's Disease. Intervention: 2 × 2 1 g gelatin capsules omega-3 free FAs providing total dose ~2.2 g/day EPA, 0.8 g/day DHA. Dose: ~3.0 g/day EPA + DHA. Control: 2 × 2 1 g capsules medium-chain triglyceride oil. RoB: moderate or high.</p>	
<p>VKM concludes: There were not data on children or adolescents. Data on adults suggested that 2.7 g EPA and 0.8 g DHA for 12 months did not cause headache or worsening of migraine. However, due to lack of ADME data and lack of certainty in evidence assessment, the findings will only be used as supporting information. VKM considers the outcome to be of relevance for children and adolescents.</p>		

Summary: The conclusions on headache and worsening migraine are based on Abdelhamid et al., 2020, which is a systematic review with low RoB. Data from four RCTs on headache and worsening migraine following exposure to EPA+DHA were summarised. No trial with exposure to DHA or EPA alone were identified.

For EPA+DHA, the doses ranged from 1.8 to 3.0 g/day. The participants mean age ranged from 38-58 years, and the exposure period was about one year. The total number of participants were 1526.

No significant effect was observed in the meta-analysis. Note that the confidence intervals were large. The RoB was low in one study and moderate to high for three studies. VKM notes that the direction of effect was not consistent within the studies at low risk of bias or those with moderate to high risk of bias. In addition, VKM notes that the inconsistencies in direction of effect cannot be explained by study size, dose or duration of intervention. No assessment of certainty in evidence was performed.

Table 4.4.4.7-2. RCTs addressing headache in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results					
Cornu et al. (2018)	Children with established diagnosis of ADHD. N=148. Children aged 6–8 years, EPA 336 mg and DHA 84 mg; for children aged 9–11 years, EPA 504 mg and DHA 126 mg, and for children aged 12–15 years EPA 672 mg and DHA 168 mg. Study duration was 3 months.	Tier 2	Eleven (14.9%) children in the DHA–EPA group experienced 13 adverse events, 2 of which were judged to be related to the study treatment by the study authors: hip pain, fatigue, headache, fever and cough (n = 2), dermatitis and allergic skin reactions (n = 4), abdominal pain, diarrhoea (n = 3), and depression. None in the placebo group experienced such effects.					
Mazahery et al. (2019)	Children with autism spectrum disorder. N=73, aged 2.5 to 8 years. Dose: 722 mg DHA. Study duration = 12 months.	Tier 2	Two events in the intervention group, none in the placebo group.					
Overall evaluation of certainty in the evidence on headache								
Initial rating	Elements triggering downgrading			Elements triggering upgrading			Overall rating	
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	

DHA+EPA	Serious	Not evaluated	Not serious	Serious	No	No	Not evaluated	Very-low
1 RCT, initial rating ++++		Serious						
DHA	Serious	Not evaluated	Not serious	Serious	No	No	Not evaluated	Very-low
1 RCT, initial rating ++++		Serious						
VKM concludes:								
<ul style="list-style-type: none"> • There is very-low certainty evidence that daily doses of 336 mg EPA and 84 mg DHA for three months do not or only to a small extent increase the risk of headache in children aged 6-8 years. • There is very-low certainty evidence that a daily dose 722 mg DHA for 12 months do not or only to a small extent increase the risk of headache in children aged 2.5-8 years. • No studies on EPA alone were included. 								

4.4.4.8 Inflammatory markers

Systematic reviews, RCTs and risk assessments/reports addressing inflammatory markers were included (Table 4.4.4.8 -1, 4.4.4.8-2 and 4.4.4.8-3).

Table 4.4.4.8-1. Systematic reviews addressing inflammatory markers.

Reference	Study/ intervention	Results, certainty in the evidence
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<p>Mocellin et al. (2016)</p>	<p><u>Studies with EPA and DHA exposure:</u></p> <p>Zhu et al., 2012. 7 postoperative days. Clinical trial, parallel design. Surgical patients, n=29 (intervention); 28 (control) Intervention: EPA+DHA, 0.2 g/kg per day (parenteral). Markers: TNF-α; IL-6. RoB: low.</p> <p>Liang et al., 2008. 7 postoperative days. Clinical trial, parallel design. Surgical patients, n=20 (intervention); 21 (control) Intervention: EPA+DHA, 0.2 g/kg per day (parenteral). Markers: TNF-α; IL-6. RoB: low.</p> <p>Mocellin et al., 2013. 9 weeks. Clinical trial, parallel design. Chemotherapy patients, n=6 (intervention); 5 (control) Intervention: EPA+DHA, 0.6 g per day (oral). Markers: TNF-α; IL-1β; CRP. RoB: low for five domains. Unclear for blinding, high for allocation concealment.</p> <p>Pastore-Silva et al., 2012. 9 weeks. Clinical trial, parallel design. Chemotherapy patients, n=10 (intervention); 8 (control) Intervention: EPA+DHA, 0.6 g/per day (oral). Markers: TNF-α; IL-6; IL-1β; CRP.</p>	<p>Benefits on some inflammatory mediators with the use of n-3 PUFA on colorectal cancer patients were suggested, but these benefits are specific to certain supplementation protocols involving duration, dose and route of administration, and also, the concomitant anti-cancer treatment adopted.</p> <p>N-3 PUFA reduce the levels of IL-6 (Standard mean differences (SMD) -2.34; 95% CI -4.37, -0.31; p ¼ 0.024) in overall analyses.</p> <p>In stratified analyses, reduction in IL-6 levels occurs in surgical patients that received 0.2 g/kg of fish oil parenterally at postoperative period (SMD -0.65; 95% CI -1.06, -0.24; p ¼ 0.002),</p> <p>In patients undergoing chemotherapy, the supplementation of 0.6 g/day of EPA + DHA during 9 week reduces CRP levels (SMD -0.95; 95% CI -1.73, -0.17; p ¼ 0.017).</p>
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	<p>RoB: low for four domains. Unclear for blinding, and high for adequate sequence generation and allocation concealment.</p> <p>Purasiri et al., 1994. 6 months. Clinical trial, parallel design. Follow-up patients, n=14 (intervention); 6 (control) Intervention: EPA+DHA, 2.4-4.8 g/per day (oral). Markers: TNF-α; IL-6; IL-1β. RoB: low for four domains. Unclear for adequate sequence generation, allocation concealment and blinding.</p> <p>Braga et al., 2002. 9 perioperative days or 5 preoperative days. Clinical trial, parallel design. Surgical patients, n=50 (intervention); 50 (control) Intervention: EPA+DHA, 3.3 g/per day (Oral on preoperative days and Enteral on postoperative days). Markers: IL-6. RoB: low for five domains. Unclear for allocation concealment and blinding.</p> <p>Sorensen et al., 2014. 14 perioperative days. Clinical trial, parallel design. Surgical patients, n=74 (intervention); 74 (control) Intervention: EPA+DHA, 3.0 g/per day (Oral on preoperative days and Enteral on postoperative days). Marker: CRP. RoB: low.</p>	
Su et al. (2021)	<p><u>Studies with EPA + DHA exposure:</u></p> <p>Overall RoB for the studies was considered serious.</p>	No significant association was found for n-3 PUFAs and CRP. The grade certainty was moderate.

	<p>Hammer et al. 2019. 3 months. RCT. N=70, all patients with lower extremity arterial disease. 2.2 g EPA + 1.8g DHA.</p> <p>Schiano et al. 2008. 3 months. RCT. N=32, all patients with lower extremity arterial disease. 2 g/day (EPA:DHA 0.9:1.5).</p> <p>Ramirez et al. 2019. 3 months. RCT. N=24, all patients with lower extremity arterial disease. 2.6 g EPA + 1.8 g DHA.</p> <p><u>Studies with EPA exposure:</u></p> <p>Gans et al. 1990. 7 weeks. RCT. 19 participants, all patients with lower extremity arterial disease. 1.8 g EPA.</p>	<p>4 trials; n=166; Effect sizes (95%CI): -0.0164 (-0.3215; 0.2887), p= 0.9162.</p>
<p>VKM concludes: VKM considers the body of evidence sufficient to conclude on EPA and DHA combined exposure, but insufficient for EPA alone due to no possibility to separate the effects from those of EPA and DHA alone and due to low number of studies. There were no studies on exposure to DHA alone. Data indicate that exposure to EPA and DHA combined do not cause inflammation in adults. However, as we lack ADME data and no assessment of certainty in evidence were performed, the data will only be used as supporting evidence.</p> <p>Summary: Mocellin et al., (2016) and Su et al., (2021), systematic reviews with unclear and low RoB, respectively, have summarized data on inflammatory markers following exposure to EPA+DHA and EPA alone.</p> <p>In Mocellin et al., (2016), data from clinical trials on inflammatory markers following exposure to EPA+DHA (seven trials) were summarised. The doses ranged from 0.6-4.8 g/day. The participants were surgical patients, chemotherapy patients and follow-up patients, and the number of patients in each trial ranged from 11-148. Mean age ranged from 50-71 years. Benefits on some inflammatory mediators with the use of n-3 PUFA on colorectal cancer patients</p>		

were suggested, but these benefits were specific to certain supplementation protocols involving duration, dose and route of administration, and also, the concomitant anti-cancer treatment adopted. No assessment of certainty in evidence was performed.

Su et al., (2021) included data from RCTs on inflammatory markers following exposure to EPA+DHA (three trials) and EPA alone (one trial). For EPA+DHA, the doses ranged from 2.0 to 4.4 g/day. For EPA alone, the dose tested was 1.8 g/day. The mean age of the participants were > 50 years, and the exposure period ranged from seven weeks to three months. Overall RoB for the studies on was considered to be serious. No significant association was found for n-3 PUFAs and CRP. The GRADE certainty was moderate. All study results, irrespective of type of intervention, were summarised combined, which limits the value of the findings.

Table 4.4.4.8-2. RCTs addressing inflammatory markers in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results
Chang et al. (2019)	Youth with Attention Deficit Hyperactivity Disorder. N = 92, age 6–18-years-old. Dose: 1.2 g/day EPA or placebo (1.2 g/day soybean oil). Study duration: 12 weeks.	Tier 2	No effect on hs-CRP.
Chase et al. (2015)	Infants with a high genetic risk for type 1 diabetes (T1D). N= 41. Group A mothers were randomized to receive DHA (800 mg/d) or corn/soy oil (800 mg/d) in the last trimester of pregnancy and continued on this same dose after delivery if breast-feeding. Formula fed infants received formula with 10.2 mg DHA/ounce (treatment) or 3.4 mg DHA/ounce (control). Formula-fed infants and infants of breast-feeding mothers in Group B (57 infants) were randomized in the first five postnatal months to receive similar dosages of DHA or corn/soy oil as their counterparts in Group A.	Tier 2	High-sensitivity C-reactive protein, was significantly lower in breast-fed DHA-treated infants compared to all formula-fed infants at the age of 12 months.
de Ferranti et al. (2014)	Hypertriglyceridemic adolescents. N=25, aged 10-19 years. Dose: ~3360 mg DHA + EPA per day vs placebo. 3 months.	Tier 2	No effect on CRP.

Engler et al. (2004)	Children with familial combined hyperlipidemia. N=20, aged 9-19 years. DHA dose: 1.2 g/day. Study duration: 6 weeks.	Tier 2	No effect on CRP.
Janczyk et al. (2015)	Children with nonalcoholic fatty liver disease. 64 completed the trial. Median age was 13 years. Dose: Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day; body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day; body weight >60 kg: 800 mg DHA and 532.5 mg EPA per day. Study duration was 24 weeks.	Tier 2	No effects reported.
Pacifico et al. (2015)	Children with biopsy-proven nonalcoholic fatty liver disease. 58 participants randomized, 51 (25 DHA, 26 placebo) completed the study. Age at study start was 10.8 (2.8) years for placebo and 11.0 (2.6) years for intervention. DHA dose: 250 mg/day. Study duration was 6 months.	Tier 1	No effect on hs-CRP.
Rodriguez et al. (2018)	Boys with Duchenne Muscular Dystrophy. N=36, between 3 and 18 years (inclusion criteria). Dose: 450 mg EPA + 2250 mg DHA + 200 mg of additional omega 3 fatty acids per day. Study duration was 6 months.	Tier 1	Omega-3 LC-PUFA intake decreased the serum IL-1 β (-59.5%; p -0.011) and IL-6 (-54.8%; p -0.041), and increased the serum IL-10 (99.9%, p<0.005), in relation to those with placebo treatment.
Smuts et al. (2015)	Healthy Fe-deficient school children. N=98, 6-11 years. Dose: 420 mg DHA + 80 mg EPA. Study duration was 8.5 months.	Tier 1	No effects on CRP (baseline: 0.57 (0.00-10.30); completed study: 0.70 (0.00-7.32)).

Overall evaluation of certainty in the evidence on inflammatory markers

Initial rating	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose-response relationship	Consistency	
DHA+EPA 4 RCTs, initial rating ++++	Serious	Not serious	Not serious	Serious	No	No	Yes	Moderate

DHA 3 RCTs, initial rating ++++	Serious	Not serious	Not serious	Serious	No	No	Yes	Moderate
EPA 1 RCT, initial rating ++++	Serious	Not evaluated Serious	Not serious	Serious	No	No	Not evaluated	Very-low
VKM concludes:								
<ul style="list-style-type: none"> • There is very-low certainty evidence that a daily dose of 1.2 g EPA for 12 weeks do not cause inflammation in children aged 6-18 years. • There is moderate certainty evidence that a daily dose of 1.2 g DHA for six weeks and 250 mg DHA for six months do not cause inflammation in infants <5 months and children aged 8-18 years. • There is moderate certainty evidence that daily doses of 1800 mg EPA + 1500 mg DHA for three months and doses up to 450 EPA + 2250 DHA for six months do not cause inflammation in children aged 3-18 years. 								

Table 4.4.4.8-3. Risk assessments addressing inflammatory markers.

Reference	Direct quotation from summary / conclusion in the risk assessment
EFSA, 2012	<i>"The Panel considers that supplemental intakes of EPA and DHA up to about 5g/day are unlikely to induce changes in immune functions which might raise concern in relation to the risk of infections or inappropriate activation of inflammatory responses. The Panel notes that the data available are insufficient to conclude on whether the same doses administered mostly as EPA or mostly as DHA would have different effects on this outcome."</i>
VKM, 2015	<i>"According to the EFSA opinion from 2012, there are no human intervention studies available that have investigated the effects of n-3 LCPUFA supplementation on the risk of infections (EFSA, 2012). There are some indications, from ex vivo and in vitro studies performed in peripheral blood white cells of human subjects consuming n-3 LCPUFA, that EPA and DHA may decrease the expression of cytokines and the</i>

proliferation of peripheral white blood cells at doses as low as 0.9g/day EPA and 0.6g/day DHA consumed as fish oil for 6-8 weeks as reviewed in IoM, 2005. However, the clinical relevance of these changes is unknown.

Furthermore, there is no available information on the effect of a high intake of n-3 LCPUFA on the risk of chronic diseases with antiinflammatory component. Some markers of the so-called low-grade systemic inflammation (e.g. high-sensitivity C-reactive protein, and some cytokines) and vascular (e.g., sICAM-1, VCAM-1, and E-selectin) have been associated with an increased risk of cardiovascular events in healthy and high-risk subjects. However, there is no evidence that changes induced by diet or drugs in any of these markers modify the risk of disease per se. Most of the intervention studies available that report on the effects of EPA and DHA on markers of systemic and vascular inflammation are small and generally not designed for the purpose. Although an increase in E-selectin and/or in sVCAM-1 has been reported in some studies at doses of EPA and DHA of about 5g/day, a recent meta-analysis of 18 randomised controlled trials found no effect of n-3 LCPUFA supplementation (dose 0.272 to 6.6g/day) on these markers of vascular inflammation nor a significant decrease in sICAM-1 (Yang et al., 2012) included in EFSA 2012). The majority of the studies report either no effect or a decrease in systemic markers of inflammation, including hs-CRP (high-sensitivity C-reactive protein) and TNF-alpha (Bloomer et al., 2009; VKM, 2011). EFSA (2012) noted that the data available are insufficient to conclude on whether the same doses administered mostly as EPA or mostly as DHA would have different effects on this outcome.”

VKM (2015) reported that none of the studies included reported on adverse effects related to immune function.

VKM concludes: The findings on inflammatory markers from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.

Summary: EFSA (2012) considered that supplemental intakes of EPA and DHA up to about 5 g/day are unlikely to induce changes in immune functions which might raise concern in relation to the risk of infections or inappropriate activation of inflammatory responses. EFSA noted that the data available are insufficient to conclude whether the same doses administered mostly as EPA or mostly as DHA would have different effects on this outcome.

4.4.4.9 Joint, lumbar and muscle pain

Systematic reviews and RCTs addressing joint, lumbar and/or muscle pain were included (Table 4.4.4.9-1 and 4.4.4.9-2).

Table 4.4.4.9-1. Systematic reviews addressing joint, lumbar and/or muscle pain.

Reference	Study/ intervention	Results, certainty in the evidence
Abdelhamid et al. (2020)	<p><u>Studies with combined EPA and DHA exposure:</u></p> <p>Dream Asbell 2018. 12 months. RCT with parallel design. N: 349 intervention randomised, 186 control randomised. Adults with dry eye. Intervention: omega-3 supplements (2000 mg EPA + 1000 mg DHA/day as 5 gel caps). Dose: 3.0 g/day LCn3. Control: olive oil supplements (5 gel caps). RoB: low.</p>	
	<p><u>Studies with EPA exposure:</u></p> <p>JELIS 2007. 5 years. N: intervention, 9326, control 9319 (analysed intervention 9326, control 9319). People with hypercholesterolaemia Intervention: 3 × 2 × 300 mg capsules/day EPA ethyl ester (total dose of 1.8 g/day EPA), after meals. Dose: 1.8 g/day EPA. Control: nothing (though all in both groups received "appropriate" dietary advice). RoB: moderate or high.</p>	<p>Joint, lumbar and muscle pain: meta-analysis of data from three trials suggested that LCn3 had little or no effect on such pain (RR 0.95, 95% CI 0.74 to 1.23; I²=61.51% > 27,000 participants, 989 people experienced pain).</p>
	<p>REDUCE-IT 2019. 4.9 years (median). RCT with parallel design N: intervention 4089 randomised, control 4090 randomised (analysed, intervention: 4083 control: 4077). People (45 years+) with hypertriglyceridaemia, and with cardiovascular disease or with diabetes mellitus and another risk factor, and on statin. Intervention: EPA ethyl ester derived from fish oil (AMR101 4 g/d, Amarin), 3.99 g/day EPA plus 8 mg/day vitamin E (2 capsules twice/day). Control: 3.73 g/day light liquid paraffin oil in 4 capsules (2 capsules twice a day). RoB: moderate or high.</p>	

Irving et al. (2006)	<p>Peet 2002: 12 weeks. 122 participants in total, only 115 participants analysed. People with schizophrenia or similar chronic mental illnesses.</p> <p>Ethyl-eicosapentenoic acid, 1 g/day, n=32. Ethyl-EPA, 2 g/day, n=32. Ethyl-EPA, 4 g/day, n=27. Placebo, n=31.</p> <p>Risk of bias assessment: Low risk of selection bias and observer bias. Unclear risk for attrition. High risk for selective reporting and source of funding.</p>	<p>Peet 2002 recorded how many people have experienced musculoskeletal adverse effects. No differences were apparent for any dose of E-EPA supplementation compared with placebo.</p>
<p>VKM concludes: There were no data on children and adolescents. There is insufficient evidence to conclude whether exposure the combination of EPA and DHA constitute a risk of joint, lumbar and muscle pain. Data on adults suggested that doses of EPA up to 3.99 g for up to five years do not cause joint, lumbar or muscle pain. However, due to lack of ADME data and lack of certainty in evidence assessment, the findings will only be used as supporting information. VKM considers the outcome to be of relevance for children and adolescents.</p> <p>Summary: Two systematic reviews have summarised effects of EPA+DHA and EPA alone (Abdelhamid 2020) and of EPA alone (Irving 2006).</p> <p>In Abdelhamid 2020, Three RCTs were identified, where two assessed effects following exposure to EPA alone with doses of 1.8-3.99 g per day for up to five years. These two studies combined covered more than 13,000 participants. For the combined exposure of EPA and DHA one study with daily doses of 2g EPA and 1g DHA for 12 months was identified. The number of participants was <400. The summary findings of the three RCTs indicate no effect of EPA+DHA and EPA alone (summarised together) on joint, lumbar and muscle pain. VKM notes that all study results, irrespective of type of intervention, were summarised combined. VKM also notes that the two studies with exposure to EPA alone has a much larger N and thus VKM considers that the summary findings is applicable for the assessment of effects of EPA alone.</p> <p>In Irving 2006, only one short-term RCT were identified. No muskoskeletal adverse effects were observed for doses up to 4 g/day for 12 weeks. VKM considers that the study duration is too short and number of participants is too small to use this study as a basis for conclusion on muscle pain.</p>		

Table 4.4.4.9-2. RCTs addressing joint, lumbar and/or muscle pain in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results
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Cornu et al. (2018)	Children with established diagnosis of ADHD. N=148. Children aged 6–8 years, EPA 336 mg and DHA 84 mg; for children aged 9–11 years, EPA 504 mg and DHA 126 mg, and for children aged 12–15 years EPA 672 mg and DHA 168 mg. Study duration was 3 months.	Tier 2	Eleven (14.9%) children in the DHA–EPA group experienced 13 adverse events, 2 of which were judged to be related to the study treatment by the study authors: hip pain, fatigue, headache, fever and cough (n = 2), dermatitis and allergic skin reaction (n = 4), abdominal pain, diarrhoea (n = 3), and depression. None in the placebo group experienced such effects.					
de Ferranti et al. (2014)	Hypertriglyceridemic adolescents. N=25, aged 10-19 years. Dose: ~3360 mg DHA + EPA per day vs placebo. 3 months.	Tier 2	No effects reported on back pain, muscle or joint ache.					
Overall evaluation of certainty in the evidence on joint, lumbar and/or muscle pain								
Initial rating	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
DHA+EPA 2 RCTs; initial rating ++++	Serious	Not serious	Not serious	Serious	No	No	Not evaluated	Low
VKM concludes:								
<ul style="list-style-type: none"> • There is low certainty evidence that daily doses up to 1800 mg EPA and 1500 mg DHA for three months do not cause or to a small extent cause joint, lumbar or muscle pain in children aged 6-18 years. • No studies on DHA alone were included. • No studies on EPA alone were included. 								

4.4.4.10 Lipid profile

Systematic reviews, RCTs and one report/risk assessment addressing effects on lipid profile were included (Table 4.4.4.10-1, 4.4.4.10-2 and 4.4.4.10-3).

Table 4.4.4.10-1. Systematic reviews addressing lipid profile effects.

Reference	Study/ intervention	Results, certainty in the evidence
Abdelhamid et al. (2020)	<p><u>Studies with EPA+DHA exposure:</u></p> <p>Ahn 2016. 12 months. RCT, parallel design N: 38 intervention, 36 control. Statin-treated coronary artery disease patients undergoing percutaneous coronary intervention. Intervention: 3 g of ω-3 PUFA containing 1395 mg of EPA and 1125 mg of DHA/day. Dose: +2.52 g/day EPA + DHA. Control: unclear whether control group were given placebo or only statins. RoB: moderate or high.</p>	<p>GRADE assessment suggests high certainty evidence that LCn3intake makes little or no difference to serum total cholesterol. Mean difference (random, 95% CI): -0.01, 95% CI -0.05 to 0.03; $I^2 = 14.34\%$; 30 trials, 35,000> participants.</p>
	<p>AlphaOmega - EPA+DHA 2010. 40 months. RCT. N: 1192 EPA/DHA intervention, 1236 control. Adults with previous myocardial infarction. Intervention: 20 g/day enriched margarine incorporating 400 mg EPA-DHA (240 mg EPA and 160 mg DHA). Dose: average achieved 376 mg/day EPA + DHA. Control: 20 g/day margarine. No additional n-3 PUFAs. Identical margarine (oleic acid) placebo. RoB: low.</p>	<p>GRADE assessment suggests high certainty evidence that LCn3intake has little or no effect on HDL cholesterol. Mean difference (random, 95% CI): 0.03, 95% CI -0.01 to 0.05; $I^2 = 50.41\%$; 30 trials, 40,000> participants.</p>
	<p>ASCEND 2018. 7.4 years. RCT, parallel design. N: 7740 intervention, 7740 control.</p>	<p>GRADE assessment suggests high certainty evidence that LCn3 intake makes little or no difference to LDL cholesterol. Mean difference (random, 95% CI): 0.01, 95% CI -0.01 to 0.03; $I^2 = 0\%$; 25 trials, 40,000> participants.</p>

	<p>Intervention: 840 mg/day EPA+DHA (460 mg/day EPA plus 380 mg/day DHA) as 1 capsule daily.</p> <ul style="list-style-type: none"> • Arm 1: omega-3 (1 g/d: 0.41 g EPA, 0.34 g DHA) and placebo tablets for aspirin. • Arm 3: omega-3 (1 g/d) and aspirin (100 mg/d) Control: 1 capsule/day of olive oil. • Arm 2: aspirin (100 mg/d) and olive oil placebo capsule. • Arm 4: olive oil placebo and placebo tablets for aspirin. <p>RoB: moderate or high.</p> <p>Brox 2001. 14 months. RCT, parallel design. N: 40 seal oil, 40 cod liver oil, 40 control. People with moderate hypercholesterolaemia. Intervention: seal oil – 15 mL/day (2.6 g, 1.1 g/day EPA + 1.5/day DHA) (total n-3 3.9 g/d, total PUFA 4.2 g/d): Seal oil dose: EPA + DHA 2.6 g/day. Cod liver oil – 15 mL/day (3.3 g, 1.5 g/day EPA + 1.8 g/day DHA) (total n-3 4.1 g/d, total PUFA 4.35 g/d): cod liver oil dose: EPA + DHA 3.3 g/day. Control: no supplement. RoB: moderate or high.</p> <p>Caldwell 2011. 12 months. RCT, parallel design. N: 20 intervention, 21 control (analysed 17 intervention, 17 control). Participants with non-cirrhotic nonalcoholic steatohepatitis. Intervention: 3 × 1 g fish oil capsules/day (Nordic Natural) for a total 2.1 g/day n-3, each capsule contained 70% of n-3 (1050 mg EPA, 750 mg DHA + 300 mg other n-3). Dose: 1.8 g/day EPA + DHA. Control: 3 × 1 g/day identical placebo (soybean) capsules containing 8% fish oils. RoB: low.</p> <p>Derosa 2016. 18 months. RCT, parallel design. N: 138 intervention, 143 control (analysed 128 intervention, 130 control). Overweight/obese adults with impaired fasting glucose or impaired glucose tolerance. Intervention: 3 × 1 g capsule/day n-3 PUFAs (ethyl esters, each 1-g capsule of n-3</p>	
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	<p>PUFAs contains highly concentrated ethyl esters of omega-3 FAs, primarily EPA, and DHA in the proportion of 0.9–1.5). Dose: unclear (approx 2-3 g/d). Control: placebo (a capsule containing sucrose, mannitol and mineral salts, magnesium stearate (a saturated fat) and silicon dioxide, used as anti-caking agents). RoB: low.</p> <p>Deslypere 1992. 12 months. RCT. N: 14 high-, 15 medium-, 15 low-dose intervention, 14 control. Healthy adults. Intervention 9 capsules (9 g vol)/day, of which 3, 6 or 9 were fish oil and any remainder were placebo (providing respectively 1.12; 2.24 or 3.37 g n-3 FA/day). Dose: 1.12 g/d; 2.24 g/day or 3.37 g/day EPA + DHA). Control: 9 placebo capsules made up of olive oil and Palmoil with the same SFA, cholesterol and vitamin E as the fish oil capsules. RoB: moderate or high.</p> <p>DIPP 2015. 24 months. RCT, parallel design. N: 104 intervention, 101 control. Adults previously polypectomised for colorectal tumours. Intervention: advice to reduce total fat intake, decrease consumption of n-6 PUFAs, increase intake of n-3 PUFAs from fish/marine foods, increase intake of n-3 PUFAs from perilla oil rich in ALA, take 8 capsules of fish oil/day (equivalent to 96 mg/day of EPA and 360 mg/day of DHA). Dose: 456 mg/day EPA + DHA and unknown dose of ALA. Control: advice to decrease intake of fats/oils as a whole. RoB: moderate or high.</p> <p>DO IT 2010. 36 months. RCT, parallel design. N: intervention 282 (140 n-3 capsules + 142 n-3 capsules and dietary advice), control 281 (142 placebo capsules + 139 placebo capsules and dietary advice). Men with longstanding dyslipidaemia or hypertension. Intervention: 2 × 2 capsules/day including 2.4 g/day of omega-3 PUFA (Pikasol, 0.84 g/day EPA plus 0.48 g/day DHA plus 8.4 mg/day tocopherols). Dose: 1.32 g/day EPA + DHA.</p>	
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	<p>Control: 2 × 2 capsules/day including 4 g/day corn oil (2.24 g/day linoleic, 1.28 g/day oleic acid, 16 mg/day tocopherols). RoB: moderate or high.</p> <p>EPE-A 2014. 12 months. RCT with parallel design. N: 86 intervention-high, 82 intervention low, 75 control. People with nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. RCT with parallel design. Comparison 1: high EPA vs low EPA (unclear what replaced EPA) Comparison 2: EPA vs unclear (placebo contents not reported). Intervention-high: EPA-E 2.7 g/d, 3 × EPA-E 300 mg capsules. Dose: 2.7 g/day EPA + DHA Intervention-low: EPA-E 1.8 g/d, 2 × EPA-E 300 mg capsules + 1 placebo capsule Dose: 1.8 g/day EPA + DHA. Control: 3 × placebo capsules. The pills were identical with respect to size, colour and smell. RoB: moderate or high.</p> <p>Franzen 1993. 12 months. RCT with parallel design. N: 15 intervention, 15 control. Adults with documented coronary heart disease. Intervention: 9 × 1 g capsules/day of fish oils (20% EPA, 15% DHA, and 3.15 g/day total omega-3). Dose: 3.15 g/day EPA + DHA. Control: 9 × 1 g capsules/day olive oil (which contains 6.3 g/day MUFA, 1.35 g/day SFA, 1.35 g/day total omega-6 fat). RoB: moderate or high.</p> <p>HARP 1995. 24 months. RCT. N: 41 intervention, 39 control. Adults with coronary heart disease. Intervention: 12 fish oil capsules/day in divided doses, preferably after meals. Each fish oil capsule contained 500 mg of n-3 polyunsaturated FAs composed of EPA (240 mg), DHA (160 mg) and other (100 mg) (mainly DPA) providing total daily dose of 6 g of n-3 FAs. Dose: 6 g/day LCn3.</p>	
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	<p>Control: olive oil capsules identical in appearance to the fish oil capsules. RoB: moderate or high.</p> <p>HEARTS 2017. 30 months. RCT. N: 143 intervention randomised, 142 control randomised. People with stable coronary artery disease on statins. Intervention: LCn3 ethyl esters from fish oil, 4 x 1000 mg capsules/day. 3.36 g/day LCn3 (1.86 g/day EPA, 1.5 g/day DHA). Control: nil (no placebo). RoB: moderate or high.</p> <p>MARINA 2011. 12 months. RCT, parallel design. N: intervention. 279 in 3 groups (G1 0.45 g/day n = 94, G2 0.9 g/day n = 93, G3 1.8 g/day n = 92); control: 88 (analysed G1 0.45 g/day n = 81, G2 0.9 g/day n = 80, G3 1.8 g/day n = 80, control 71). Non-smoking men and women aged 45-70 years. Intervention: 3 x 1 g oil gelatin capsule/day consisting of blend of EPA concentrate, DHA concentrate, refined olive oil and 0.1% peppermint oil. Providing a daily dose of: 0.45 g, 0.9 g, or 1.8 g/day (all with EPA/ DHA ratio of 1.51). Dose: 1.8 g/day EPA + DHA (G3 used for outcomes). Control: 3 gelatin capsules/ day containing refined olive oil + 0.1% peppermint oil. RoB: low.</p> <p>NAT2 2013. 36 months. N: 150 intervention, 150 control. People with early age-related macular degeneration. Intervention: 3 daily fish oil capsules containing 1110 total n-3 FAs (EPA: 270 mg/day DHA: 840 mg/d) and vit E: 6 mg/day. Dose: 1.1 g/day EPA + DHA. Control: 3 x 602 mg/day olive oil capsules containing 0.2 g total PUFA and vitamin E: 0.09 g/day. RoB: low.</p> <p>OFAMI 2001. 2 years. RCT with parallel design.</p>	
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	<p>N: 150 intervention, 150 control. Participants recruited 4-8 days after confirmed myocardial infarction. Intervention: 4 gelatin capsules of omega-3-acid ethyl esters 90, each is 1 g containing 850-882 mg EPA and DHA as concentrated ethylesters. Dose ~3.4- 3.5 g/day EPA + DHA. Control: corn oil capsules, 4/d, each contains 1 g of corn oil. RoB: moderate or high.</p> <p>ORIGIN 2012. 72 months. RCT, 2x2 factorial. N: 6319 intervention, 6292 control (analysed, intervention: 6281 control: 6255). People at high risk of cardiovascular events with impaired fasting glucose, impaired glucose tolerance or diabetes. Intervention: 1 gelatin capsule/day Omacor containing at least 900 mg ethyl esters of n-3 fats (465 mg EPA + 375 mg DHA). Dose: 0.84 g/day EPA + DHA. Control: 1 × 1 g gelatin capsule/day olive oil. RoB: low.</p> <p>ORL 2013. 12 months. N: 171 intervention (4 g TAK), 165 control (2 g TAK). Adults with hypertriglyceridaemia. Intervention: 1 × 2/day capsule each containing 2 g of TAK-085 (1 g of FA in TAK-085 capsules contains approximately 465 mg of EPA-E plus 375 mg of DHA-E). Total dose of 1.86 g/day EPA + 1.5 g/day DHA. Dose: ~3.4 g/day EPA + DHA (difference of +1.7 g/day from control arm). Control: 1 capsule/day containing 2 g of TAK-085 (1 g of FA in TAK-085 capsules contains approximately 465 mg of EPA-E plus 375 mg of DHA-E). Total dose of 0.93 g/day EPA + 0.75 g/day DHA. Dose: 1.7 g/day EPA + DHA. RoB: moderate or high.</p> <p>Rossing 1996. 12 months. N: 18 intervention, 18 control (analysed, 17 intervention, 15 control). Adults with insulin-dependent diabetes mellitus. Intervention: cod-liver oil emulsion. EPA 2 g, DHA 2.6 g, total PUFA 4.6 g/day. Dose: 4.6 g/day EPA + DHA.</p>	
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	<p>Control: olive oil emulsion. RoB: moderate or high.</p> <p>Sandhu 2016. 24 months. RCT with parallel design. N: 54 + 53 intervention, 53 + 53 control. Healthy postmenopausal women. Intervention: group 4, Lovaza 4 g/day. Lovaza is the FDA-approved n-3 FA formulation containing 465 mg of EPA + 375 mg of DHA per gram, total dose; 1860 mg/day EPA, 1500 mg/day DHA. Group 5 as group 4 plus 30 mg raloxifene/day. Dose: 3.36 g/day EPA + DHA. Control: group 1, no treatment; group 3, 30 mg raloxifene/day. RoB: moderate or high.</p> <p>SCIMO 1999. 2 years. RCT with parallel design. N: 112 intervention, 111 control (analysed 82 intervention, 80 control). People with angiographically proven coronary artery disease. Intervention: concentrated fish oil capsules, 6 x 1 g capsules/day for first 3 months, 3 x 1 g/day for rest of trial (4 g/day EPA +DHA + DPA + ALA for first 3 months, then 2 g/d). Dose: ~2 g/day LCn3. Control: capsules containing fat that replicated the fat composition of the average European diet, 6/day for first 3 months, 3/day for rest of trial, opaque soft gelatin capsules identical to fish capsules in identical screw-top containers. RoB: low.</p> <p>SHOT 1996. 1 year. RCT with parallel design. N: 317 intervention, 293 control. People admitted for coronary artery bypass graft. Intervention: 4 fish-oil concentrate soft gelatin capsules/day containing 51% EPA and 32% DHA ethyl esters and 3.7 mg vitamin E as an antioxidant. Dose: 3.3 g/day EPA + DHA. Control: no treatment. RoB: moderate or high.</p>	
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	<p>SMART 2013. 12 months. RCT. N: fish + S intervention 41, fish 43, control 42. Overweight adults. Intervention, fish + S: hypocaloric diet aiming at 30% E from fat, 25% E from protein, 45% E from CHO, plus 180 g fish/week plus capsules including 420 mg/day EPA + 210 mg/day DHA. Dose: 0.63 g/day EPA + DHA. Intervention, fish: hypocaloric diet aiming at 30% E from fat, 25% E from protein, 45% E from CHO, plus 180 g fish/week plus capsules including 1 g olive oil/day. Control: hypocaloric diet aiming at 30% E from fat, 25% E from protein, 45% E from CHO, plus capsules including 1 g olive oil/day. RoB: moderate or high.</p> <p>Sofi 2010. 12 months. RCT with parallel design. N: 6 intervention, 5 control. People with non-alcoholic fatty liver disease. Comparison: EPA + DHA vs MUFA Intervention: 6.5 mL/day olive oil enriched with n-3 containing 0.47 g EPA, 0.24 g DHA plus dietary recommendations. Dose: 0.83 g/day EPA + DHA. Control: 6.5 mL/day olive oil plus dietary recommendations. RoB: moderate or high.</p> <p>Tande 2016. 12 months. RCT with parallel design. N: 64 intervention, 63 control (50 intervention, 50 control analysed). Healthy males and females. Intervention: 2 × 500 mg Calanus oil capsules twice daily to provide a daily dose of 2 g. The Calanus oil contained approximately 85% wax ester with a sum of neutral lipids > 90%. Dose: 2 g/day EPA + DHA. Control: identical capsules of olive oil. Compositional analysis indicated that the FA content of the olive oil was primarily oleic acid (76.9%), palmitic acid (10.2%), and linoleic acid (7.7%). RoB: moderate or high.</p>	
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	<p>THIS DIET 2008. 24 months. RCT with parallel design. N: 51 intervention, 50 control. Recent survivors of 1st MI (within < 6 weeks). Intervention: Mediterranean-style diet high in n-3. Dose: ~1.5 g/day omega-3 fat, or 0.31% E by intake assessment. Control: dietary advice (to follow the American Heart Association Step II diet). RoB: moderate or high.</p> <p>Weinstock-Guttman 2005. 12 months. RCT with parallel design. N: 15 intervention, 16 control (analysed, intervention: 13, control: 14). Adults with MS. Intervention: 1.98 g/day EPA, 1.32 g/day DHA supplements + low fat diet (< 15% total calories). Dose: 3.3 g/day EPA + DHA. Control: one 1 g olive oil placebo capsules 6 times/d, moderate-fat diet (< 30% total calories) (AHA Step 1 diet). RoB: moderate or high.</p> <p>WELCOME 2015. 15-18 months. RCT with parallel design. N: 51 intervention, 52 control (analysed, 47 intervention, 48 control). Patients with non-alcoholic fatty liver disease. Intervention: 4 g OMACOR/day (providing 1.84 g EPA, 1.52 g DHA as ethyl esters). Dose: 3.36 g/day EPA + DHA. Control: 4 g olive oil capsules/day (providing; ALA 1%, oleic acid 67%, palmitic acid 15%, stearic acid 2%, n-6 fat: 15%). RoB: low.</p> <p><u>Studies with DHA exposure:</u></p> <p>Berson 2004. 48 months. RCT, parallel design. N: 221 randomised overall, analysed 105 intervention, 103 control. People with retinitis pigmentosa. Intervention: 6 × 500 mg capsules/day of DHA (1.2 day DHA plus 1.8 g vegetable oil)</p>	
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plus < 0.0006 mg/day tocopherols plus 15,000 IU retinyl palmitate (vitamin A). Dose: 1.2 g/day DHA.

Control: 6 × 500 mg capsules/day of soy and corn oils (half each) with 120 mg/day ALA, plus < 0.0006 mg/day tocopherols plus 15000 IU retinyl palmitate (vitamin A).

RoB: low.

Studies with EPA exposure:

DART 1989. 2 years.

RCT, parallel design.

N: 1015 intervention, 1018 control. Men recovering from myocardial infarction.

Intervention: advised to eat at least 2 weekly portions of 200-400 g fatty fish (mackerel, herring, kipper, pilchard, sardine, salmon, trout). If this was not possible, given MaxEPA capsules, 3/day (0.5 g EPA/d). 191/883 participants were taking MaxEPA at 2 years.

Advice was reinforced 3-monthly. Dose: aimed for 0.5 g/day EPA.

Control: no such dietary advice or capsules.

RoB: moderate or high.

JELIS 2007. 5 years.

N: intervention, 9326, control 9319 (analysed intervention 9326, control 9319). People with hypercholesterolaemia

Intervention: 3 × 2 × 300 mg capsules/day EPA ethyl ester (total dose of 1.8 g/day EPA), after meals. Dose: 1.8 g/day EPA.

Control: nothing (though all in both groups received "appropriate" dietary advice).

RoB: moderate or high.

Nodari 2011 HF. 12 months.

RCT with parallel design.

N: 67 intervention, 66 control (analysed, intervention: 67 control: 66). People with heart failure.

Intervention: MaxEPA capsules 12/day (2.2 g EPA). Dose: 2.2 g/day EPA.

Control: olive oil capsules, 12/d, identical to MaxEPA.

RoB: moderate or high.

	<p>Nye 1990. 1 year. RCT with parallel design. N: 36 intervention, 37 control (also 35 allocated to arm 3, aspirin and dipyridamole). People undergoing percutaneous transluminal coronary artery angioplasty. Intervention: MaxEPA capsules 12/day (2.2 g EPA). Dose: 2.2 g/day EPA. Control: olive oil capsules, 12/d, identical to MaxEPA. RoB: moderate or high.</p> <p>REDUCE-IT 2019. 4.9 years (median). RCT with parallel design. N: intervention 4089 randomised, control 4090 randomised (analysed, intervention: 4083 control: 4077). People (45 years+) with hypertriglyceridaemia, and with cardiovascular disease or with diabetes mellitus and another risk factor, and on statin. Intervention: EPA ethyl ester derived from fish oil (AMR101 4 g/d, Amarin), 3.99 g/day EPA plus 8 mg/day vitamin E (2 capsules twice/day). Control: 3.73 g/day light liquid paraffin oil in 4 capsules (2 capsules twice a day). RoB: moderate or high.</p>	
Su et al. (2021)	<p>Overall RoB for studies on total cholesterol, LDL and HDL was considered serious.</p> <p><u>Studies with EPA and DHA exposure:</u></p> <p>Mori et al. 1992. 1 month. RCT. N=29. 2.8 g. EPA + 1.8 g DHA.</p> <p>Ramirez et al. 2019. 3 months. RCT. N=24, all patients with lower extremity arterial disease. 2.6 g EPA + 1.8 g DHA.</p> <p>Schiano et al. 2008. 3 months. RCT.</p>	No significant association was found for n-3 PUFAs and HDL or LDL. The grade certainty was moderate.

	<p>N=32, all patients with lower extremity arterial disease. 2g/day (EPA:DHA 0.9:1.5).</p> <p>Woodcock et al. 1984. 7 weeks. RCT. N=19. 1.8 g EPA.</p> <p>Hammer et al. 2019. 3 months. RCT. N=70, all patients with lower extremity arterial disease. 2.2 g EPA + 1.8g DHA.</p> <p>Ramirez et al. 2019. 3 months. RCT. N=24. 2.6 g EPA + 1.8 g DHA.</p> <p><u>Studies with EPA exposure:</u></p> <p>Gans et al. 1990. 7 weeks. RCT. 19 participants, all patients with lower extremity arterial disease. 1.8 g EPA.</p> <p>Ishikawa et al. 2010. 5 years. RCT. N=173. EPA 1.8 g/day.</p> <p>Grenon et al. 2015. 1 month. RCT. N=72. 2.6 g. EPA + 1.8 g DHA.</p>	
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VKM concludes: It is not possible to conclude on the effects of EPA and DHA or EPA alone on lipid metabolism in adults since all studies irrespective of intervention are summarised together.

Summary: Abdelhamid et al. (2020) and Su et al. (2021), both systematic reviews with low RoB, have summarized data on lipid profile following exposure to EPA+DHA, DHA alone and EPA alone.

Abdelhamid et al., (2020) included data from RCTs on lipid profile following exposure to EPA+DHA (25 trials), DHA alone (one trial) and EPA alone (five trials) were summarised. For EPA+DHA, the doses ranged from 0.376 – 6 g/day. For EPA alone, the doses ranged from 0.5-3.99 g/day. For DHA, the dose was 1.2 g/day. The participants were adults, and the exposure period ranged from 1.0 to 7.4 years. The total number of participants were high. GRADE assessment suggested high certainty evidence that LCn3 intake makes little or no difference to serum total cholesterol, has little or no effect on HDL cholesterol, and little or no difference to LDL cholesterol. VKM notes that all study results, irrespective of type of intervention, were summarised combined, which limits the value of the findings.

Su et al., (2021) included data from RCTs on lipid profile following exposure to EPA+DHA (five trials) and EPA alone (four trials). For EPA+DHA, the doses ranged from 2.2-4.6 g/day. For EPA alone, the dose tested in all trials was 1.8 g/day. The mean age of the participants were > 50 years, and the exposure period ranged from one month to five years. Overall RoB for studies on total cholesterol, LDL and HDL was considered serious. No significant association was found for n-3 PUFAs and HDL or LDL. The GRADE certainty was moderate. VKM notes that all study results, irrespective of type of intervention, were summarised combined, which limits the value of the findings.

Table 4.4.4.10-2. RCTs addressing lipid profile effects in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results
de Ferranti et al. (2014)	Hypertriglyceridemic adolescents. N=25, aged 10-19 years. Dose: ~3360 mg DHA + EPA per day vs placebo. 3 months.	Tier 2	LDL levels increased significantly in the omega-3 FA group by 14 ± 6 mg/dL (p=0.02) at 3 months but not at 6 months (7 ± 8 mg/dl; p=0.37) and were unchanged in the placebo group at both timepoints. No significant changes were seen in any of the other lipid parameters within or between groups at any other time points.
Engler et al. (2004)	Children with familial combined hyperlipidemia. N=20, aged 9-19	Tier 2	DHA supplementation was associated with increased levels of total cholesterol, LDL- and HDL cholesterol concentrations.

	years. DHA dose: 1.2 g/day. Study duration: 6 weeks.							
Janczyk et al. (2015)	Children with nonalcoholic fatty liver disease. 64 completed the trial. Median age was 13 years. Dose: Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day; body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day; body weight >60 kg: 800 mg DHA and 532.5 mg EPA per day. Study duration was 24 weeks.	Tier 2	No effects were reported.					
Verduci et al. (2014)	Children with primary hyperlipidemia. N=36, 8-13 years. After an 8-week stabilization period on the Step I diet, participants were randomized to additionally receive for a 16-week period one capsule (500 mg) daily of DHA alone or a DHA plus EPA mixture (45.6% DHA; 41.6% EPA) or wheat germ oil (control).	Tier 1	An effect size (as percentage change from baseline) of +8%, -12% and -16% for high-density lipoprotein cholesterol (HDLC), total cholesterol/HDL-C ratio and triglycerides was observed in children supplemented with DHA, compared to +2%, -8% and -12%, respectively, in children supplemented with DHA plus EPA.					
Overall evaluation of certainty in the evidence on lipid profile								
Initial rating	Elements triggering downgrading			Elements triggering upgrading			Overall rating	
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose-response relationship	Consistency	
DHA+EPA	Serious	Serious	Not serious	Serious	No	No	No	Very-low
3 RCTs; initial								

rating ++++								
DHA 2 RCTs, initial rating ++++	Serious	Not serious	Not serious	Serious	No	No	Not evaluated	Low
VKM concludes: <ul style="list-style-type: none"> • There is very-low certainty evidence that daily doses up to 1800 mg EPA and 1500 mg DHA for three months causes a small increase in LDL in children and adolescents aged 8-18 years. • There is low certainty evidence that a daily dose of 1.2 g DHA for six weeks and 500 mg DHA for 16 weeks causes a small increase in LDL in children and adolescents aged 8-18 years. • No studies on EPA alone were included. 								

Table 4.4.4.10-3. Risk assessments addressing lipid profile effects.

Reference	Direct quotation from summary / conclusion in the risk assessment
EFSA, 2012	<i>"The Panel notes that supplemental intakes of EPA and DHA combined of 2-6 g/day, and supplemental intakes of mostly DHA of 2-4 g/day, increase blood concentrations of LDL-cholesterol by about 3%, and that such increase is accompanied by a decrease in TG with no changes in total (or non-HDL) cholesterol concentrations. The Panel also notes that supplemental intakes of mostly EPA at doses up to 4 g/day have no significant effect on LDL cholesterol concentrations. The Panel considers that the small increase in LDL-cholesterol concentrations associated with combined EPA and DHA supplementation or with DHA supplementation alone at the doses mentioned above may not be adverse in relation to CVD risk"</i>
VKM concludes: The findings on lipid profile from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.	

Summary: EFSA reported that supplemental intakes of EPA and DHA combined of 2-6 g/day, and supplemental intakes of mostly DHA of 2-4 g/day, increase blood concentrations of LDL-cholesterol by about 3%, and that such increase is accompanied by a decrease in TG with no changes in total (or non-HDL) cholesterol concentrations. EFSA considers that the small increase in LDL-cholesterol concentrations associated with combined EPA and DHA supplementation or with DHA supplementation alone at the doses mentioned above may not be adverse in relation to CVD risk. EFSA also reported that supplemental intakes of mostly EPA at doses up to 4 g/day have no significant effect on LDL cholesterol concentrations.

4.4.4.11 Liver and biliary tract

Systematic reviews and RCTs addressing liver and/or biliary effects were included (4.4.4.11-1 and 4.4.4.11-2).

Table 4.4.4.11-1. Systematic reviews addressing liver and biliary tract effects.

Reference	Study/ intervention	Results and certainty in the evidence as reported
Irving et al. (2006)	Peet 2002: 12 weeks. 122 participants in total, only 115 participants analysed. People with schizophrenia or similar chronic mental illnesses. Ethyl-eicosapentenoic acid, 1 g/day, n=32. Ethyl-EPA, 2 g/day, n=32. Ethyl-EPA, 4 g/day, n=27. Placebo, n=31. RoB: Low risk of selection bias and observer bias. Unclear risk for attrition. High risk for selective reporting and source of funding.	Peet 2002 recorded how many people have experienced liver and biliary tract problems. No differences were apparent for any dose of E-EPA supplementation compared with placebo.
<p>VKM concludes: There were no data on children and adolescents. There is insufficient evidence to conclude whether EPA alone increases the risk for liver and biliary effects. No studies on EPA+DHA or DHA alone were included. VKM considers this outcome of relevance for children and adolescents.</p> <p>Summary: A short-term study found no liver or biliary tract effects following exposure to up to 4 g of ethyl-EPA. No assessment of certainty in evidence was conducted. The duration of intervention was short, and the number of participants was low. VKM considers that these findings cannot be used as a basis for conclusion on liver and biliary tract effects.</p>		

Table 4.4.4.11-2. RCTs addressing liver effects in children and adolescents.

Reference	Participants/ intervention/duration				RoB	Results		
Janczyk et al. (2015)	Children with nonalcoholic fatty liver disease. 64 completed the trial. Dose: Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day; body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day; body weight >60 kg: 800 mg DHA and 532.5 mg EPA per day. Study duration was 24 weeks.				Tier 2	No differences in intervention and placebo in effect on ALT. AST levels was significantly lower in the intervention group.		
Pacifico et al. (2015)	Children with biopsy-proven nonalcoholic fatty liver disease. 58 participants randomized, 51 (25 DHA, 26 placebo) completed the study. Age at study start was 10.8 (2.8) years for placebo and 11.0 (2.6) years for intervention. DHA dose: 250 mg/day. Study duration was 6 months.				Tier 1	ALT changes – p=0.06.		
Overall evaluation of certainty in the evidence on liver effects								
Initial rating	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
DHA+EPA, 1 RCTs, initial rating +++++	Serious	Not evaluated Serious	Not serious	Serious	No	No	Not evaluated	Very-low
DHA 1 RCT, initial rating +++++	Serious	Not evaluated Serious	Not serious	Serious	No	No	Not evaluated	Very-low
VKM concludes:								

- There is very-low certainty evidence that daily doses of DHA 267-800 mg and 177.5-532.5 mg EPA for 24 weeks do not cause adverse liver effects in children and adolescents (median age of 13 years).
- There is low certainty evidence that a daily dose of 250 mg DHA for six months do not cause adverse liver effects in children aged 8-13 years.
- No studies on EPA alone were included.

4.4.4.12 *Metabolic and nutritional effects (e.g. weight gain)*

One systematic review addressing metabolic effects was included (4.4.4.12-1).

Table 4.4.4.12-1. Systematic review addressing metabolic effects.

Reference	Study/ intervention	Results, certainty in the evidence
Irving et al. (2006)	<p>Peet 2002: 12 weeks. 122 participants in total, only 115 participants analysed. People with schizophrenia or similar chronic mental illnesses. Ethyl-eicosapentenoic acid, 1 g/day, n=32. Ethyl-EPA, 2 g/day, n=32. Ethyl-EPA, 4 g/day, n=27. Placebo, n=31.</p> <p>Risk of bias assessment: Low risk of selection bias and observer bias. Unclear risk for attrition. High risk for selective reporting and source of funding.</p>	<p>Peet 2002 recorded how many people have experienced metabolic difficulties (for example weight gain). No differences were apparent for any dose of E-EPA supplementation compared with placebo.</p>
<p>VKM concludes: There were not data on children and adolescents. There is insufficient evidence to conclude whether exposure to E-EPA constitute a risk for metabolic effects in adults. No studies on DHA+EPA or DHA alone were included. It is unclear what the study authors have measured except for weight gain, and therefore the findings are of limited value.</p>		

Summary: The conclusions on metabolic effects are based on Irving et al., 2006, which is a systematic review with unclear RoB. Data from one study on metabolic effects following exposure to E-EPA alone (one trial) were summarised. No trial with exposure to EPA+DHA or DHA alone were identified.

For E-EPA, the doses were 1, 2 and 4 g/day. The participants' age ranged from 18-65 years, and the exposure period was 12 weeks. The total number of participants included in the analysis was 115. The selection bias and observer bias were low, the attrition bias was unclear, whereas the risk for selective reporting bias and source of funding bias were high.

No differences were apparent for any dose of E-EPA supplementation compared with placebo. Note that the number of participants were low, and that only one trial reporting on this outcome was included.

4.4.4.13 *Movement disorders*

One systematic review addressing movement disorders was included (Table 4.4.4.13-1).

Table 4.4.4.13-1. Systematic review addressing movement disorders.

Reference	Study/ intervention	Results, certainty in the evidence
Irving et al. (2006)	Fenton 2001. 16 weeks. 90 participants in total, only data from 87 participants. People with schizophrenia or similar chronic mental illnesses. Ethyl-EPA, 500 mg/day + vitamin. Mineral oil placebo + vitamin E. RoB: low for three domains. Unclear for adequate sequence generation, allocation concealment and other bias.	One study (Fenton 2001, RCT) reported data for movement disorders as measured by the AIMS and Simpson and Angus scales. The data were skewed and did not suggest any effect of omega-3 supplementation.

VKM concludes: There were not data on children and adolescents. There is insufficient evidence to conclude whether exposure to E-EPA constitute a risk for movement disorders in adults. No studies on DHA+EPA or DHA alone were included. VKM considers this outcome as relevant for children and adolescents.

Summary: The conclusions on metabolic effects are based on Irving et al., 2006, which is a systematic review with unclear RoB. Data from one study on movement disorders following exposure to E-EPA alone (one trial) were summarised. No trial with exposure to EPA+DHA or DHA alone were identified.

For E-EPA, the dose was 500 mg/day. The study duration was 16 weeks and number of participants was low. Findings suggested no effect of EPA on movement disorders. Assessment of certainty in evidence was not performed. VKM considers that the study duration is too short, and number of participants is too small to use this study as a basis for conclusion on movement disorders in adults.

4.4.4.14 Other side effects

Systematic reviews and RCTs addressing other side effects were included (Table 4.4.4.14-1 and 4.4.4.14-2).

Table 4.4.4.14-1. Systematic reviews addressing other side effects.

Reference	Study/ intervention	Results, certainty in the evidence
Sarmiento et al. (2016)	Yuen 2005. 12 weeks. 58 participants. RCT. Adults with a diagnosis of drug-resistant epilepsy. PUFA group: 1000 mg fish oil capsules, total daily dose 1.7 g omega-3 PUFAs (1 g EPA and 0.7 g DHA). Each capsule contained 171 mg EPA and 112 mg DHA, and < 100 IU vitamin A and < 40 IU vitamin D). Placebo: matching capsules containing mixed oils (palm olein 70%, rapeseed oil 15%, sunflower oil 15%). RoB: low.	There were a few reports of other adverse events, all in one study. In the PUFA group there was one case of sleepiness, one participant complained of fatigue and breathlessness and one had recurrence of depression and paranoia. In the placebo group there were two cases of sleepiness and one case of aggression and fatigue.

Irving et al. (2006)	<p>Peet 2002: 12 weeks. 122 participants in total, only 115 participants analysed. People with schizophrenia or similar chronic mental illnesses.</p> <p>Ethyl-eicosapentenoic acid, 1 g/day, n=32. Ethyl-EPA, 2 g/day, n=32. Ethyl-EPA, 4 g/day, n=27. Placebo, n=31.</p> <p>Risk of bias assessment: Low risk of selection bias and observer bias. Unclear risk for attrition. High risk for selective reporting and source of funding.</p>	<p>Psychosexual difficulties, infections: No statistically significant differences were found, however it appears as though there is an effect. Based on one short-term study only.</p> <p>Urinary problems and "any other" adverse effects: These effects were rare and not different for any dose. Based on one short-term study.</p>
<p>VKM concludes: It is not possible to conclude for adults with regard to other side effects, as the data are too scarce.</p>		

Table 4.4.4.14-2. RCTs addressing other side effects in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results
Crippa et al. (2019)	Children with ADHD. N=50, aged 7-14 years. Dose: 500 DHA/day. Study duration = 6 months.	Tier 2	No adverse effects reported.
Mazahery et al. (2019)	Children with autism spectrum disorder. N=73, aged 2.5 to 8 years. Dose: 722 mg DHA. Study duration = 12 months.	Tier 2	Five events were reported in the intervention group, 3 in the placebo group.
Pacifico et al. (2015)	Children with biopsy-proven nonalcoholic fatty liver disease. 58 participants randomized, 51 (25 DHA, 26 placebo) completed the study. Age at study start was 10.8 (2.8) years for placebo and 11.0 (2.6) years for intervention. DHA dose: 250 mg/day. Study duration was 6 months.	Tier 2	No adverse effects reported.

Overall evaluation of certainty in the evidence on other side effects								
Initial rating	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
DHA 3 RCTs, initial rating ++++	Serious	Not serious*	Not serious	Serious	No	No	No	Low
<p>VKM concludes:</p> <ul style="list-style-type: none"> No studies on EPA alone were included. No studies on EPA + DHA were included. There is low certainty evidence that daily doses of DHA up to 722 mg for up to 12 months causes little or no side effects for children aged 2.5-14 years. <p>*The study that reported events of side effects had longer duration, higher dose and lower age of the participants.</p>								

4.4.4.15 Oxidative stress and lipid peroxidation

One RCT (Table 4.4.4.15-1) and two risk assessments (Table 4.4.4.15-2) addressing oxidative stress and lipid peroxidation were included.

Table 4.4.4.15-1. RCT addressing oxidative stress in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results
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Engler et al. (2004)	Children with familial combined hyperlipidemia. N=20, aged 9-19 years. DHA dose: 1.2 g/day. Study duration: 6 weeks.	Tier 2	No effect on biomarkers for oxidative stress (F2 isoprostanes or 8-OH-2-dG).					
Overall evaluation of certainty in the evidence on oxidative stress								
Initial rating	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose-response relationship	Consistency	
DHA	Serious	Not evaluated	Not serious	Serious	No	No	Not evaluated	
1 RCT, initial rating +++++		Serious						
VKM concludes:								
<ul style="list-style-type: none"> • No studies on DHA+EPA were included. • There is very-low certainty evidence that a daily dose of 1.2 g DHA for six weeks do not cause oxidative stress in children and adolescents aged 9-18 years. • No studies on EPA alone were included. 								

Table 4.4.4.15-2. Risk assessments addressing lipid peroxidation.

Reference	Direct quotation from summary / conclusion in the risk assessment
EFSA, 2012	<i>"The Panel considers that supplemental intakes of EPA and DHA consumed either alone or in combination at doses up to about 5 g/day for up to 16 weeks do not induce changes in lipid peroxidation which might raise concern in relation to CVD risk as long as the oxidative stability of these n-3 LCPUFAs is guaranteed".</i>
VKM, 2015	<i>"PUFAs are generally more prone to peroxidation compared with saturated fatty acids. Enhanced oxidative stress and increased lipid peroxidation either locally in the vessel wall or systemically have been associated with the pathogenesis of atherosclerosis in humans and the</i>

relation to adverse effects (IOM, 2005; Steinberg et al., 1989; VKM, 2011). The majority of the human intervention studies used fish oils stabilised with antioxidants, but some studies did not report whether sources of EPA, DHA, or both, contained antioxidants. Only a few studies reported on the concentration of primary and secondary oxidation products in the supplements administered. The addition of antioxidants to food supplements containing n-3 LCPUFA to ensure product stability appears to be optional (EFSA, 2012). F2-isoprostanes measured in urine or plasma are reliable markers of in vivo lipid peroxidation. In 2011, VKM identified nine controlled human intervention studies that used n-3 LCPUFA-rich oils stabilised with antioxidants, mostly with vegetable oils as control (olive, maize, sunflower, safflower or soy oil), and reported on plasma or urinary F2-isoprostanes (VKM, 2011). Studies were conducted in newborns (following maternal supplementation with 4 g/day EPA and DHA from fish oil from 20 weeks of gestation until delivery) (Barden et al., 2004), pre-term infants (EPA and DHA were incorporated to the pre-term formula; 5.25-8.75 mg/100mL of formula) (Stier et al., 2001) or children/adolescents with familial hypercholesterolaemia (9-19 years, 1.2 g/day DHA) (Engler et al., 2004). The remaining studies had recruited a variety of adults who were either healthy (e.g. young men, post-menopausal women) or with various disease conditions (e.g. obesity, non-insulin-dependent diabetes mellitus, hypertension, end-stage renal disease), and used either DHA alone (0.8-4 g/day), EPA alone (1.6-4 g/day) or EPA and DHA in combination as fish oil (2-4 g/day) for three to six weeks. The studies of longer duration (six weeks) used the highest doses of EPA and DHA, both alone and in combination. Half of the studies reported a significant decrease in plasma or urinary concentrations of F2-isoprostanes in the n-3 LCPUFA group compared with the controls (Barden et al., 2004; Higdon et al., 2000; Mas et al., 2010; Mori et al., 2000; Mori et al., 2003), whereas the remaining studies did not observe significant changes between groups (Engler et al., 2004; Himmelfarb et al., 2007; Stier et al., 2001; Tholstrup et al., 2004; Wu et al., 2006). Susceptibility of LDL to oxidation has in a number of studies been reported to be increased, decreased or unchanged during consumption of EPA and DHA either from fish oil or as ethyl esters. Whereas an increased susceptibility of LDL to oxidation has been reported in some short-term studies (4-6 weeks), longer-term interventions (6-16 weeks) showed no effect (of EPA and DHA) in comparison with control (mostly vegetable oils) at doses up to about 5g/day (VKM, 2011). In two studies in which the diet was supplemented with salmon providing EPA +DHA, 1.5 g/day or 2.9 g/day (Seierstad et al., 2005) or herring providing EPA +DHA, 1.2 g/day (Lindqvist et al., 2009) the intervention had no effect of on plasma oxidised LDL concentrations in comparison with controls (EFSA, 2012). EFSA concluded that intakes of EPA and DHA consumed alone or in combination at doses up to 4 g/day for six weeks do not induce lipid peroxidation as assessed by F2-isoprostanes".

VKM (2015) reported that none of the studies included reported on adverse effects related to lipid peroxidation.

VKM concludes: The findings on lipid peroxidation from previous risk assessments is of limited value due to lack of systematic approach in the assessment and will only be used as supporting information.

4.4.4.16 *Preterm birth and birth weight*

Two systematic reviews addressing preterm birth and birth weight were included (Table 4.4.4.16-1).

Table 4.4.4.16-1. Systematic reviews addressing preterm birth and birth weight.

Reference	Study/ intervention	Results, certainty in the evidence
Newberry et al. (2016)	7 RCTs on healthy pregnant women. Intervention: Algal DHA or DHA-enriched fish oil supplementation.	Risk for preterm birth, 7 RCTs on healthy pregnant women. No significant effects on the incidence of preterm birth compared with placebo. Meta-analysis of 7 RCTs: OR 0.87 (95% CI 0.66, 1.15).
	9 RCTs and 2 observational studies on at-risk pregnant women. Intervention: EPA+DHA fish oil supplementation.	Risk for preterm birth, 9 RCTs and 2 observational studies. RCTs: No significant effects on the incidence of preterm birth compared with placebo. Meta-analysis of 9 RCTs: 0.86 (95% CI 0.65, 1.15). Observational studies showed mixed results.
	16 RCTs and 10 observational studies on healthy pregnant women. Intervention: n-3 FA supplementation.	Birth weight, 16 RCTs and 10 observational studies. RCTs: Significant increase in birth weight compared with placebo. Meta-analysis of 16 RCTs in update: WMD 74.8 (95% CI 12.4, 137.17) grams. Observational studies of dietary intake, supplement use, and biomarkers generally showed positive associations with birth weight. Original report: Mixed findings.
	12 RCTs and 3 observational studies on healthy pregnant women. Intervention: Algal DHA or DHA-enriched fish oil supplementation.	Birth weight, 12 RCTs and 3 observational studies. RCTs: Significant Increase in birth weight compared with placebo. Meta-analysis of 12 RCTs: WMD 90.12 (95% CI 2.62, 177.62) grams. Observational studies showed associations between DHA intake and biomarkers and birth weight. Original report: mixed findings.
	5 RCTs and 4 observational studies on healthy pregnant women. Intervention: EPA+DHA fish oil supplementation.	Birth weight, 5 RCTs and 4 observational studies. RCTs: No significant effects on birth weight compared with placebo. Meta-analysis of 5 RCTs: WMD 37.89 (95% CI -19.53, 95.31) grams. Observational studies showed mixed associations with birth weight. Original report: no effects.
	4 RCTs on healthy pregnant women. Intervention: Algal DHA or DHA-enriched fish oil supplementation.	Original report: no effects.

		Low birth weight, 4 RCTs. RCTs: No significant effects on risk of low birth weight compared with placebo. Meta-analysis of 4 RCTs: OR 0.72 (95% CI 0.43, 1.11).
Ren et al. (2021)	19 included RCTs; 6 RCTs had low risk of bias, 6 RCTs had unclear risk of bias, and 7 RCTs had high risk of bias.	<p>A total of 6,408 infants were investigated in relation to birth weight. There was no indication of publication bias for the outcome birth weight.</p> <p>The meta-analysis showed significant overall higher birth weight in the n-3 LCPUFA-supplemented compared to the control groups, irrespective of intake level [mean differences (MD) 57.5 g, 95% CI 26.2–88.9, n=6,408, I²=19%].</p> <p>Stratified analysis of the effects of n-3 LCPUFA intervention doses showed a higher birth weight among the supplemented individual with doses higher than 650 mg/day compared to the control groups, but not among individuals supplied with lower doses (MD 87.5 g, 95% CI 52.3–122.6, n=3,831).</p> <p>The overall certainty of the meta-analysis results for birth weight was rated as moderate.</p>
<p>VKM concludes: For preterm birth there were no data on EPA alone. Data from RCTs indicated no effect on preterm birth for both intake of DHA alone and EPA+DHA. The certainty of the evidence was low. For birth weight, the findings varied from no effect to a significant increase. Certainty of the evidence was low to moderate for one of the systematic reviews, whereas for the other systematic review assessment of the certainty in evidence was not performed. VKM considers that this outcome is of minor relevance for the children and adolescents.</p> <p>Summary: Newberry et al. 2016 summarized data on the effect of DHA alone and DHA+EPA on the risk of preterm birth. For DHA alone, 7 RCTs were included, and the summary findings indicated no effect. The certainty of the evidence was low. For DHA+EPA, 9 RCTs and 2 observational studies were included. Summary findings of RCTs indicated no effect, whereas the observational studies showed mixed effects. The certainty of the evidence was low. The effect of EPA+DHA on birth weight was summarised and the effect varied from no effect to a significant increase compared with placebo. The certainty of the evidence varied from low to moderate.</p> <p>Ren et al. 2021 summarised the effect of n-3 LCPUFA supplementation on birth weight. Summarised findings from 19 RCTs, indicated that intake of n-3 LCUPFA in pregnant women at doses higher than 650 mg/day leads to higher birth weight. Certainty of the evidence was not assessed. VKM notes that 7 of the included RCTs had high risk of bias.</p>		

4.4.4.17 Prostate cancer

Two systematic reviews addressing prostate cancer were included (Table 4.4.4.17-1).

Table 4.4.4.17-1. Systematic reviews addressing prostate cancer.

Reference	Study/ intervention	Results, certainty in the evidence
Chua et al. (2012)	<p><u>Studies with EPA and DHA exposure:</u></p> <p>Schuurman et al., 1999. 6.3 years. Cohort study. 58,279 participants in total. Intervention: Omega 3 (ALA, EPA, DHA). RoB: the score was 8/13.</p> <p>Leitzmann et al., 2004. 14 years. Cohort study. 58,279 participants in total. Intervention: Omega 3. RoB: the score was 8/13.</p> <p>Koralek et al., 2006. 5.1 years (average years). Cohort study. 29,592 participants in total. Intervention: Omega 3. RoB: the score was 8/13.</p> <p>Giovannucci et al., 2007. 16 years. Cohort study. 51,529 participants in total.</p>	<p>A slightly positive association was noted on dietary long-chain n-3 PUFA, composed of EPA and DHA with prostate cancer risk (pooled RR: 1.135;95% CI: 1.008, 1.278 ;P=0.036; 2 trials; n=30,731); however, when two other cohort studies with data of EPA and DHA, both analysed separately, were included into the pool, the association became not significant (RR: 1.034; 95% CI: 0.973, 1.096;P=0.2780; 4 trials; n=82,483).</p> <p>EPA: 4 trials, n=196,192, RR: RR: 0.996; 95% CI: 0.921, 1.076; P = 0.911. EPA (adjusted): 3 trials, n= 151,326, RR: 1.049; 95% CI: 0.955, 1.152; P = 0.317.</p> <p>DHA: 4 trials, n=196,192, RR: 0.990; 95% CI: 0.918, 1.068; P = 0.804.</p>

	<p>Intervention: Omega 3. RoB: the score was 8/13.</p> <p>Park et al., 2007. 8 years. Cohort study. 82,483 participants. Intervention: Omega 3 (DHA and EPA). RoB: the score was 10/13.</p> <p>Wallstrom et al., 2007. 11 years. Cohort study. 10,564 participants in total. Intervention: Omega 3. RoB: the score was 10/13.</p> <p>Chavarro et al., 2008. 19 years. Cohort study. 20,167 participants in total. Intervention: Omega 3 (long-chain n-3). RoB: the score was 8/13.</p>	
Fu et al. (2015)	<p><i>Shuurman et al., 1999, Wallstrom et al., 2007, Park et al., 2007, and Leitzmann et al., 2004 were included in Chua et al., 2012.</i></p> <p><u>Studies with EPA and DHA exposure:</u></p> <p>Pelser et al., 2013. 9 years. Prospective cohort study. 288,268 participants in total.</p>	<p>Blood concentration of DHA, but not alpha-linolenic acid or EPA, showed marginal positive association with prostate cancer (PCa) risk (relative risk for 1% increase in blood DHA concentration: 1.02; 95% confidence interval, 1.00–1.05; I²= 26%;P= 0.05 for linear trend), while dietary DHA intake showed a non-linear positive association with PCa risk (P< 0.01).</p>

	<p>Intervention: EPA, DHA, EPA+DHA. RoB: the score was 7/13.</p> <p>Männistö 2003. 5-8 years. Nested case-control study. 290,406 participants in total. Intervention: DHA, EPA. RoB: the score was 6/10.</p> <p>Bassett et al., 2013. 8.9 years. Case-cohort study. 17,045 participants in total. Intervention: EPA, DHA. RoB: the score was 6/10.</p>	<p>Subgroup analyses indicated that blood EPA concentration and blood DHA concentration were positively associated with aggressive PCa risk and nonaggressive PCa risk, respectively. Among studies with nested case-control study designs, a 0.2% increase in blood docosapentaenoic acid concentration was associated with a 3% reduced risk of PCa (relative risk 0.97; 95% confidence interval, 0.94–1.00; I²= 44%; P= 0.05 for linear trend).</p>
<p>VKM concludes: No conclusion as outcome is to be of minor relevance for children and adolescents.</p>		

4.4.4.18 Pulmonary embolus or deep vein thrombosis (DVT)

One systematic review addressing pulmonary embolus or DVT was included (Table 4.4.4.18-1).

Table 4.4.4.18-1. Systematic review addressing pulmonary embolus or DVT.

Reference	Study/ intervention	Results, certainty in the evidence
Abdelhamid et al. (2020)	<p><u>Studies with EPA and DHA exposure:</u></p> <p>Dream Asbell 2018. 12 months. RCT with parallel design. N: 349 intervention randomised, 186 control randomised. Adults with dry eye.</p>	<p>Pulmonary embolus or DVT (RR 1.15, 95% CI 0.44 to 2.98; I² =0%; 5 trials, > 3000 participants, 20 events). Assessed as being very-low certainty evidence (GRADE), so</p>

	<p>Intervention: omega-3 supplements (2000 mg EPA + 1000 mg DHA/day as 5 gel caps). Dose: 3.0 g/day LCn3. Control: olive oil supplements (5 gel caps). RoB: low.</p> <p>NAT2 2013. 36 months. N: 150 intervention, 150 control. People with early age-related macular degeneration. Intervention: 3 daily fish oil capsules containing 1110 total n-3 FAs (EPA: 270 mg/day DHA: 840 mg/d) and vit E: 6 mg/day. Dose: 1.1 g/day EPA + DHA. Control: 3 × 602 mg/day olive oil capsules containing 0.2 g total PUFA and vitamin E: 0.09 g/day. RoB: low.</p> <p>OFAMI 2001. 2 years. RCT with parallel design. N: 150 intervention, 150 control. Participants recruited 4-8 days after confirmed myocardial infarction. Intervention: 4 gelatin capsules of omega-3-acid ethyl esters 90, each is 1 g containing 850-882 mg EPA and DHA as concentrated ethylesters. Dose ~3.4- 3.5 g/day EPA + DHA. Control: corn oil capsules, 4/d, each contains 1 g of corn oil. RoB: moderate or high.</p> <p><u>Studies with DHA exposure:</u></p> <p>ADCS 2010. 18 months. N: 238 intervention, 164 control. Individuals with mild to moderate Alzheimer's disease. Intervention: 2 × 1 g algal-derived DHA capsules/day, each capsule contain 45%-55% of (950 mg soft-gel capsules, which contain approximately 510 mg DHA). Dose: +DHA 1.02 g/day. Control: 2 × 1 g placebo capsules/day (made up of corn or soy oil). RoB: low.</p> <p><u>Studies with EPA exposure:</u></p>	<p>the effect of LCn3 on pulmonary embolus or DVT is unclear.</p>
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	<p>DART 1989. 2 years. RCT, parallel design. N: 1015 intervention, 1018 control. Men recovering from myocardial infarction. Intervention: advised to eat at least 2 weekly portions of 200-400 g fatty fish (mackerel, herring, kipper, pilchard, sardine, salmon, trout). If this was not possible, given MaxEPA capsules, 3/day (0.5 g EPA/d). 191/883 participants were taking MaxEPA at 2 years. Advice was reinforced 3-monthly. Dose: aimed for 0.5 g/day EPA. Control: no such dietary advice or capsules. RoB: moderate or high.</p>	
<p>VKM concludes: There is insufficient evidence to conclude whether EPA and DHA in combination or alone increases may have an effect on pulmonary embolus.</p> <p>Summary: The conclusions on pulmonary embolus or DVT are based on Abdelhamid et al., 2020 alone, which is a systematic review with low RoB. Data from five RCTs on pulmonary embolus following exposure to EPA+DHA (three trials), DHA alone (one trial) and EPA alone (one trial) were summarised.</p> <p>For EPA+DHA, the doses ranged from 1.1 to 3.5 g/day. For EPA alone, the dose was 0.5 g/day, and for DHA alone the dose was 1.02 g/day. The participants mean age ranged from 56-76 years, and the exposure period ranged from 1.0 to 3.0 years. The total number of participants were >3,000.</p> <p>No significant effect on pulmonary embolus or DVT was observed in the meta-analysis. Note that the confidence intervals were large. For EPA+DHA, the RoB was low for two studies and moderate to high for one study. For EPA alone, the RoB was moderate to high. For DHA alone, the RoB was low. VKM notes that the direction of effect was not consistent within the studies at low risk of bias or those with moderate to high risk of bias. In addition, VKM notes that the inconsistencies in direction of effect cannot be explained by the dose or duration of intervention. VKM considers that the certainty in the evidence should be downgraded for the inconsistent results, and for the large confidence intervals (imprecision); resulting in moderate certainty in the evidence on pulmonary embolus.</p>		

4.4.4.19 Reflux

One systematic review addressing reflux was included (Table 4.4.4.19-1).

Table 4.4.4.19-1. Systematic review addressing reflux.

Reference	Study/ intervention	Results, certainty in the evidence
Abdelhamid et al. (2020)	<p><u>Studies with EPA and DHA exposure:</u></p> <p>Dream Asbell 2018. 12 months. RCT with parallel design. N: 349 intervention randomised, 186 control randomised. Adults with dry eye. Intervention: omega-3 supplements (2000 mg EPA + 1000 mg DHA/day as 5 gel caps). Dose: 3.0 g/day LCn3. Control: olive oil supplements (5 gel caps). RoB: low.</p> <p>Fostar 2016. 24 months. RCT with parallel design. N: 101 intervention, 101 control. Intervention: 1-3 × a day drink of fruit juice mixed with day total = 15 mL of fish oil supplement (18% EPA, 12% DHA, 4.5 g/day total omega-3). Dose: 4.5 g/day EPA + DHA. Control: liquid oral oil 15 mL Sunola oil/day (which contains fish oil 2 mL plus 13 mL canola oil) (total omega-3 fat: ≥ 0.45 g EPA + DHA from 15 mL). RoB: low.</p> <p><u>Studies with EPA exposure:</u></p> <p>REDUCE-IT 2019. 4.9 years (median). RCT with parallel design N: intervention 4089 randomised, control 4090 randomised (analysed, intervention: 4083 control: 4077). People (45 years+) with hypertriglyceridaemia, and with cardiovascular disease or with diabetes mellitus and another risk factor, and on statin. Intervention: EPA ethyl ester derived from fish oil (AMR101 4 g/d, Amarin), 3.99 g/day EPA plus 8 mg/day vitamin E (2 capsules twice/day). Control: 3.73 g/day light liquid paraffin oil in 4 capsules (2 capsules twice a day). RoB: moderate or high.</p>	<p>Reflux: there were limited data (RR 1.23, 95% CI 0.79 to 1.91; I² =32%; 3 trials, > 8000 participants, 282 events).</p>
<p>VKM concludes: There is insufficient evidence to conclude whether EPA and DHA in combination or alone increases may have an effect on reflux.</p>		

Summary: The conclusions on reflux are based on Abdelhamid et al., 2020, which is a systematic review with low RoB. Data from three RCTs on reflux following exposure to EPA+DHA (two trials) and EPA alone (one trial) were summarised by Abdelhamid et al., 2020. No trial with exposure to DHA alone were identified.

For EPA+DHA, the doses ranged from ≥ 0.45 g to 3 g/day. For EPA alone, the dose was 3.99 g/day. The participants mean age were > 50 years, and the exposure period ranged from 1.0 to 4.9 years. The total number of participants were > 8000 .

No significant effect on reflux was observed in the meta-analysis. Note that the data were limited, and the confidence intervals were large. For EPA+DHA, the RoB was low for both studies. For the study on EPA alone, the RoB was moderate to high. VKM notes that in both studies on DHA+EPA the direction of effect was towards favouring lower omega 3. VKM considers that the certainty in the evidence should be downgraded for the limited number of studies, and for the large confidence intervals (imprecision); resulting in moderate certainty in the evidence on reflux.

4.4.4.20 *Skin problems, including dermatitis/eczema/hypersensitivity/skin allergy*

One systematic review and two RCTs addressing skin problems were included (Table 4.4.4.20-1 and 4.4.4.20-2).

Table 4.4.4.20-1. Systematic review addressing skin problems.

Reference	Study/ intervention	Results, certainty in the evidence
Abdelhamid et al. (2020)	<p><u>Studies with combined EPA and DHA exposure:</u></p> <p>AREDS2 2014: 5 years. RCT with parallel design. People aged 50-85 years at high risk of progression to advanced age-related macular degeneration. N: 2147 intervention (1068 DHA/EPA, 1079 DHA/EPA + lutein/zeaxanthin), 2056 control (1012 placebo, 1044 lutein/zeaxanthin). Intervention 350 mg/day DHA plus 650 mg/day EPA added to the standard AREDS</p>	<p>Skin problems, including itching or rashes: these were not affected by LCn3 in a meta-analysis with high heterogeneity (RR1.11, 95% CI 0.52 to 2.37; $I^2 = 68\%$; 9 trials, $> 36,000$ participants, 293 events).</p>

	<p>supplement of Vitamin C (500 mg/day), Vitamin E (440 IU/day), beta-carotene (15 mg/d), zinc oxide (80 mg/day) and cupric oxide (2 mg/day). Dose: +1 g/day EPA + DHA. Control: standard AREDS supplement. RoB: low.</p> <p>Dream Asbell 2018. 12 months. RCT with parallel design. N: 349 intervention randomised, 186 control randomised. Adults with dry eye. Intervention: omega-3 supplements (2000 mg EPA + 1000 mg DHA/day as 5 gel caps). Dose: 3.0 g/day LCn3. Control: olive oil supplements (5 gel caps). RoB: low.</p> <p>EPE-A 2014. 12 months. RCT with parallel design. N: 86 intervention-high, 82 intervention low, 75 control. People with nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. RCT with parallel design. Comparison 1: high EPA vs low EPA (unclear what replaced EPA) Comparison 2: EPA vs unclear (placebo contents not reported). Intervention-high: EPA-E 2.7 g/d, 3 × EPA-E 300 mg capsules. Dose: 2.7 g/day EPA + DHA Intervention-low: EPA-E 1.8 g/d, 2 × EPA-E 300 mg capsules + 1 placebo capsule Dose: 1.8 g/day EPA + DHA. Control: 3 × placebo capsules. The pills were identical with respect to size, colour and smell. RoB: moderate or high.</p> <p>Lorenz-Meyer 1996. 12 months. N: 70 intervention, 63 control. People with Crohn's disease in remission. Intervention: 2 × 3 1 g gelatin capsules/day of ethylester fish oil concentrate (3.3 g/day EPA + 1.8 g/day DHA). Dose: 5.1 g/day EPA + DHA. Control: 2 × 3 1 g gelatin capsules/day of corn oil. RoB: low.</p>	
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	<p>Risk and prevention 2013. 60 months. RCT with parallel design. N: 6244 intervention, 6269 control (analysed, intervention: 6239 control: 6266). Patients with multiple cardiovascular risk factors (59.9% had diabetes). Intervention: 1 g/day n-3 capsules polyunsaturated FA ethyl esters (EPA and DHA content 850-882 mg with an average ratio of 1.0:1.2). Dose: ~0.87 g/day EPA + DHA. Control: 1 g/day olive oil capsules. Rob: moderate or high.</p> <p>Sandhu 2016. 24 months. RCT with parallel design. N: 54 + 53 intervention, 53 + 53 control. Healthy postmenopausal women. Intervention: group 4, Lovaza 4 g/day. Lovaza is the FDA-approved n-3 FA formulation containing 465 mg of EPA + 375 mg of DHA per gram, total dose; 1860 mg/day EPA, 1500 mg/day DHA. Group 5 as group 4 plus 30 mg raloxifene/day. Dose: 3.36 g/day EPA + DHA. Control: group 1, no treatment; group 3, 30 mg raloxifene/day. RoB: moderate or high.</p> <p>SCIMO 1999. 2 years. RCT with parallel design. N: 112 intervention, 111 control (analysed 82 intervention, 80 control). People with angiographically proven coronary artery disease. Intervention: concentrated fish oil capsules, 6 x 1 g capsules/day for first 3 months, 3 x 1 g/day for rest of trial (4 g/day EPA + DHA + DPA + ALA for first 3 months, then 2 g/d). Dose: ~2 g/day LCn3. Control: capsules containing fat that replicated the fat composition of the average European diet, 6/day for first 3 months, 3/day for rest of trial, opaque soft gelatin capsules identical to fish capsules in identical screw-top containers. RoB: low.</p> <p>Tande 2016. 12 months. RCT with parallel design.</p>	
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N: 64 intervention, 63 control (50 intervention, 50 control analysed). Healthy males and females.
 Intervention: 2 × 500 mg Calanus oil capsules twice daily to provide a daily dose of 2 g. The Calanus oil contained approximately 85% wax ester with a sum of neutral lipids > 90%. Dose: 2 g/day EPA + DHA.
 Control: identical capsules of olive oil. Compositional analysis indicated that the FA content of the olive oil was primarily oleic acid (76.9%), palmitic acid (10.2%), and linoleic acid (7.7%).
 RoB: moderate or high.

Studies with EPA exposure:

JELIS 2007. 5 years.
 RCT with parallel design.
 N: intervention, 9326, control 9319 (analysed intervention 9326, control 9319).
 People with hypercholesterolaemia.
 Intervention: 3 × 2 × 300 mg capsules/day EPA ethyl ester (total dose of 1.8 g/day EPA), after meals. Dose: 1.8 g/day EPA.
 Control: nothing (though all in both groups received "appropriate" dietary advice).
 RoB: moderate or high.

VKM concludes: There is low certainty evidence that EPA+DHA and EPA alone does not cause skin problems.

Summary: The conclusions on skin problems are based on Abdelhamid et al., 2020, which is a systematic review with low RoB. Data from nine RCTs on skin problems following exposure to EPA+DHA (eight trials) and EPA alone (one trial) were summarised. No trial with exposure to DHA alone were identified.

For EPA+DHA, the doses ranged from 0.87- 3.36 g/day. For EPA alone, the dose was 1.8 g/day. The participants mean age ranged from 36-79 years, and the exposure period ranged from 1.0 to 7.4 years. The total number of participants were > 36,000.

No significant effect on skin problems was observed in the meta-analysis. Note that the heterogeneity was high, and the confidence intervals were large. For EPA+DHA, the RoB was low for four studies and moderate to high for four studies. For EPA alone, the RoB was moderate to high. VKM notes that the

direction of effect was not consistent within the studies at low risk of bias or those with moderate to high risk of bias. In addition, VKM notes that the inconsistencies in direction of effect cannot be explained by study size, dose or duration of intervention. VKM considers that the certainty in the evidence should be downgraded for the number of studies with moderate to high RoB, for the inconsistent results, and for the large confidence intervals (imprecision); resulting in low confidence in the evidence on skin problems.

Table 4.4.4.20-2. RCTs addressing skin problems in children and adolescents.

Reference	Participants/ intervention/duration	RoB	Results					
Cornu et al. (2018)	Children with established diagnosis of ADHD. N=148. Children aged 6–8 years, EPA 336 mg and DHA 84 mg; for children aged 9–11 years, EPA 504 mg and DHA 126 mg, and for children aged 12–15 years EPA 672 mg and DHA 168 mg. Study duration was 3 months.	Tier 2	Eleven (14.9%) children in the DHA–EPA group experienced 13 adverse events, 2 of which were judged to be related to the study treatment by the study authors: hip pain, fatigue, headache, fever and cough (n = 2), dermatitis and allergic skin reaction (n = 4), abdominal pain, diarrhoea (n = 3), and depression. Eight children (10.7%) in the placebo group experienced 10 adverse events: fatigue, influenza (n = 2), abdominal pain, dermatitis, swollen eyes, vomiting, and diarrhoea (n = 3).					
de Ferranti et al. (2014)	Hypertriglyceridemic adolescents. N=25, aged 10-19 years. Dose: ~3360 mg DHA + EPA per day vs placebo. 3 months.	Tier 2	No effects reported.					
Mazahery et al. (2019)	Children with autism spectrum disorder. N=73, aged 2.5 to 8 years. Dose: 722 mg DHA. Study duration = 12 months.	Tier 2	Five events reported by the participants in the intervention group, none in the placebo group.					
Overall evaluation of certainty in the evidence on skin problems								
Initial rating	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	

DHA+EPA RCTs, initial rating ++++	Serious	Serious	Not serious	Serious	No	No	Not evaluated	Very-low
DHA 1 RCT initial rating ++++	Serious	Not evaluated Serious	Not serious	Serious	No	No	Not evaluated	Very-low
VKM concludes:								
<ul style="list-style-type: none"> • There is very-low certainty evidence that daily doses of 1800 mg EPA + 1500 mg DHA for three months do not cause skin problems in children aged 6-18 years. • There is very-low certainty evidence that 722 mg of DHA for 12 months caused a small increase in allergic skin reactions in children aged 2.5 to 8 years. • No studies on EPA alone were included. 								

4.5 Summary and conclusions of the hazard identification and characterisation

NFSA requested VKM to evaluate if daily intake of food supplements containing 1100 mg DHA, 1550 mg EPA, or DHA+EPA combined (1100 mg + 1550 mg) may constitute a health risk for Norwegian children and adolescents (both sexes) from 3 to 18 years. If these daily doses may constitute a health risk, NFSA need to know which DHA and EPA doses that are safe.

Endogenous production and intake from food

The food supplement doses included in the request from NFSA are higher than the EPA and DHA levels produced endogenously and are also higher than the intake from food (Section 3).

Absorption, distribution, metabolism and excretion (ADME)

No ADME data for children and adolescents were identified, and VKM can therefore not rule out that there are important differences with regard to ADME in adults, and children and adolescents. A literature search for ADME data was not performed due to the limited time available.

ADME data for adults:

N-3 PUFAs are almost completely absorbed (IOM, 2005). EPA and DHA are incorporated into cell membranes, and thus may impact cellular metabolism, signal transduction, and regulation of gene expression (IOM, 2005). EPA can be transformed to eicosanoids, including prostaglandins, prostacyclin, and leukotrienes, which participate in the regulation of blood pressure, renal function, blood coagulation, inflammatory and immunological reactions and other functions in tissues (EFSA, 2012). DHA is a component of membrane structural lipids, especially of phospholipids in nervous tissue and the retina (EFSA, 2012). Other metabolites of DHA and EPA includes resolvins, poxytrins, neuroprotectins and maresins, which are thought to be involved in resolution of inflammatory responses (EFSA, 2012).

Literature for the hazard identification and characterisation

To identify reports (including risk- or safety assessments), systematic reviews and RCTs addressing potential adverse health effects related to DHA and EPA, websites of relevant international organisations and electronic databases were searched.

Five risk assessments were included. No quality assessment of the included studies was performed in these reports; therefore, these reports were only used as supporting information.

Twelve systematic reviews of sufficient quality were included. Most of the studies included in the systematic reviews addressed negative effects in adults and lacked data on effects in children and adolescents. Since VKM did not have data on ADME for children and adolescents, it is not possible to know whether ADME in children and adolescents is similar to

that of adults. Therefore, data on adults were used as supporting information only. For several outcomes, the findings are of limited value as data on DHA alone, EPA alone, and/or DHA+EPA combined are summarised without distinctions between them.

Fourteen RCTs of sufficient quality were included.

Hazard identification and characterisation

According to VKM (2015), genotoxicity has been assessed earlier and no concerns for genotoxicity were raised. No data on genotoxicity were included in the systematic reviews or the RCTs.

The possible adverse outcomes included in the RCTs were bleeding, level of fasting glucose and insulin and/or insulin resistance (glucose/insulin homeostasis), gastrointestinal effects, headache, inflammatory markers (inflammation), joint and lumbar and muscle pain, lipid profile (lipid homeostasis), liver effects, other side effects, oxidative stress, and skin problems. VKM considered that bleeding, glucose/insulin homeostasis, inflammation, lipid homeostasis, and liver effects were the most important outcomes. An overview of the results and the certainty in the evidence are given in Table 4.5-1.

Table 4.5-1. RCTs on children and adolescents: an overview of the results for the adverse outcomes assessed and the certainty in the evidence for the reported effect/no effect.

Important/less important	Outcome	DHA+EPA combined (daily intake) (Certainty in the evidence/ RCTs/ participants (n)/ dose / duration/ age/ effect)	DHA alone (daily intake) (Certainty in the evidence/ RCTs/ participants (n)/ dose / duration/ age/ effect)	EPA alone (daily intake) (Certainty in the evidence/ RCTs/ participants (n)/ dose / duration/ age/ effect)
Important	Bleeding	-	Very-low certainty evidence/ 1 RCT/ 73/ 722 mg/ 12 months/ 2.5-8 years/ no or very little increase.	-
	Glucose/insulin homeostasis	Moderate certainty evidence/ 3 RCTs/ 117/ 450 mg EPA+2250 mg DHA/ 6 months/ 6-18 years/ no negative effect.	Low certainty evidence/ 1 RCT/ 51/ 250 mg/ 6 months/ 8-13 years/ no negative effect.	-
	Inflammation	Moderate certainty evidence/ 4 RCTs/ 223/ 1800 mg EPA+1500 mg DHA for 3 months/ 450 EPA+2250 DHA for 6 months/ 3-18 years/ no negative effect.	Moderate certainty evidence/ 3 RCTs/ 118/ 1.2 g for 6 weeks/ 250 mg /6 months/ 8-18 years/ no negative effect.	Very-low certainty evidence/ 1 RCT/ 92/ 1.2 g/ 12 weeks/ 6-18 years/ no negative effect.
	Lipid metabolism	Very-low certainty evidence/ 3 RCTs/ 125/ 1800 mg EPA+1500 mg DHA/ 3 months/ 8-18 years/ small increase in LDL.	Low certainty evidence/ 2 RCTs/ 56/ 1.2 g / 6 weeks/ 500 mg/16 weeks/ 8-18 years/ small increase in LDL.	-
	Liver effects	Very-low certainty evidence/ 1 RCT/ 64/ 267-800 mg DHA+ 177.5-532.5 mg EPA/ 24 weeks/ median age 13 years/ no negative effect.	Low certainty evidence/ 1 RCT/ 51/ 250 mg/ 6 months/ 8-13 years/ no negative effect.	-
	Gastrointestinal effects	Low certainty evidence/ 2 RCTs/ 173/ 1800 mg EPA+1500 mg DHA/ 3 months/ 6-18 years/ no negative effect.	Low certainty evidence/ 2 RCTs/ 445/ 722 mg/ 12 months / 2.5-9 years/ no negative effect.	-

Important/less important	Outcome	DHA+EPA combined (daily intake) (Certainty in the evidence/ RCTs/ participants (n)/ dose / duration/ age/ effect)	DHA alone (daily intake) (Certainty in the evidence/ RCTs/ participants (n)/ dose / duration/ age/ effect)	EPA alone (daily intake) (Certainty in the evidence/ RCTs/ participants (n)/ dose / duration/ age/ effect)
Less important	Headache	Very-low certainty evidence/ 1 RCT/ 148/ 336 mg EPA+84 mg DHA/ 3 months/ 6-8 years/ no or a small increase.	Very-low certainty evidence/ 1 RCT/ 73/ 722 mg/ 12 months/ 2.5-8 years/ no or a small increase.	-
	Joint, lumbar and muscle pain	Low certainty evidence/ 2 RCTs/ 173/ 1800 mg EPA+1500 mg DHA/ 3 months/ 6-18 years/ no or a small increase.	-	-
	Other side effects	-	Low certainty evidence/ 3 RCTs/ 174/ 722 mg/ 12 months/ 2.5-14 years/ little or no side effects.	-
	Oxidative stress	-	Very-low certainty evidence/ 1 RCT/ 20/ 1.2 g/ 6 weeks/ 9-19 years/ no effect.	-
	Skin problems	Very-low certainty evidence/ 3 RCTs/ 173/ 1800 mg EPA+1500 mg DHA/ 3 months/ 6-18 years/ no negative effects.	Very-low certainty evidence/ 1 RCT/ 73/ 722 mg DHA/ 12 months/ 2.5-8 years/ small increase in allergic skin reactions.	-

Conclusions and answers to the terms of reference

Daily intake of food supplement containing 1100 mg DHA

VKM concludes that a health-based guidance value or a point of departure for DHA for 3-18-year-olds cannot be established or identified. Therefore, it is not possible to conclude on the safety of the suggested daily dose of 1100 mg DHA for children and adolescents.

Justification: The suggested dose of 1100 mg DHA is 7.4 to 13.5-fold higher than the median dietary intakes. There were few RCTs addressing potential adverse effects in children and adolescents. In addition, the studies had small number of participants and the exposure length varied from 16 weeks to 12 months. No adverse effects or a small increase in adverse effects were reported in the RCTs, and for all five outcomes considered to be most important, some data were available. The certainty in the evidence was considered moderate for inflammation, and for the other important outcomes the certainty in the evidence was considered low or very-low. For three of the five important outcomes the daily doses tested ranged from 250 to 722 mg, for two the doses were 1200 mg. For the important outcomes identified in the current assessment, supporting information from studies on adults in the systematic reviews was available only for lipid metabolism. VKM considers, therefore, that it is not possible to pinpoint safe or unsafe DHA doses for 3-18-year-olds above the average dietary intake.

Daily intake of food supplement containing 1550 mg EPA

VKM concludes that a health-based guidance value or a point of departure for EPA for 3-18-year-olds cannot be established or identified. Therefore, it is not possible to conclude on the safety of the suggested daily dose of 1550 mg EPA for children and adolescents.

Justification: The suggested dose of 1550 mg EPA is 20.7 to 38.8-fold higher than the median dietary intakes. There were only data for four of the five outcomes that was considered most important. One RCT with sufficient quality addressed effects of EPA on inflammatory markers in children and adolescents. The certainty in this evidence for no effect on inflammation was very-low. VKM considers, therefore, that it is not possible to pinpoint safe or unsafe EPA doses for 3-18-year-olds above the average dietary intake.

Daily intake of food supplements containing 1100 mg DHA and 1550 mg EPA

VKM concludes that a health-based guidance value or a point of departure for DHA+EPA for 3-18-year-olds cannot be established or identified. Therefore, it is not possible to conclude on the safety of the suggested daily dose of 1100 mg DHA and 1550 mg EPA for children and adolescents.

Justification: The suggested dose of 1100 mg DHA and 1550 mg EPA is 12.1 to 22.1-fold higher than the median dietary intakes. There were few RCTs addressing the potential adverse effects in children and adolescents. In addition, the studies had small number of participants and the exposure length varied from three to six months. No RCT addressing bleeding was included. In addition, supporting information from studies on adults included in

the systematic reviews could not be used as results from different interventions were summarised together. For the four other outcomes considered important, some data were identified for EPA and DHA, and no adverse effects or a small increase in adverse effects were reported. The certainty in the evidence on glucose/insulin homeostasis and inflammation was moderate, and the certainty in the evidence on lipid metabolism and liver effects was very-low. VKM considers, therefore, that it is not possible to pinpoint safe or unsafe DHA+EPA doses for 3-18-year-olds above the average dietary intake.

DHA and EPA doses that are safe

From the exposure estimation VKM notes that for Norwegian 4-year-olds, 8-9-year-olds, 12-13-year-olds and 18-27-year-olds, the daily dietary median intake ranged from 81 to 148 mg, 43 to 75 mg, and 120 to 219 mg for DHA, EPA, and DHA+EPA combined.

EFSA has established an Adequate Intake (AI), which is a type of Dietary Reference Value (DRV), for EPA and DHA (EFSA, 2010). An AI is estimated in cases where a Population Reference Intake (PRI), also a type of DRV, cannot be established. A PRI is the level of (nutrient) intake that is adequate for virtually all people in a population group. The AI is the average observed or experimentally determined approximations or estimates of nutrient intake by a population group (or groups) of apparently healthy people that is assumed to be adequate. In practice, both PRI and AI describe the level of intake that is considered adequate for health reasons.

For the age group 2-3 years, 4-17 years and ≥ 18 years EFSA has set an AI of 250 mg/day of EPA+DHA (combined) (EFSA, 2010). VKM considers that it is likely that these intakes are safe. From the included literature, it is not possible for VKM to pinpoint the highest safe doses of DHA, EPA or DHA and EPA combined.

5 Risk characterisation

VKM was not able to identify a health-based guidance value or a point of departure for DHA, EPA or DHA+EPA combined for 3-18-year-olds (Section 4.5). Therefore, it is not possible to characterise the risk related to daily intake of 1100 mg DHA, 1550 mg EPA or 1100 mg DHA and 1550 mg EPA combined, for these age groups.

6 Uncertainty

Due to the limited time available for the preparation of this risk assessment, a literature search for ADME-data for children and adolescents was not performed. No information on ADME for children and adolescents were available in the included literature and VKM can therefore not rule out that there are important differences with regard to ADME in adults, and children and adolescents. As a consequence, data on adverse effects in adults were only used as supporting information.

VKM (2015) performed a search for RCTs on adverse effects related to EPA and DHA. Therefore, the present literature search for RCTs was limited to the period 2014 to 2021. However, VKM (2015) used a closed search strategy where only a few of the adverse effects included in the present evaluation were included. In the present literature search, an open search strategy was used where no search words for adverse effects were included. This was done to be able to identify as many relevant studies as possible. Due to restrictions in the search strategy by VKM (2015), and the choice in the current literature search to apply a restriction on time period, relevant publications published before 2014 might not have been identified. In addition, in the current literature search, several other filters were applied to refine the search; more specifically filters on language (English only), study design (RCT) and age groups (0-18 years). These type of restrictions in the search is dependent on that all relevant publications have been tagged with these filters. Therefore, it is possible that relevant RCTs were missed due to the application of filters in the search. The restrictions to the search strategy were applied to make it possible to prepare the present risk assessment within the limited time frame.

7 Summary, discussion and conclusions

NFSA requested VKM to assess if daily intake of a food supplement containing i) 1100 mg docosahexaenoic acid (DHA), ii) 1550 mg eicosapentaenoic acid (EPA), or iii) both 1550 mg EPA and 1100 mg DHA may constitute a health risk for Norwegian children and adolescents (both sexes) from 3 to 18 years. If these daily doses may constitute a health risk, NFSA need to know which DHA and EPA doses that are safe.

The food supplement doses included in the request from NFSA are higher than the EPA and DHA levels produced endogenously, and also higher than the intake from food (Section 3).

Exposure

The intake from food is shown in Table 3-1, 3-2 and 3-3.

With the exception of the 95th percentile intake from food for 18-27-years-olds, where the DHA dose is lower than the intake from food, the doses of 1100 mg DHA and 1550 mg EPA are higher than the mean, median and 95 percentile intakes from food for all age groups. This is also the case for the combined exposure of DHA and EPA.

ADME

A literature search for ADME data was not performed due to the limited time available. No ADME data for children and adolescents were identified in the included literature, and VKM can therefore not rule out that there are important differences with regard to ADME in adults, and children and adolescents. Therefore, data from studies on adults were only used as supporting information.

Hazard literature

To identify reports (including risk- or safety assessments), systematic reviews and RCTs addressing potential adverse health effects related to DHA and EPA, websites of relevant international organisations and electronic databases were searched. Due to the limited time available, several restrictions were applied to the literature search for RCTs, and it is therefore possible that not all relevant RCTs were identified.

Five risk assessments were included. No quality assessment of the included studies was performed in these reports; therefore, these were used as supporting information.

Twelve systematic reviews of sufficient quality were included in this risk assessment. These were used as supporting information as most of the studies included in the systematic reviews addressed negative effects in adults and there were no or few data on effects in children and adolescents. For several outcomes, the findings were of limited value as data on DHA alone, EPA alone, and/or DHA+EPA combined were summarised together.

Fourteen RCTs of sufficient quality were included.

The primary aim of the identified RCTs and systematic reviews was predominantly assessing beneficial effects and to a lesser extent adverse effects. Since beneficial effects were not in focus in the current assessment, studies only addressing beneficial effects were excluded.

Hazard identification and characterisation

According to VKM (2015), genotoxicity has been assessed earlier and no concerns for genotoxicity were raised. No data on genotoxicity were included in the systematic reviews or the RCTs.

Several possible adverse outcomes were included in the RCTs. The outcomes considered to be the important were bleeding, glucose/insulin homeostasis, inflammation, lipid homeostasis, and liver effects.

The majority of the RCTs reported no or small effects, and the certainty in the evidence was very-low to moderate (see an overview in Table 7-1). In general, the number of RCTs addressing the different outcomes were limited, the number of participants were limited, for several of the studies the doses were lower than the doses evaluated in the present assessment, chronic exposure was not addressed, and the age group 3-18 years was only partly covered.

Table 7-1. An overview of the RCTs and the reported effects and the certainty in the evidence for daily intake of 1100 mg DHA, 1550 mg EPA, and 1100 mg DHA and 1550 mg EPA combined.

Important/ less important	Outcome	DHA+EPA combined (Certainty in the evidence/ effect)	DHA alone (Certainty in the evidence/ effect)	EPA alone (Certainty in the evidence/ effect)
Important	Bleeding	-	Very-low certainty evidence/ no or very small increase.	-
	Glucose/insulin homeostasis	Moderate certainty evidence/ no negative effect.	Low certainty evidence/ no negative effect.	-
	Inflammatory markers	Moderate certainty evidence/ no negative effect.	Moderate certainty evidence/ no negative effect.	Very-low certainty evidence/ no negative effect.
	Lipid profile	Very-low certainty evidence/ small increase in LDL.	Low certainty evidence/ small increase in LDL.	-
	Liver effects	Very-low certainty evidence/ no negative effect.	Low certainty evidence/ no negative effect.	-
	Gastrointestinal effects	Low certainty evidence/ no negative effect.	Low certainty evidence/ no negative effect.	-

Important/ less important	Outcome	DHA+EPA combined (Certainty in the evidence/ effect)	DHA alone (Certainty in the evidence/ effect)	EPA alone (Certainty in the evidence/ effect)
Less important	Headache	Very-low certainty evidence/ no or a small increase.	Very-low certainty evidence/ no or a small increase.	-
	Joint, lumbar and muscle pain	Low certainty evidence/ no or a small increase.	-	-
	Other side effects	-	Low certainty evidence/ little or no side effects.	-
	Skin problems	Very-low certainty evidence/ no negative effects.	Very-low certainty evidence/ small increase in allergic skin reactions.	-

Conclusions and answers to the terms of reference

Daily intake of food supplement containing 1100 mg DHA

VKM concludes that a health-based guidance value or a point of departure for DHA for 3-18-year-olds cannot be established or identified. Therefore, it is not possible to conclude on the safety of the suggested daily dose of 1100 mg DHA for children and adolescents.

Daily intake of food supplement containing 1550 mg EPA

VKM concludes that a health-based guidance value or a point of departure for EPA for 3-18-year-olds cannot be established or identified. Therefore, it is not possible to conclude on the safety of the suggested daily dose of 1550 mg EPA for children and adolescents.

Daily intake of food supplements containing 1100 mg DHA and 1550 mg EPA

VKM concludes that a health-based guidance value or a point of departure for DHA+EPA for 3-18-year-olds cannot be established or identified. Therefore, it is not possible to conclude on the safety of the suggested daily dose of 1100 mg DHA and 1550 mg EPA for children and adolescents.

DHA and EPA doses that are safe

From the exposure estimation VKM notes that for Norwegian 4-27-year-olds, the daily dietary median intake ranged from 81 to 148 mg, 43 to 75 mg, and 120 to 219 mg for DHA, EPA, and DHA+EPA combined, respectively.

EFSA has established an Adequate Intake (AI), which is a type of Dietary Reference Value (DRV), for EPA and DHA (EFSA, 2010). An AI is estimated in cases where a Population Reference Intake (PRI), also a type of DRV, cannot be established. A PRI is the level of

(nutrient) intake that is adequate for virtually all people in a population group. The AI is the average observed or experimentally determined approximations or estimates of nutrient intake by a population group (or groups) of apparently healthy people that is assumed to be adequate. In practice, both PRI and AI describe the level of intake that is considered adequate for health reasons.

For the age group 2-3 years, 4-17 years and ≥ 18 years EFSA has set an AI of 250 mg/day of EPA+DHA (combined) (EFSA, 2010). VKM considers that it is likely that these intakes are safe. From the included literature, it is not possible for VKM to pinpoint the highest safe doses of DHA, EPA or DHA and EPA combined.

8 Data gaps

More data from high quality studies with larger sample size and longer exposure time, on DHA and EPA alone and in combination, are needed.

9 References

- Abdelhamid A.S., Brown T.J., Brainard J.S., Biswas P., Thorpe G.C., Moore H.J., Deane K.H., Summerbell C.D., Worthington H.V., Song F., Hooper L. (2020) Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. Cochrane Database of Systematic Reviews 3:CD003177. DOI: <https://dx.doi.org/10.1002/14651858.CD003177.pub5>.
- Abdullah M., Jowett B., Whittaker P.J., Patterson L. (2019) The effectiveness of omega-3 supplementation in reducing ADHD associated symptoms in children as measured by the Conners' rating scales: A systematic review of randomized controlled trials. *Journal of Psychiatric Research* 110. DOI: <http://dx.doi.org/10.1016/j.jpsychires.2018.12.002>.
- Abdulrazaq M., Innes J.K., Calder P.C. (2017) Effect of omega-3 polyunsaturated fatty acids on arthritic pain: A systematic review. *Nutrition* 39-40:57-66. DOI: <https://dx.doi.org/10.1016/j.nut.2016.12.003>.
- Abeywardena M.Y., Patten G.S. (2011) Role of omega3 long-chain polyunsaturated fatty acids in reducing cardio-metabolic risk factors. *Endocrine, Metabolic & Immune Disorders Drug Targets* 11:232-46.
- AbuMweis S., Abu Omran D., Al-Shami I., Jew S. (2021) The ratio of eicosapentaenoic acid to docosahexaenoic acid as a modulator for the cardio-metabolic effects of omega-3 supplements: A meta-regression of randomized clinical trials. *Complementary Therapies in Medicine* 57. DOI: <http://dx.doi.org/10.1016/j.ctim.2021.102662>.
- AbuMweis S., Jew S., Tayyem R., Agraib L. (2018) Eicosapentaenoic acid and docosahexaenoic acid containing supplements modulate risk factors for cardiovascular disease: a meta-analysis of randomised placebo-control human clinical trials. *Journal of Human Nutrition & Dietetics* 31:67-84. DOI: <https://dx.doi.org/10.1111/jhn.12493>.
- AESAN. (2012) Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the use conditions for certain substances other than vitamins, minerals and plants in food supplements. Reference number: AESAN-2012-008. , The Scientific Committee of the Spanish Agency for Food Safety and Nutrition, https://www.aesan.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/evaluacion_riesgos/informes_cc_ingles/FOOD_SUPPLEMENTS.pdf.
- Akter K., Gallo D.A., Martin S.A., Myronyuk N., Roberts R.T., Stercula K., Raffa R.B. (2012) A review of the possible role of the essential fatty acids and fish oils in the aetiology, prevention or pharmacotherapy of schizophrenia. *Journal of Clinical Pharmacy and Therapeutics* 37:132-139. DOI: <http://dx.doi.org/10.1111/j.1365-2710.2011.01265.x>.
- AlAmmar W.A., Albeesh F.H., Ibrahim L.M., Algindan Y.Y., Yamani L.Z., Khattab R.Y. (2019) Effect of omega-3 fatty acids and fish oil supplementation on multiple sclerosis: a systematic review. *Nutritional Neuroscience*. DOI: <http://dx.doi.org/10.1080/1028415X.2019.1659560>.

- Alexander D.D., Miller P.E., Van Elswyk M.E., Kuratko C.N., Bylsma L.C. (2017) A Meta-Analysis of Randomized Controlled Trials and Prospective Cohort Studies of Eicosapentaenoic and Docosahexaenoic Long-Chain Omega-3 Fatty Acids and Coronary Heart Disease Risk. *Mayo Clinic Proceedings* 92:15-29. DOI: <https://dx.doi.org/10.1016/j.mayocp.2016.10.018>.
- Allaire J., Vors C., Tremblay A.J., Marin J., Charest A., Tchernof A., Couture P., Lamarche B. (2018) High-Dose DHA Has More Profound Effects on LDL-Related Features Than High-Dose EPA: The ComparED Study. *Journal of Clinical Endocrinology & Metabolism* 103:2909-2917. DOI: <https://dx.doi.org/10.1210/jc.2017-02745>.
- Anthony R., Macartney M.J., Peoples G.E. (2021) The influence of long-chain omega-3 fatty acids on eccentric exercise-induced delayed muscle soreness: Reported outcomes are compromised by study design issues. *International Journal of Sport Nutrition and Exercise Metabolism* 31:143-153. DOI: <http://dx.doi.org/10.1123/IJSNEM.2020-0238>.
- Asfaw A., Minhas S., Khouzam A.R., Khouzam N.R., Khouzam R.N. (2021) Fish Oil Dilemma: Does It Increase the Risk of Ventricular Arrhythmias and Death? Can Fish Oil Kill You? *Current Problems in Cardiology* 46. DOI: <http://dx.doi.org/10.1016/j.cpcardiol.2020.100718>.
- Astill Wright L., Sijbrandij M., Sinnerton R., Lewis C., Roberts N.P., Bisson J.I. (2019) Pharmacological prevention and early treatment of post-traumatic stress disorder and acute stress disorder: a systematic review and meta-analysis. *Translational Psychiatry* 9. DOI: <http://dx.doi.org/10.1038/s41398-019-0673-5>.
- Astorg P. (2004) Dietary n - 6 and n - 3 polyunsaturated fatty acids and prostate cancer risk: A review of epidemiological and experimental evidence. *Cancer Causes and Control* 15:367-386. DOI: <http://dx.doi.org/10.1023/B:CACO.0000027498.94238.a3>.
- Atlantis E., Cochrane B. (2016) The association of dietary intake and supplementation of specific polyunsaturated fatty acids with inflammation and functional capacity in chronic obstructive pulmonary disease: a systematic review. *International Journal of Evidence-Based Healthcare* 14:53-63. DOI: <https://dx.doi.org/10.1097/XEB.0000000000000056>.
- Aung T., Halsey J., Kromhout D., Gerstein H.C., Marchioli R., Tavazzi L., Geleijnse J.M., Rauch B., Ness A., Galan P., Chew E.Y., Bosch J., Collins R., Lewington S., Armitage J., Clarke R., Omega-3 Treatment Trialists C. (2018) Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease Risks: Meta-analysis of 10 Trials Involving 77917 Individuals. *JAMA Cardiology* 3:225-234. DOI: <https://dx.doi.org/10.1001/jamacardio.2017.5205>.
- Azzi D.V., Viafara J.A.S., Zangeronimo M.G., Ribeiro Lima R., Marques L.S., Pereira L.J. (2018) n-3 Ingestion may modulate the severity of periodontal disease? Systematic review. *Critical Reviews in Food Science & Nutrition* 58:1937-1942. DOI: <https://dx.doi.org/10.1080/10408398.2017.1278677>.
- Bai Z.G., Bo A., Wu S.J., Gai Q.Y., Chi I. (2018) Omega-3 polyunsaturated fatty acids and reduction of depressive symptoms in older adults: A systematic review and meta-

- analysis. *Journal of Affective Disorders* 241:241-248. DOI: <https://dx.doi.org/10.1016/j.jad.2018.07.057>.
- Bakouei F., Delavar M.A., Mashayekh-Amiri S., Esmailzadeh S., Taheri Z. (2020) Efficacy of n-3 fatty acids supplementation on the prevention of pregnancy induced-hypertension or preeclampsia: A systematic review and meta-analysis. *Taiwanese Journal of Obstetrics and Gynecology* 59:8-15. DOI: <http://dx.doi.org/10.1016/j.tjog.2019.11.002>.
- Balachandar R., Soundararajan S., Bagepally B.S. (2020) Docosahexaenoic acid supplementation in age-related cognitive decline: a systematic review and meta-analysis. *European Journal of Clinical Pharmacology* 76:639-648. DOI: <https://dx.doi.org/10.1007/s00228-020-02843-x>.
- Balk E.M., Adams G.P., Langberg V., Halladay C., Chung M., Lin L., Robertson S., Yip A., Steele D., Smith B.T., Lau J., Lichtenstein A.H., Trikalinos T.A. (2016) Omega-3 Fatty Acids and Cardiovascular Disease: An Updated Systematic Review. *Evidence Report/Technology Assessment:1-1252*. DOI: <https://dx.doi.org/10.23970/AHRQEPERTA223>.
- Bath-Hextall F.J., Jenkinson C., Humphreys R., Williams H.C. (2012) Dietary supplements for established atopic eczema. *Cochrane Database of Systematic Reviews:CD005205*. DOI: <https://dx.doi.org/10.1002/14651858.CD005205.pub3>.
- Beatriz L.F.S., Alcantara S.T., De Jesus Leija Martinez J., Del Rio Navarro B.E., Del Carmen Castillo Hernandez M., Huang F. (2019) Effect of N-3 polyunsaturated fatty acids on oxidative stress markers and resolvin E1 in obese asthmatic adolescents with hypertriglyceridemia. *Endocrine Practice Conference:16th Annual World Congress on Insulin Resistance, Diabetes and Cardiovascular Disease, WCIRDC 2018*. Los Angeles, CA United States. 25 (1) (pp 2A).
- Becic T., Studenik C. (2018) Effects of omega-3 supplementation on adipocytokines in prediabetes and type 2 diabetes mellitus: Systematic review and meta-analysis of randomized controlled trials. *Diabetes and Metabolism Journal* 42:101-116. DOI: <http://dx.doi.org/10.4093/dmj.2018.42.2.101>.
- Bellino S., Bozzatello P., Brignolo E., Brunetti C., Bogetto F. (2012) Omega-3 fatty acids supplementation in psychiatric disorders: A systematic review. *Current Psychopharmacology* 1:353-364. DOI: <http://dx.doi.org/10.2174/2211556011201040353>.
- Benedetto U., Angeloni E., Melina G., Danesi T.H., Di Bartolomeo R., Lechiancole A., Refice S., Roscitano A., Comito C., Sinatra R. (2013) N-3 Polyunsaturated fatty acids for the prevention of postoperative atrial fibrillation: A meta-analysis of randomized controlled trials. *Journal of Cardiovascular Medicine* 14:104-109. DOI: <http://dx.doi.org/10.2459/JCM.0b013e32834a13c1>.
- Bernasconi A.A., Wiest M.M., Lavie C.J., Milani R.V., Laukkanen J.A. (2021) Effect of Omega-3 Dosage on Cardiovascular Outcomes: An Updated Meta-Analysis and Meta-Regression of Interventional Trials. *Mayo Clinic Proceedings* 96:304-313. DOI: <https://dx.doi.org/10.1016/j.mayocp.2020.08.034>.

- Bernstein A.M., Ding E.L., Willett W.C., Rimm E.B. (2012) A meta-analysis shows that docosahexaenoic acid from algal oil reduces serum triglycerides and increases hdl-cholesterol and ldl-cholesterol in persons without coronary heart disease. *Journal of Nutrition* 142:99-104. DOI: <http://dx.doi.org/10.3945/jn.111.148973>.
- Bloch M.H., Hannestad J. (2012) Omega-3 fatty acids for the treatment of depression: Systematic review and meta-analysis. *Molecular Psychiatry* 17:1272-1282. DOI: <http://dx.doi.org/10.1038/mp.2011.100>.
- Bloch M.H., Qawasmi A. (2011) Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: Systematic review and meta-analysis. *Journal of the American Academy of Child and Adolescent Psychiatry* 50:991-1000. DOI: <http://dx.doi.org/10.1016/j.jaac.2011.06.008>.
- Bourre J.M. (2007) Dietary omega-3 fatty acids for women. *Biomedicine and Pharmacotherapy* 61:105-112. DOI: <http://dx.doi.org/10.1016/j.biopha.2006.09.015>.
- Breslow J.L. (2006) n-3 Fatty acids and cardiovascular disease. *American Journal of Clinical Nutrition* 83.
- Brito-Garcia N., Del Pino-Sedeno T., Trujillo-Martin M.M., Coco R.M., Rodriguez de la Rúa E., Del Cura-Gonzalez I., Serrano-Aguilar P. (2017) Effectiveness and safety of nutritional supplements in the treatment of hereditary retinal dystrophies: a systematic review. *Eye* 31:273-285. DOI: <https://dx.doi.org/10.1038/eye.2016.286>.
- Brouwer I.A. (2008) Omega-3 PUFA: Good or bad for prostate cancer? *Prostaglandins Leukotrienes and Essential Fatty Acids* 79:97-99. DOI: <http://dx.doi.org/10.1016/j.plefa.2008.09.006>.
- Brownawell A.M., Harris W.S., Hibbeln J.R., Klurfeld D.M., Newton I., Yates A. (2009) Assessing the environment for regulatory change for eicosapentaenoic acid and docosahexaenoic acid nutrition labeling. *Nutrition Reviews* 67:391-397. DOI: <http://dx.doi.org/10.1111/j.1753-4887.2009.00212.x>.
- Buhling K., Schumacher A., Eulenbug C.Z., Laakmann E. (2019) Influence of oral vitamin and mineral supplementation on male infertility: a meta-analysis and systematic review. *Reproductive Biomedicine Online* 39:269-279. DOI: <https://dx.doi.org/10.1016/j.rbmo.2019.03.099>.
- Burgess V., Dushianthan A., Cusack R., Grocott M. (2018) Immunonutrition for acute respiratory distress syndrome (ARDS) in adults; a cochrane meta-analysis. *Journal of the Intensive Care Society Conference: Intensive Care Society State of the Art, ICS 2017*. Liverpool United Kingdom. 19 (2 Supplement 1) (pp 10-11). DOI: <http://dx.doi.org/10.1177/1751143718772957>.
- Calder P.C. (2013) Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *British Journal of Clinical Pharmacology* 75:645-62. DOI: <https://dx.doi.org/10.1111/j.1365-2125.2012.04374.x>.

- Campoy C., Escolano-Margarit V., Anjos T., Szajewska H., Uauy R. (2012) Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. *British Journal of Nutrition* 107:S85-S106. DOI: <http://dx.doi.org/10.1017/S0007114512001493>.
- Carlson S.E. (2009) Docosahexaenoic acid supplementation in pregnancy and lactation. *American Journal of Clinical Nutrition* 89:678S-84S. DOI: <https://dx.doi.org/10.3945/ajcn.2008.26811E>.
- Carr J.A. (2018) Role of Fish Oil in Post-Cardiotomy Bleeding: A Summary of the Basic Science and Clinical Trials. *Annals of Thoracic Surgery* 105:1563-1567. DOI: <http://dx.doi.org/10.1016/j.athoracsur.2018.01.041>.
- Casula M., Olmastroni E., Gazzotti M., Galimberti F., Zambon A., Catapano A.L. (2020) Omega-3 polyunsaturated fatty acids supplementation and cardiovascular outcomes: do formulation, dosage, and baseline cardiovascular risk matter? An updated meta-analysis of randomized controlled trials. *Pharmacological Research* 160. DOI: <http://dx.doi.org/10.1016/j.phrs.2020.105060>.
- Cederholm T. (2017) Fish consumption and omega-3 fatty acid supplementation for prevention or treatment of cognitive decline, dementia or Alzheimer's disease in older adults-any news? *Current Opinion in Clinical Nutrition and Metabolic Care* 20:104-109. DOI: <http://dx.doi.org/10.1097/MCO.0000000000000350>.
- Chang C.H., Tseng P.T., Chen N.Y., Lin P.C., Lin P.Y., Chang J.P.C., Kuo F.Y., Lin J., Wu M.C., Su K.P. (2018a) Safety and tolerability of prescription omega-3 fatty acids: A systematic review and meta-analysis of randomized controlled trials. *Prostaglandins Leukotrienes and Essential Fatty Acids* 129. DOI: <http://dx.doi.org/10.1016/j.plefa.2018.01.001>.
- Chang J.P.C., Su K.P., Mondelli V., Pariante C.M. (2018b) Omega-3 Polyunsaturated Fatty Acids in Youths with Attention Deficit Hyperactivity Disorder: A Systematic Review and Meta-Analysis of Clinical Trials and Biological Studies. *Neuropsychopharmacology* 43:534-545. DOI: <http://dx.doi.org/10.1038/npp.2017.160>.
- Chang J.P.C., Su K.P., Mondelli V., Satyanarayanan S.K., Yang H.T., Chiang Y.J., Chen H.T., Pariante C.M. (2019) High-dose eicosapentaenoic acid (EPA) improves attention and vigilance in children and adolescents with attention deficit hyperactivity disorder (ADHD) and low endogenous EPA levels. *Translational Psychiatry* 9. DOI: <http://dx.doi.org/10.1038/s41398-019-0633-0>.
- Chase H.P., Boulware D., Rodriguez H., Donaldson D., Chritton S., Rafkin-Mervis L., Krischer J., Skyler J.S., Clare-Salzler M., Type 1 Diabetes TrialNet Nutritional Intervention to Prevent Type 1 Diabetes Study G. (2015) Effect of docosahexaenoic acid supplementation on inflammatory cytokine levels in infants at high genetic risk for type 1 diabetes. *Pediatric Diabetes* 16:271-9. DOI: <https://dx.doi.org/10.1111/pedi.12170>.
- Chemical Book. (2021a) 6217-54-5(Docosahexaenoic Acid) Product Description, https://www.chemicalbook.com/ChemicalProductProperty_US_CB4128690.aspx.

- Chemical Book. (2021b) EPA, https://www.chemicalbook.com/ProductChemicalPropertiesCB9426441_EN.htm.
- Chen B., Zhou Y., Yang P., Wan H.W., Wu X.T. (2010) Safety and efficacy of fish oil-enriched parenteral nutrition regimen on postoperative patients undergoing major abdominal surgery: A meta-analysis of randomized controlled trials. *Journal of Parenteral and Enteral Nutrition* 34:387-394. DOI: <http://dx.doi.org/10.1177/0148607110362532>.
- Chen C., Yu X., Shao S. (2015) Effects of omega-3 fatty acid supplementation on glucose control and lipid levels in type 2 diabetes: A meta-analysis. *PLoS ONE* 10. DOI: <http://dx.doi.org/10.1371/journal.pone.0139565>.
- Chen G.C., Yang J., Eggersdorfer M., Zhang W., Qin L.Q. (2016) N-3 long-chain polyunsaturated fatty acids and risk of all-cause mortality among general populations: a meta-analysis. *Scientific Reports* 6:28165. DOI: <https://dx.doi.org/10.1038/srep28165>.
- Chen H.C., Tsai P.C., Tsai M.J., Lee M.H., Tsai J.R., Sheu C.C. (2013) Anti-inflammatory immuno-modulating enteral nutrition in patients with acute respiratory distress syndrome. *Journal of Internal Medicine of Taiwan* 24:95-106.
- Chen M., Ding Y., Tong Z. (2020) Efficacy and Safety of *Sophora flavescens* (Kushen) Based Traditional Chinese Medicine in the Treatment of Ulcerative Colitis: Clinical Evidence and Potential Mechanisms. *Frontiers in Pharmacology* 11:603476. DOI: <https://dx.doi.org/10.3389/fphar.2020.603476>.
- Chowdhury M.H., Ghosh S., Kabir M.R., Mamun M.A.A., Islam M.S. (2020) Effect of supplementary omega-3 fatty acids on pregnant women with complications and pregnancy outcomes: review from literature. *Journal of Maternal Fetal and Neonatal Medicine*. DOI: <http://dx.doi.org/10.1080/14767058.2020.1786522>.
- Chua M., Christina Sio M., Sorongon M., Dy J. (2012) Relationship of dietary intake of omega-3 and omega-6 fatty acids with risk of prostate cancer development: A meta-analysis of prospective studies and review of literature. *Journal of Urology Conference:2012 Annual Meeting of the American Urological Association, AUA*. Atlanta, GA United States. Conference Publication: (var.pagings). 187 (4 SUPPL. 1) (pp e139-e140). DOI: <http://dx.doi.org/10.1016/j.juro.2012.02.404>.
- Chua M.E., Sio M.C., Sorongon M.C., Morales M.L., Jr. (2013) The relevance of serum levels of long chain omega-3 polyunsaturated fatty acids and prostate cancer risk: A meta-analysis. *Canadian Urological Association Journal* 7:E333-43. DOI: <https://dx.doi.org/10.5489/cuaj.1056>.
- Ciappolino V., Delvecchio G., Agostoni C., Mazzocchi A., Altamura A.C., Brambilla P. (2017) The role of n-3 polyunsaturated fatty acids (n-3PUFAs) in affective disorders. *Journal of Affective Disorders* 224. DOI: <http://dx.doi.org/10.1016/j.jad.2016.12.034>.
- Clayton E.H., Hanstock T.L., Garg M.L., Hazell P.L. (2007) Long chain omega-3 polyunsaturated fatty acids in the treatment of psychiatric illnesses in children and adolescents. *Acta Neuropsychiatrica* 19:92-103. DOI: <https://dx.doi.org/10.1111/j.1601-5215.2007.00189.x>.

- Cochrane Glossary. (2020) <https://community.cochrane.org/glossary#letter-S>.
- Cohen J.T., Bellinger D.C., Connor W.E., Shaywitz B.A. (2005) A quantitative analysis of prenatal intake of n-3 polyunsaturated fatty acids and cognitive development. *American Journal of Preventive Medicine* 29:366-74.
- Colomer R., Moreno-Nogueira J.M., Garcia-Luna P.P., Garcia-Peris P., Garcia-de-Lorenzo A., Zarazaga A., Quecedo L., del Llano J., Usan L., Casimiro C. (2007) N-3 fatty acids, cancer and cachexia: a systematic review of the literature. *British Journal of Nutrition* 97:823-31.
- Cornu C., Mercier C., Ginhoux T., Masson S., Mouchet J., Nony P., Kassai B., Laudy V., Berquin P., Franc N., Le Heuzey M.F., Desombre H., Revol O. (2018) A double-blind placebo-controlled randomised trial of omega-3 supplementation in children with moderate ADHD symptoms. *European Child and Adolescent Psychiatry* 27:377-384. DOI: <http://dx.doi.org/10.1007/s00787-017-1058-z>.
- Couce M.L., De Castro M.J., De Lamas C., Leis R. (2019) Effects of LC-PUFA supplementation in patients with phenylketonuria: A systematic review of controlled trials. *Nutrients* 11. DOI: <http://dx.doi.org/10.3390/nu11071537>.
- Craddock J.C., Neale E.P., Probst Y.C., Peoples G.E. (2017) Algal supplementation of vegetarian eating patterns improves plasma and serum docosahexaenoic acid concentrations and omega-3 indices: a systematic literature review. *Journal of Human Nutrition & Dietetics* 30:693-699. DOI: <https://dx.doi.org/10.1111/jhn.12474>.
- Crawford M.A., Costeloe K., Ghebremeskel K., Phylactos A., Skirvin L., Stacey F. (1997) Are deficits of arachidonic and docosahexaenoic acids responsible for the neural and vascular complications of preterm babies? *American Journal of Clinical Nutrition* 66. DOI: <http://dx.doi.org/10.1093/ajcn/66.4.1032S>.
- Crippa A., Agostoni C., Mauri M., Molteni M., Nobile M. (2018) Polyunsaturated Fatty Acids Are Associated With Behavior But Not With Cognition in Children With and Without ADHD: An Italian study. *Journal of attention disorders* 22:971-983. DOI: <http://dx.doi.org/10.1177/1087054716629215>.
- Crippa A., Tesei A., Sangiorgio F., Salandi A., Trabattoni S., Grazioli S., Agostoni C., Molteni M., Nobile M. (2019) Behavioral and cognitive effects of docosahexaenoic acid in drug-naive children with attention-deficit/hyperactivity disorder: a randomized, placebo-controlled clinical trial. *European Child and Adolescent Psychiatry* 28:571-583. DOI: <http://dx.doi.org/10.1007/s00787-018-1223-z>.
- de Aguiar Pastore Silva J., Emilia de Souza Fabre M., Waitzberg D.L. (2015) Omega-3 supplements for patients in chemotherapy and/or radiotherapy: A systematic review. *Clinical Nutrition* 34:359-66. DOI: <https://dx.doi.org/10.1016/j.clnu.2014.11.005>.
- de Ferranti S.D., Milliren C.E., Denhoff E.R., Steltz S.K., Selamet Tierney E.S., Feldman H.A., Osganian S.K. (2014) Using high-dose omega-3 fatty acid supplements to lower triglyceride levels in 10- to 19-year-olds. *Clinical Pediatrics* 53:428-38. DOI: <https://dx.doi.org/10.1177/0009922814528032>.

- de Souza Fernandes D.P., Canaan Rezende F.A., Pereira Rocha G., De Santis Filgueiras M., Silva Moreira P.R., Goncalves Alfenas Rde C. (2015) Effect of Eicosapentaenoic Acid and Docosahexaenoic Acid Supplementations to Control Cognitive Decline in Dementia and Alzheimer's Disease: A Systematic Review. *Nutricion Hospitalaria* 32:528-33. DOI: <https://dx.doi.org/10.3305/nh.2015.32.2.9111>.
- Delarue J., Le Guen V., Allain G., Corporeau C., Guillerme S. (2007) Can marine omega 3 fatty acids prevent and/or treat metabolic syndrome? *Current Nutrition and Food Science* 3:151-156. DOI: <http://dx.doi.org/10.2174/157340107780598663>.
- Delgado-Lista J., Perez-Martinez P., Lopez-Miranda J., Perez-Jimenez F. (2012) Long chain omega-3 fatty acids and cardiovascular disease: A systematic review. *British Journal of Nutrition* 107:S201-S213. DOI: <http://dx.doi.org/10.1017/S0007114512001596>.
- Delgado-Noguera M.F., Calvache J.A., Bonfill Cosp X., Kotanidou E.P., Galli-Tsinopoulou A. (2015) Supplementation with long chain polyunsaturated fatty acids (LCPUFA) to breastfeeding mothers for improving child growth and development. *Cochrane Database of Systematic Reviews*:CD007901. DOI: <https://dx.doi.org/10.1002/14651858.CD007901.pub3>.
- Dewey A., Baughan C., Dean T., Higgins B., Johnson I. (2007) Eicosapentaenoic acid (EPA, an omega-3 fatty acid from fish oils) for the treatment of cancer cachexia. *Cochrane Database of Systematic Reviews*. DOI: <http://dx.doi.org/10.1002/14651858.CD004597.pub2>.
- DiNicolantonio J.J., O'Keefe J.H. (2020) The Importance of Marine Omega-3s for Brain Development and the Prevention and Treatment of Behavior, Mood, and Other Brain Disorders. *Nutrients* 12:04. DOI: <https://dx.doi.org/10.3390/nu12082333>.
- Downie L.E., Ng S.M., Lindsley K.B., Akpek E.K. (2019) Omega-3 and omega-6 polyunsaturated fatty acids for dry eye disease. *Cochrane Database of Systematic Reviews*. DOI: <http://dx.doi.org/10.1002/14651858.CD011016.pub2>.
- Dushianthan A., Cusack R., Burgess V.A., Grocott M.P., Calder P. (2020) Immunonutrition for Adults With ARDS: Results From a Cochrane Systematic Review and Meta-Analysis. *Respiratory Care* 65:99-110. DOI: <https://dx.doi.org/10.4187/respcare.06965>.
- Dushianthan A., Cusack R., Burgess V.A., Grocott M.P., Calder P.C. (2019) Immunonutrition for acute respiratory distress syndrome (ARDS) in adults. *Cochrane Database of Systematic Reviews* 1:CD012041. DOI: <https://dx.doi.org/10.1002/14651858.CD012041.pub2>.
- EFSA. (2010) Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA). *EFSA Journal* 8:1461. DOI: <https://doi.org/10.2903/j.efsa.2010.1461>.
- EFSA. (2012) Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 10:2815. DOI: <https://doi.org/10.2903/j.efsa.2012.2815>.

EFSA Glossary. European Food Safety Authority, <https://www.efsa.europa.eu/en/glossary-taxonomy-terms>.

Elagizi A., Lavie C.J., O'Keefe E., Marshall K., O'Keefe J.H., Milani R.V. (2021) An update on omega-3 polyunsaturated fatty acids and cardiovascular health. *Nutrients* 13:1-12. DOI: <http://dx.doi.org/10.3390/nu13010204>.

Elia M., Van Bokhorst-de van der Schueren M.A., Garvey J., Goedhart A., Lundholm K., Nitenberg G., Stratton R.J. (2006) Enteral (oral or tube administration) nutritional support and eicosapentaenoic acid in patients with cancer: a systematic review. *International Journal of Oncology* 28:5-23.

Emery S., Haberling I., Berger G., Walitza S., Schmeck K., Albert T., Baumgartner N., Strumberger M., Albermann M., Drechsler R. (2020) Omega-3 and its domain-specific effects on cognitive test performance in youths: A meta-analysis. *Neuroscience and Biobehavioral Reviews* 112. DOI: <http://dx.doi.org/10.1016/j.neubiorev.2020.02.016>.

Engler M.M., Engler M.B., Malloy M., Chiu E., Besio D., Paul S., Stuehlinger M., Morrow J., Ridker P., Rifai N., Mietus-Snyder M. (2004) Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY study. *Int J Clin Pharmacol Ther* 42:672-9. DOI: 10.5414/cpp42672.

Eslick G.D., Howe P.R.C., Smith C., Priest R., Bensoussan A. (2009) Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. *International Journal of Cardiology* 136:4-16. DOI: <http://dx.doi.org/10.1016/j.ijcard.2008.03.092>.

Fassett R.G., Gobe G.C., Peake J.M., Coombes J.S. (2010) Omega-3 polyunsaturated fatty acids in the treatment of kidney disease. *American Journal of Kidney Diseases* 56:728-742. DOI: <http://dx.doi.org/10.1053/j.ajkd.2010.03.009>.

Firth J., Teasdale S.B., Allott K., Siskind D., Marx W., Cotter J., Veronese N., Schuch F., Smith L., Solmi M., Carvalho A.F., Vancampfort D., Berk M., Stubbs B., Sarris J. (2019) The efficacy and safety of nutrient supplements in the treatment of mental disorders: a meta-review of meta-analyses of randomized controlled trials. *World Psychiatry* 18:308-324. DOI: <http://dx.doi.org/10.1002/wps.20672>.

Fogacci F., Strocchi E., Veronesi M., Borghi C., Cicero A.F.G. (2020) Effect of omega-3 polyunsaturated fatty acids treatment on lipid pattern of hiv patients: A meta-analysis of randomized clinical trials. *Marine Drugs* 18. DOI: <http://dx.doi.org/10.3390/md18060292>.

Freeman M.P., Hibbeln J.R., Wisner K.L., Davis J.M., Mischoulon D., Peet M., Keck P.E., Jr., Marangell L.B., Richardson A.J., Lake J., Stoll A.L. (2006) Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. *Journal of Clinical Psychiatry* 67:1954-67.

Fu Y.Q., Zheng J.S., Yang B., Li D. (2015) Effect of individual omega-3 fatty acids on the risk of prostate cancer: a systematic review and dose-response meta-analysis of prospective cohort studies. *Journal of Epidemiology* 25:261-74. DOI: <https://dx.doi.org/10.2188/jea.JE20140120>.

- Fusar-Poli P., Berger G. (2012) Eicosapentaenoic acid interventions in schizophrenia: Meta-analysis of randomized, placebo-controlled studies. *Journal of Clinical Psychopharmacology* 32:179-185. DOI: <http://dx.doi.org/10.1097/JCP.0b013e318248b7bb>.
- Gawlik N.R., Anderson A.J., Makrides M., Kettler L., Gould J.F. (2020) The influence of DHA on language development: A review of randomized controlled trials of DHA supplementation in pregnancy, the neonatal period, and infancy. *Nutrients* 12:1-31. DOI: <http://dx.doi.org/10.3390/nu12103106>.
- Gil-Campos M., Sanjurjo Crespo P. (2012) Omega 3 fatty acids and inborn errors of metabolism. *British Journal of Nutrition* 107:S129-S136. DOI: <http://dx.doi.org/10.1017/S0007114512001523>.
- Glaser C., Koletzko B. (2009) Long-chain omega-3 fatty acids in the perinatal period: Recommendations for intake. *Aktuelle Ernährungsmedizin* 34:240-245. DOI: <http://dx.doi.org/10.1055/s-0029-1220446>.
- Goh K.K., Chen C.Y.A., Chen C.H., Lu M.L. (2021) Effects of omega-3 polyunsaturated fatty acids supplements on psychopathology and metabolic parameters in schizophrenia: A meta-analysis of randomized controlled trials. *Journal of Psychopharmacology* 35:221-235. DOI: <http://dx.doi.org/10.1177/0269881120981392>.
- Gonzalez-Becerra K., Ramos-Lopez O., Barron-Cabrera E., Riezu-Boj J.I., Milagro F.I., Martinez-Lopez E., Martinez J.A. (2019) Fatty acids, epigenetic mechanisms and chronic diseases: A systematic review. *Lipids in Health and Disease* 18. DOI: <http://dx.doi.org/10.1186/s12944-019-1120-6>.
- Gould J.F., Roberts R.M., Makrides M. (2021) The influence of omega-3 long-chain polyunsaturated fatty acid, docosahexaenoic acid, on child behavioral functioning: A review of randomized controlled trials of dha supplementation in pregnancy, the neonatal period and infancy. *Nutrients* 13:1-30. DOI: <http://dx.doi.org/10.3390/nu13020415>.
- Gow R.V., Hibbeln J.R., Parletta N. (2015) Current evidence and future directions for research with omega-3 fatty acids and attention deficit hyperactivity disorder. *Current Opinion in Clinical Nutrition and Metabolic Care* 18:133-138. DOI: <http://dx.doi.org/10.1097/MCO.000000000000140>.
- Grosso G., Micek A., Marventano S., Castellano S., Mistretta A., Pajak A., Galvano F. (2016) Dietary n-3 PUFA, fish consumption and depression: A systematic review and meta-analysis of observational studies. *Journal of Affective Disorders* 205. DOI: <http://dx.doi.org/10.1016/j.jad.2016.08.011>.
- Grosso G., Pajak A., Marventano S., Castellano S., Galvano F., Bucolo C., Drago F., Caraci F. (2014) Role of omega-3 fatty acids in the treatment of depressive disorders: A comprehensive meta-analysis of randomized clinical trials. *PLoS ONE* 9. DOI: <http://dx.doi.org/10.1371/journal.pone.0096905>.

- Grynberg A. (2005) Hypertension prevention: From nutrients to (fortified) foods to dietary patterns. Focus on fatty acids. *Journal of Human Hypertension* 19:S25-S33. DOI: <http://dx.doi.org/10.1038/sj.jhh.1001957>.
- Guo X.F., Li K.L., Li J.M., Li D. (2019) Effects of EPA and DHA on blood pressure and inflammatory factors: a meta-analysis of randomized controlled trials. *Critical Reviews in Food Science & Nutrition* 59:3380-3393. DOI: <https://dx.doi.org/10.1080/10408398.2018.1492901>.
- Guo X.F., Li X., Shi M., Li D. (2017) N-3 polyunsaturated fatty acids and metabolic syndrome risk: A meta-analysis. *Nutrients* 9. DOI: <http://dx.doi.org/10.3390/nu9070703>.
- Guo X.F., Yang B., Tang J., Li D. (2018) Fatty acid and non-alcoholic fatty liver disease: Meta-analyses of case-control and randomized controlled trials. *Clinical Nutrition* 37:113-122. DOI: <http://dx.doi.org/10.1016/j.clnu.2017.01.003>.
- Guo X.Y., Yan X.L., Chen Y.W., Tang R.B., Du X., Dong J.Z., Ma C.S. (2014) Omega-3 fatty acids for postoperative atrial fibrillation: Alone or in combination with antioxidant vitamins? *Heart Lung and Circulation* 23:743-750. DOI: <http://dx.doi.org/10.1016/j.hlc.2014.02.018>.
- Guu T.W., Mischoulon D., Sarris J., Hibbeln J., McNamara R.K., Hamazaki K., Freeman M.P., Maes M., Matsuoka Y.J., Belmaker R.H., Jacka F., Pariante C., Berk M., Marx W., Su K.P. (2019) International Society for Nutritional Psychiatry Research Practice Guidelines for Omega-3 Fatty Acids in the Treatment of Major Depressive Disorder. *Psychotherapy and Psychosomatics* 88:263-273. DOI: <http://dx.doi.org/10.1159/000502652>.
- Haberling I., Berger G., Schmeck K., Held U., Walitza S. (2019) Omega-3 Fatty Acids as a Treatment for Pediatric Depression. A Phase III, 36 Weeks, Multi-Center, Double-Blind, Placebo-Controlled Randomized Superiority Study. *Frontiers in Psychiatry* 10. DOI: <http://dx.doi.org/10.3389/fpsy.2019.00863>.
- Hadders-Algra M., Kikkert H.K., De Jong C. (2010) Effect of an omega-3 enriched diet on development. *Developmental Medicine and Child Neurology Conference: 22nd Annual Meeting of the European Academy of Childhood Disability, EACD. Brussels Belgium. Sponsor: Medtronic . Conference Publication: (var.pagings). 52 (SUPPL. 4) (pp 45-46)*. DOI: <http://dx.doi.org/10.1111/j.1469-8749.2010.03682.x>.
- Hammad S., Pu S., Jones P.J. (2016) Current Evidence Supporting the Link Between Dietary Fatty Acids and Cardiovascular Disease. *Lipids* 51:507-517. DOI: <http://dx.doi.org/10.1007/s11745-015-4113-x>.
- Han J., Kang L., Liang D., Li H., Su Y., Zhang Y., Yang Y. (2019) Composition requirements of follow-up formula for 6-12-month-old infants: recommendations of a Chinese expert group. *Asia Pacific Journal of Clinical Nutrition* 28:347-355. DOI: [https://dx.doi.org/10.6133/apjcn.201906_28\(2\).0017](https://dx.doi.org/10.6133/apjcn.201906_28(2).0017).
- Hansen L.B., Myhre J.B., Johansen A.M.W., Paulsen M.M., Andersen L.F. (2016) UNGKOST 3 Landsomfattende kostholdsundersøkelse blant elever i 4. -og 8. klasse i Norge, 2015,

<https://www.fhi.no/globalassets/dokumenterfiler/rapporter/2016/ungkost-rapport-24.06.16.pdf>.

- Hansen S.N., Harris W.S. (2007) New evidence for the cardiovascular benefits of long chain omega-3 fatty acids. *Current Atherosclerosis Reports* 9:434-440. DOI: <http://dx.doi.org/10.1007/s11883-007-0058-8>.
- Hanssens L., Thiebaut I., Lefevre N., Malroot A., Knoop C., Duchateau J., Casimir G. (2016) The clinical benefits of long-term supplementation with omega-3 fatty acids in cystic fibrosis patients - A pilot study. *Prostaglandins Leukotrienes & Essential Fatty Acids* 108:45-50. DOI: <https://dx.doi.org/10.1016/j.plefa.2016.03.014>.
- Harper C.R., Jacobson T.A. (2005) Usefulness of omega-3 fatty acids and the prevention of coronary heart disease. *American Journal of Cardiology* 96:1521-9.
- Harris W.S., Dayspring T.D., Moran T.J. (2013) Omega-3 fatty acids and cardiovascular disease: new developments and applications. *Postgraduate Medicine* 125:100-13. DOI: <https://dx.doi.org/10.3810/pgm.2013.11.2717>.
- Harris W.S., Poston W.C., Haddock C.K. (2007) Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis* 193:1-10.
- Harris W.S., Zotor F.B. (2019) N-3 Fatty acids and risk for fatal coronary disease. *Proceedings of the Nutrition Society* 78:526-531. DOI: <http://dx.doi.org/10.1017/S0029665118002902>.
- Hartweg J., Farmer A.J., Holman R.R., Neil A. (2009) Potential impact of omega-3 treatment on cardiovascular disease in type 2 diabetes. *Current Opinion in Lipidology* 20:30-38. DOI: <http://dx.doi.org/10.1097/MOL.0b013e328321b3be>.
- Hegarty B., Parker G. (2013) Fish oil as a management component for mood disorders - An evolving signal. *Current Opinion in Psychiatry* 26:33-40. DOI: <http://dx.doi.org/10.1097/YCO.0b013e32835ab4a7>.
- Hendrich S. (2010) (n-3) Fatty Acids: Clinical Trials in People with Type 2 Diabetes. *Advances in Nutrition* 1:3-7. DOI: <https://dx.doi.org/10.3945/an.110.1003>.
- Hibbeln J.R., Gow R.V. (2014) The potential for military diets to reduce depression, suicide, and impulsive aggression: a review of current evidence for omega-3 and omega-6 fatty acids. *Military Medicine* 179:117-28. DOI: <https://dx.doi.org/10.7205/MILMED-D-14-00153>.
- Hidayat K., Yang J., Zhang Z., Chen G.C., Qin L.Q., Eggersdorfer M., Zhang W. (2018) Effect of omega-3 long-chain polyunsaturated fatty acid supplementation on heart rate: A meta-Analysis of randomized controlled trials. *European Journal of Clinical Nutrition* 72:805-817. DOI: <http://dx.doi.org/10.1038/s41430-017-0052-3>.
- Hosseini B., Nourmohamadi M., Hajipour S., Taghizadeh M., Asemi Z., Keshavarz S.A., Jafarnejad S. (2019) The Effect of Omega-3 Fatty Acids, EPA, and/or DHA on Male Infertility: A Systematic Review and Meta-analysis. *Journal of Dietary Supplements* 16:245-256. DOI: <https://dx.doi.org/10.1080/19390211.2018.1431753>.

- Hou R., Yao S.S., Liu J., Wang L.L., Wu L., Jiang L. (2017) Dietary n-3 polyunsaturated fatty acids, fish consumption, and endometrial cancer risk: A meta-analysis of epidemiological studies. *Oncotarget* 8:91684-91693. DOI: <http://dx.doi.org/10.18632/oncotarget.18295>.
- Hsu M.C., Tung C.Y., Chen H.E. (2018) Omega-3 polyunsaturated fatty acid supplementation in prevention and treatment of maternal depression: Putative mechanism and recommendation. *Journal of Affective Disorders* 238. DOI: <http://dx.doi.org/10.1016/j.jad.2018.05.018>.
- Hu C., Yang M., Zhu X., Gao P., Yang S., Han Y., Chen X., Xiao L., Yuan S., Liu F., Kanwar Y.S., Sun L. (2018) Effects of Omega-3 Fatty Acids on Markers of Inflammation in Patients With Chronic Kidney Disease: A Controversial Issue. *Therapeutic Apheresis and Dialysis* 22:124-132. DOI: <http://dx.doi.org/10.1111/1744-9987.12611>.
- Hu Y., Hu F.B., Manson J.E. (2019) Marine Omega-3 Supplementation and Cardiovascular Disease: An Updated Meta-Analysis of 13 Randomized Controlled Trials Involving 127 477 Participants. *Journal of the American Heart Association* 8. DOI: <http://dx.doi.org/10.1161/JAHA.119.013543>.
- Huang F., Del Rio Navarro B., Saucedo Ramirez O., Hall Mondragon M., Perez Ontiveros J., Torres Alcantara S. (2014) Effect of N-3 polyunsaturated fatty acids on adipokines and biomarkers of endothelial dysfunction in obese asthmatic adolescents with hypertriglyceridemia. *Obesity Surgery Conference:19th World Congress of the International Federation for the Surgery of Obesity and Metabolic Disorders, IFSO 2014. Montreal, QC Canada. Conference Publication: (var.pagings). 24 (8) (pp 1281)*. DOI: <http://dx.doi.org/10.1007/s11695-014-1292-0>.
- Huang F., Del Rio Navarro B.E., Ramirez O.J.S., Mondragon M.S.H., Ontiveros J.A.P., Alcantara S.T. (2015) Effect of N-3 polyunsaturated fatty acids on adipokines and biomarkers of endothelial dysfunction in obese asthmatic adolescents with hypertriglyceridemia. *Endocrine Practice Conference:12th Annual World Congress on Insulin Resistance, Diabetes and Cardiovascular Disease, WCIRDC 2014. Los Angeles, CA United States. Conference Publication: (var.pagings). 21 (1) (pp 8A-9A)*.
- Hughbanks-Wheaton D.K., Birch D.G., Fish G.E., Spencer R., Pearson N.S., Takacs A., Hoffman D.R. (2014) Safety assessment of docosahexaenoic acid in X-linked retinitis pigmentosa: the 4-year DHAX trial. *Investigative Ophthalmology & Visual Science* 55:4958-66. DOI: <https://dx.doi.org/10.1167/iovs.14-14437>.
- Innes J.K., Calder P.C. (2018) The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: A systematic review. *International Journal of Molecular Sciences* 19. DOI: <http://dx.doi.org/10.3390/ijms19020532>.
- Innes J.K., Calder P.C. (2020) Marine omega-3 (N-3) fatty acids for cardiovascular health: An update for 2020. *International Journal of Molecular Sciences* 21. DOI: <http://dx.doi.org/10.3390/ijms21041362>.
- IOM. (2005) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids, Institute of Medicine, Washington DC.

- Irving C.B., Mumby-Croft R., Joy L.A. (2006) Polyunsaturated fatty acid supplementation for schizophrenia. Cochrane Database of Systematic Reviews:CD001257.
- Jacobson T.A. (2008) Role of n-3 fatty acids in the treatment of hypertriglyceridemia and cardiovascular disease. *American Journal of Clinical Nutrition* 87:1981S-90S.
- Janczyk W., Lebensztejn D., Wierzbicka-Rucinska A., Mazur A., Neuhoff-Murawska J., Matusik P., Socha P. (2015) Omega-3 Fatty acids therapy in children with nonalcoholic Fatty liver disease: a randomized controlled trial. *Journal of Pediatrics* 166:1358-63.e1-3. DOI: <https://dx.doi.org/10.1016/j.jpeds.2015.01.056>.
- Jans L.A.W., Giltay E.J., Willem Van Der Does A.J. (2010) The efficacy of n-3 fatty acids DHA and EPA (fish oil) for perinatal depression. *British Journal of Nutrition* 104:1577-1585. DOI: <http://dx.doi.org/10.1017/S0007114510004125>.
- Jasani B., Simmer K., Patole S.K., Rao S.C. (2017) Long chain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database of Systematic Reviews. DOI: <http://dx.doi.org/10.1002/14651858.CD000376.pub4>.
- Jenkins D.J.A., Josse A.R., Dorian P., Burr M.L., Trangmar R.L., Kendall C.W.C., Cunnane S.C. (2008) Heterogeneity in randomized controlled trials of long chain (fish) omega-3 fatty acids in restenosis, secondary prevention and ventricular arrhythmias. *Journal of the American College of Nutrition* 27:367-378.
- Jho D.H., Cole S.M., Lee E.M., Espat N.J. (2004) Role of omega-3 fatty acid supplementation in inflammation and malignancy. *Integrative Cancer Therapies* 3:98-111. DOI: <http://dx.doi.org/10.1177/1534735404264736>.
- Jia X., Koh S., Al Rifai M., Blumenthal R.S., Virani S.S. (2020) Spotlight on icosapent ethyl for cardiovascular risk reduction: Evidence to date. *Vascular Health and Risk Management* 16. DOI: <http://dx.doi.org/10.2147/VHRM.S210149>.
- Jiao J., Li Q., Chu J., Zeng W., Yang M., Zhu S. (2014) Effect of n-3 PUFA supplementation on cognitive function throughout the life span from infancy to old age: a systematic review and meta-analysis of randomized controlled trials. *American Journal of Clinical Nutrition* 100:1422-36. DOI: <https://dx.doi.org/10.3945/ajcn.114.095315>.
- Jung J.Y., Kwon H.H., Hong J.S., Yoon J.Y., Park M.S., Jang M.Y., Suh D.H. (2014) Effect of dietary supplementation with omega-3 fatty acid and gamma-linolenic acid on acne vulgaris: a randomised, double-blind, controlled trial. *Acta Dermato-Venereologica* 94:521-5. DOI: <https://dx.doi.org/10.2340/00015555-1802>.
- Kalkman H.O., Hersberger M., Walitza S., Berger G.E. (2021) Disentangling the molecular mechanisms of the antidepressant activity of omega-3 polyunsaturated fatty acid: A comprehensive review of the literature. *International Journal of Molecular Sciences* 22. DOI: <http://dx.doi.org/10.3390/ijms22094393>.
- Kar S., Wong M., Rogozinska E., Thangaratinam S. (2016) Effects of omega-3 fatty acids in prevention of early preterm delivery: A systematic review and meta-analysis of randomized studies. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 198. DOI: <http://dx.doi.org/10.1016/j.ejogrb.2015.11.033>.

- Keenan K., Hipwell A.E. (2015) Modulation of prenatal stress via docosahexaenoic acid supplementation: Implications for child mental health. *Nutrition Reviews* 73:166-174. DOI: <http://dx.doi.org/10.1093/nutrit/nuu020>.
- Kidd P.M. (2007) Omega-3 DHA and EPA for cognition, behavior, and mood: Clinical findings and structural-functional synergies with cell membrane phospholipids. *Alternative Medicine Review* 12:207-227.
- Kim Y., Kim J. (2020) Intake or blood levels of n-3 polyunsaturated fatty acids and risk of colorectal cancer: A systematic review and meta-analysis of prospective studies. *Cancer Epidemiology Biomarkers and Prevention* 29:288-299. DOI: <http://dx.doi.org/10.1158/1055-9965.EPI-19-0931>.
- Kimmig L.M., Karalis D.G. (2013) Do omega-3 polyunsaturated fatty acids prevent cardiovascular disease? A review of the randomized clinical trials. *Lipid Insights* 6. DOI: <http://dx.doi.org/10.4137/LPI.S10846>.
- Klosiewicz-Latoszek L., Cybulska B., Tyszko P. (2020) Current state-of-the-art knowledge on the role of omega-3 fatty acids in the prevention of cardiovascular disease. *Annals of Agricultural and Environmental Medicine* 27:519-525. DOI: <http://dx.doi.org/10.26444/aaem/126674>.
- Koekkoek W.A.C., Panteleon V., van Zanten A.R.H. (2018) Data on effects, tolerability and safety of Omega-3 Fatty Acids in Enteral Nutrition in the Critically ill. *Data in Brief* 21:604-615. DOI: <https://dx.doi.org/10.1016/j.dib.2018.10.017>.
- Koletzko B., Boey C.C.M., Campoy C., Carlson S.E., Chang N., Guillermo-Tuazon M.A., Joshi S., Prell C., Quak S.H., Sjarif D.R., Su Y., Supapannachart S., Yamashiro Y., Osendarp S.J.M. (2014) Current information and asian perspectives on long-chain polyunsaturated fatty acids in pregnancy, lactation, and infancy: Systematic review and practice recommendations from an early nutrition academy workshop. *Annals of Nutrition and Metabolism* 65:49-80. DOI: <http://dx.doi.org/10.1159/000365767>.
- Koletzko B., Cremer M., Flothkotter M., Graf C., Hauner H., Hellmers C., Kersting M., Krawinkel M., Przyrembel H., Robl-Mathieu M., Schiffner U., Vetter K., Weibenborn A., Wockel A. (2018) Diet and Lifestyle before and during Pregnancy - Practical Recommendations of the Germany-wide Healthy Start - Young Family Network. *Geburtshilfe und Frauenheilkunde* 78:1262-1282. DOI: <http://dx.doi.org/10.1055/a-0713-1058>.
- Koletzko B., Uauy R., Palou A., Kok F., Hornstra G., Eilander A., Moretti D., Osendarp S., Zock P., Innis S. (2010) Dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in children-A workshop report. *British Journal of Nutrition* 103:923-928. DOI: <http://dx.doi.org/10.1017/S0007114509991851>.
- Kromhout D. (2012) Omega-3 fatty acids and coronary heart disease. The final verdict? *Current Opinion in Lipidology* 23:554-9. DOI: <https://dx.doi.org/10.1097/MOL.0b013e328359515f>.

- Kromhout D., De Goede J. (2014) Update on cardiometabolic health effects of omega-3 fatty acids. *Current Opinion in Lipidology* 25:85-90. DOI: <http://dx.doi.org/10.1097/MOL.0000000000000041>.
- Kuratko C.N., Barrett E.C., Nelson E.B., Salem N., Jr. (2013) The relationship of docosahexaenoic acid (DHA) with learning and behavior in healthy children: a review. *Nutrients* 5:2777-810. DOI: <https://dx.doi.org/10.3390/nu5072777>.
- Kwak S.M., Myung S.K., Lee Y.J., Seo H.G., Korean Meta-analysis Study G. (2012) Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: a meta-analysis of randomized, double-blind, placebo-controlled trials. *Archives of Internal Medicine* 172:686-94. DOI: <https://dx.doi.org/10.1001/archinternmed.2012.262>.
- Lachance L., McKenzie K., Taylor V.H., Vigod S.N. (2016) Omega-6 to omega-3 fatty acid ratio in patients with ADHD: A meta-analysis. *Journal of the Canadian Academy of Child and Adolescent Psychiatry* 25:87-96.
- Langlois P.L., D'Aragon F., Hardy G., Manzanares W. (2019) Omega-3 polyunsaturated fatty acids in critically ill patients with acute respiratory distress syndrome: A systematic review and meta-analysis. *Nutrition* 61. DOI: <http://dx.doi.org/10.1016/j.nut.2018.10.026>.
- Lapillonne A., Jensen C.L. (2009) Reevaluation of the DHA requirement for the premature infant. *Prostaglandins Leukotrienes & Essential Fatty Acids* 81:143-50. DOI: <https://dx.doi.org/10.1016/j.plefa.2009.05.014>.
- Larque E., Gil-Sanchez A., Prieto-Sanchez M.T., Koletzko B. (2012) Omega 3 fatty acids, gestation and pregnancy outcomes. *British Journal of Nutrition* 107:S77-S84. DOI: <http://dx.doi.org/10.1017/S0007114512001481>.
- Lehner A., Staub K., Aldakak L., Eppenberger P., Ruhli F., Martin R.D., Bender N. (2021) Impact of omega-3 fatty acid DHA and EPA supplementation in pregnant or breast-feeding women on cognitive performance of children: Systematic review and meta-analysis. *Nutrition Reviews* 79:585-598. DOI: <http://dx.doi.org/10.1093/nutrit/nuaa060>.
- Lenighan Y.M., McNulty B.A., Roche H.M. (2019) Dietary fat composition: Replacement of saturated fatty acids with PUFA as a public health strategy, with an emphasis on alpha-linolenic acid. *Proceedings of the Nutrition Society* 78:234-245. DOI: <http://dx.doi.org/10.1017/S0029665118002793>.
- Leon H., Shibata M.C., Sivakumaran S., Dorgan M., Chatterley T., Tsuyuki R.T. (2008) Effect of fish oil on arrhythmias and mortality: Systematic review. *BMJ* 338:149-151. DOI: <http://dx.doi.org/10.1136/bmj.a2931>.
- Leslie M.A., Cohen D.J.A., Liddle D.M., Robinson L.E., Ma D.W.L. (2015) A review of the effect of omega-3 polyunsaturated fatty acids on blood triacylglycerol levels in normolipidemic and borderline hyperlipidemic individuals. *Lipids in Health and Disease* 14. DOI: <http://dx.doi.org/10.1186/s12944-015-0049-7>.

- Liao Y., Xie B., Zhang H., He Q., Guo L., Subramaniapillai M., Fan B., Lu C., McLntyre R.S. (2019) Efficacy of omega-3 PUFAs in depression: A meta-analysis. *Translational Psychiatry* 9. DOI: <http://dx.doi.org/10.1038/s41398-019-0515-5>.
- Lohner S., Fekete K., Marosvolgyi T., Decsi T. (2013) Gender differences in the long-chain polyunsaturated fatty acid status: Systematic review of 51 publications. *Annals of Nutrition and Metabolism* 62:98-112. DOI: <http://dx.doi.org/10.1159/000345599>.
- Lopez-Frias S.B., Alcantara S.T., De Jesus Leija Martinez J., Del Rio Navarro B.E., Del Carmen Castillo Hernandez M., Huang F. (2018) Effect of N-3 polyunsaturated fatty acids on oxidative stress markers and resolvin e1 in obese asthmatic adolescents with hypertriglyceridemia. *Atherosclerosis Supplements Conference:18th International Symposium on Atherosclerosis, ISA 2018*. Toronto, ON Canada. 32 (pp 133).
- Lopez-Huertas E. (2012) The effect of EPA and DHA on metabolic syndrome patients: A systematic review of randomised controlled trials. *British Journal of Nutrition* 107:S185-S194. DOI: <http://dx.doi.org/10.1017/S0007114512001572>.
- Lorente-Cebrian S., Costa A.G.V., Navas-Carretero S., Zabala M., Martinez J.A., Moreno-Aliaga M.J. (2013) Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: A review of the evidence. *Journal of Physiology and Biochemistry* 69:633-651. DOI: <http://dx.doi.org/10.1007/s13105-013-0265-4>.
- Lovegrove C., Ahmed K., Challacombe B., Khan M.S., Popert R., Dasgupta P. (2015) Systematic review of prostate cancer risk and association with consumption of fish and fish-oils: Analysis of 495,321 participants. *International Journal of Clinical Practice* 69:87-105. DOI: <http://dx.doi.org/10.1111/ijcp.12514>.
- Maki K.C., Palacios O.M., Bell M., Toth P.P. (2017) Use of supplemental long-chain omega-3 fatty acids and risk for cardiac death: An updated meta-analysis and review of research gaps. *Journal of Clinical Lipidology* 11:1152-1160.e2. DOI: <https://dx.doi.org/10.1016/j.jacl.2017.07.010>.
- Manos B.E., Bravender T.D., Harrison T.M., Lange H.L.H., Cottrill C.B., Abdel-Rasoul M., Bonny A.E. (2018) A pilot randomized controlled trial of omega-3 fatty acid supplementation for the treatment of anxiety in adolescents with anorexia nervosa. *International Journal of Eating Disorders* 51:1367-1372. DOI: <https://dx.doi.org/10.1002/eat.22964>.
- Marc I., Piedboeuf B., Lacaze-Masmonteil T., Fraser W., Masse B., Mohamed I., Qureshi M., Afifi J., Lemyre B., Caouette G., Bartholomew J., Nuyt A.M., Julien P., Synnes A., Lucas M., Perreault T., Strueby L., Cieslak Z., Yusuf K., Pelligra G., Masse E., Larsen B., de Cabo C., Ruth C., Khurshid F., Lavoie P.M. (2020) Effect of Maternal Docosahexaenoic Acid Supplementation on Bronchopulmonary Dysplasia-Free Survival in Breastfed Preterm Infants: A Randomized Clinical Trial. *JAMA* 324:157-167. DOI: <https://dx.doi.org/10.1001/jama.2020.8896>.
- Marik P.E., Varon J. (2009) Omega-3 dietary supplements and the risk of cardiovascular events: A systematic review. *Clinical Cardiology* 32:365-372. DOI: <http://dx.doi.org/10.1002/clc.20604>.

- Marti Del Moral A., Fortique F. (2019) Omega-3 fatty acids and cognitive decline: a systematic review. *Nutricion Hospitalaria* 36:939-949. DOI: <https://dx.doi.org/10.20960/nh.02496>.
- Mazahery H., Conlon C., Beck K.L., Kruger M.C., Stonehouse W., Camargo C.A., Meyer B.J., Tsang B., Mugridge O., von Hurst P.R. (2016) Vitamin D and omega-3 fatty acid supplements in children with autism spectrum disorder: A study protocol for a factorial randomised, double-blind, placebo-controlled trial. *Trials* 17. DOI: <http://dx.doi.org/10.1186/s13063-016-1428-8>.
- Mazahery H., Conlon C.A., Beck K.L., Mugridge O., Kruger M.C., Stonehouse W., Camargo C.A., Jr., Meyer B.J., Tsang B., Jones B., von Hurst P.R. (2019) A Randomised-Controlled Trial of Vitamin D and Omega-3 Long Chain Polyunsaturated Fatty Acids in the Treatment of Core Symptoms of Autism Spectrum Disorder in Children. *Journal of Autism & Developmental Disorders* 49:1778-1794. DOI: <https://dx.doi.org/10.1007/s10803-018-3860-y>.
- Mazahery H., Conlon C.A., Beck K.L., Mugridge O., Kruger M.C., Stonehouse W., Camargo C.A., Jr., Meyer B.J., Tsang B., von Hurst P.R. (2020) Inflammation (IL-1beta) Modifies the Effect of Vitamin D and Omega-3 Long Chain Polyunsaturated Fatty Acids on Core Symptoms of Autism Spectrum Disorder-An Exploratory Pilot Study++. *Nutrients* 12:28. DOI: <https://dx.doi.org/10.3390/nu12030661>.
- McNamara R., Asch R., Schurdak J., Weber W., Tallman M., Blom T., Moore L., Patino L.R., Epstein J. (2017) Docosahexaenoic acid supplementation increases cortical white matter microstructural integrity in medication-free youth with ADHD: A placebo-controlled diffusion tensor imaging study. *Biological Psychiatry Conference:72nd Annual Scientific Convention and Meeting of the Society of Biological Psychiatry, SOBP 2017*. San Diego, CA United States. 81 (10 Supplement 1) (pp S349).
- McNamara R., Li W., Lei D., Tallman M., Patino L.R., DelBello M. (2019) Fish oil supplementation alters emotion-generated functional connectivity within corticolimbic networks of depressed bipolar offspring: A 12-week placebo-controlled fMRI trial. *Neuropsychopharmacology Conference:58th Annual Meeting of the American College of Neuropsychopharmacology, ACNP 2019*. Orlando, FL United States. 44 (Supplement 1) (pp 312). DOI: <http://dx.doi.org/10.1038/s41386-019-0546-x>.
- McNamara R., Strawn J., Stahl L., Weber W., Welge J., Patino R., Strakowski S., DelBello M. (2014a) Effects of fish oil monotherapy on emotion-generated cortical activity in depressed bipolar offspring: A double-blind placebo-controlled fMRI study. *Neuropsychopharmacology Conference:53rd Annual Meeting of the American College of Neuropsychopharmacology, ACNP 2014*. Phoenix, AZ United States. Conference Publication: (var.pagings). 39 (SUPPL. 1) (pp S228). DOI: <http://dx.doi.org/10.1038/npp.2014.280>.
- McNamara R.K., Li W., Lei D., Tallman M.J., Welge J.A., Strawn J.R., Patino L.R., DelBello M.P. (2021) Fish oil supplementation alters emotion-generated corticolimbic functional connectivity in depressed adolescents at high-risk for bipolar I disorder: A 12-week placebo-controlled fMRI trial. *Bipolar Disorders*. DOI: <http://dx.doi.org/10.1111/bdi.13110>.

- McNamara R.K., Strawn S.R., Stahl L., Weber W., Welge J., Patino R., Strakowski S.M., DelBello M.P. (2014b) Effects of long-chain omega-3 fatty acid monotherapy on cortical biochemistry in depressed bipolar offspring: A double-blind placebo-controlled 1H MRS study. *Biological Psychiatry Conference:69th Annual Scientific Convention and Meeting of the Society of Biological Psychiatry, SOBP 2014*. New York, NY United States. Conference Publication: (var.pagings). 75 (9 SUPPL. 1) (pp 121S). DOI: <http://dx.doi.org/10.1016/j.biopsych.2014.03.014>.
- McNamara R.K., Strimpfel J., Jandacek R., Rider T., Tso P., Welge J.A., Strawn J.R., DelBello M.P. (2014c) Detection and treatment of long-chain omega-3 fatty acid deficiency in adolescents with SSRI-resistant major depressive disorder. *PharmaNutrition* 2:38-46. DOI: <http://dx.doi.org/10.1016/j.phanu.2014.02.002>.
- Meguid N., Effat S., Hussien H., Azzam H., Gouda A.S., Anwar M., Hashem H.S., Hassan H. (2016) Role of plasma fatty acids in Egyptian children with attention deficit hyperactivity disorder. *International Journal of Pharmaceutical and Clinical Research* 8:671-675.
- Meldrum S., D'Vaz N., Heaton A., Reischl E., Demmelmair H., Koletzko B., Prescott S., Simmer K. (2015) Polymorphisms in the fatty acid desaturase (FADS) gene cluster alter the effects of fish oil supplementation on erythrocyte DHA levels. *Journal of Paediatrics and Child Health Conference:19th Annual Meeting of the Perinatal Society of Australia and New Zealand, PSANZ 2015*. Melbourne, VIC Australia. Conference Publication: (var.pagings). 51 (SUPPL. 1) (pp 58). DOI: <http://dx.doi.org/10.1111/jpc.12884-4>.
- Meldrum S.J., Li Y., Zhang G., Heaton A.E.M., D'Vaz N., Manz J., Reischl E., Koletzko B.V., Prescott S.L., Simmer K. (2018) Can polymorphisms in the fatty acid desaturase (FADS) gene cluster alter the effects of fish oil supplementation on plasma and erythrocyte fatty acid profiles? An exploratory study. *European Journal of Nutrition* 57:2583-2594. DOI: <https://dx.doi.org/10.1007/s00394-017-1529-5>.
- Miles E.A., Calder P.C. (2012) Influence of marine n-3 polyunsaturated fatty acids on immune function and a systematic review of their effects on clinical outcomes in rheumatoid arthritis. *British Journal of Nutrition* 107:S171-S184. DOI: <http://dx.doi.org/10.1017/S0007114512001560>.
- Miller P.E., Van Elswyk M., Alexander D.D. (2014) Long-chain Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: A meta-analysis of randomized controlled trials. *American Journal of Hypertension* 27:885-896. DOI: <http://dx.doi.org/10.1093/ajh/hpu024>.
- Milte C.M., Parletta N., Buckley J.D., Coates A.M., Young R.M., Howe P.R. (2015) Increased Erythrocyte Eicosapentaenoic Acid and Docosahexaenoic Acid Are Associated With Improved Attention and Behavior in Children With ADHD in a Randomized Controlled Three-Way Crossover Trial. *Journal of Attention Disorders* 19:954-64. DOI: <https://dx.doi.org/10.1177/1087054713510562>.
- Mischoulon D. (2007) Update and Critique of Natural Remedies as Antidepressant Treatments. *Psychiatric Clinics of North America* 30:51-68. DOI: <http://dx.doi.org/10.1016/j.psc.2006.12.003>.

- Mischoulon D. (2009) Update and critique of natural remedies as antidepressant treatments. *Obstetrics & Gynecology Clinics of North America* 36:789-807, x. DOI: <https://dx.doi.org/10.1016/j.ogc.2009.10.005>.
- Mocellin M.C., Camargo C.Q., Nunes E.A., Fiates G.M.R., Trindade E.B.S.M. (2016) A systematic review and meta-analysis of the n-3 polyunsaturated fatty acids effects on inflammatory markers in colorectal cancer. *Clinical Nutrition* 35:359-369. DOI: <http://dx.doi.org/10.1016/j.clnu.2015.04.013>.
- Moher D., Liberati A., Tetzlaff J., Altman D.G., The PRISMA Group. (2009) Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Plos Medicine* 6. DOI: ARTN e1000097 10.1371/journal.pmed.1000097.
- Montgomery P., Burton J.R., Sewell R.P., Spreckelsen T.F., Richardson A.J. (2014) Fatty acids and sleep in UK children: Subjective and pilot objective sleep results from the DOLAB study - a randomized controlled trial. *Journal of Sleep Research* 23:364-388. DOI: <http://dx.doi.org/10.1111/jsr.12135>.
- Montgomery P., Spreckelsen T.F., Burton A., Burton J.R., Richardson A.J. (2018) Docosahexaenoic acid for reading, working memory and behavior in UK children aged 7-9: A randomized controlled trial for replication (the DOLAB II study). *PLoS ONE* 13. DOI: <http://dx.doi.org/10.1371/journal.pone.0192909>.
- Moreno C., Calvo-Escalona R., Gutierrez S., Graell M., Romo J., Dorado M.L., Giraldez M.L., Llorente C., Arango C., Parellada M. (2014) Effect of omega-3 polyunsaturated fatty acids on oxidative stress in children and adolescents with autism spectrum disorders. *European Neuropsychopharmacology Conference: 27th European College of Neuropsychopharmacology, ECNP Congress. Berlin Germany. Conference Publication: (var.pagings). 24 (SUPPL. 2) (pp S725)*.
- Morsy S., Khalil S.M., Doheim M.F., Kamel M.G., El-Basiony D.S.M., Ahmed Hassan H.I., Eisa A.A., Anh Ngoc C.T., Dang N.P., Hirayama K., Huy N.T. (2019) Efficacy of ethyl-EPA as a treatment for Huntington disease: a systematic review and meta-analysis. *Acta Neuropsychiatrica* 31:175-185. DOI: <https://dx.doi.org/10.1017/neu.2019.11>.
- Mossaheb N., Schafer M.R., Schlogelhofer M., Klier C.M., Smesny S., McGorry P.D., Berger M., Amminger G.P. (2018) Predictors of longer-term outcome in the Vienna omega-3 high-risk study. *Schizophrenia Research* 193. DOI: <http://dx.doi.org/10.1016/j.schres.2017.08.010>.
- Myhrstad M.C., Ulven S.M., Gunther C.C., Ottestad I., Holden M., Ryeng E., Borge G.I., Kohler A., Bronner K.W., Thoresen M., Holven K.B. (2014) Fish oil supplementation induces expression of genes related to cell cycle, endoplasmic reticulum stress and apoptosis in peripheral blood mononuclear cells: a transcriptomic approach. *Journal of Internal Medicine* 276:498-511. DOI: <https://dx.doi.org/10.1111/joim.12217>.
- Newberry S.J., Chung M., Booth M., Maglione M.A., Tang A.M., O'Hanlon C.E., Wang D.D., Okunogbe A., Huang C., Motala A., Trimmer M., Dudley W., Shanman R., Coker T.R., Shekelle P.G. (2016) Omega-3 Fatty Acids and Maternal and Child Health: An Updated Systematic Review. *Evidence Report/Technology Assessment: 1-826*. DOI: <https://dx.doi.org/10.23970/AHRQEPERTA224>.

- O'Connor M.G., Thomsen K., Brown R.F., Laposata M., Seegmiller A. (2016) Elevated prostaglandin E metabolites and abnormal plasma fatty acids at baseline in pediatric cystic fibrosis patients: a pilot study 113:46-49.
- OHAT. (2019) Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration, Office of Health Assessment and Translation (OHAT) Division of the National Toxicology Program National Institute of Environmental Health Sciences, https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookmarch2019_508.pdf.
- Ostadrahimi A., Mohammad-Alizadeh S., Mirgafourvand M., Yaghoubi S., Shahrissa E., Farshbaf-Khalili A. (2016) Effects of fish oil supplementation on gestational diabetes mellitus (GDM): A systematic review. Iranian Red Crescent Medical Journal 18. DOI: <http://dx.doi.org/10.5812/ircmj.24690>.
- Pacifico L., Bonci E., Di Martino M., Versacci P., Andreoli G., Silvestri L.M., Chiesa C. (2015) A double-blind, placebo-controlled randomized trial to evaluate the efficacy of docosahexaenoic acid supplementation on hepatic fat and associated cardiovascular risk factors in overweight children with nonalcoholic fatty liver disease 25:734-41.
- Panahi Y., Dashti-Khavidaki S., Farnood F., Noshad H., Lotfi M., Gharekhani A. (2016) Therapeutic effects of omega-3 fatty acids on chronic kidney disease-associated pruritus: A literature review. Advanced Pharmaceutical Bulletin 6:509-514. DOI: <http://dx.doi.org/10.15171/apb.2016.064>.
- Parellada M., Llorente C., Calvo R., Gutierrez S., Lazaro L., Graell M., Alvarez M., Guisasola M., Dulin E., Dorado M.L., Romo J., Arango C., Moreno C. (2015) Double-blind crossed-over randomized controlled-trial with omega-3 fatty acids for autism spectrum disorders: 28th European College of Neuropsychopharmacology, ECNP Congress. Amsterdam Netherlands. Conference Publication: (var.pagings). 25 (SUPPL. 2) (pp S138).
- Patel A.A., Budoff M.J. (2016) Effects of eicosapentaenoic acid and docosahexaenoic acid on lipoproteins in hypertriglyceridemia. Current Opinion in Endocrinology, Diabetes and Obesity 23:145-149. DOI: <http://dx.doi.org/10.1097/MED.000000000000233>.
- Pawelczyk T., Grancow-Grabka M., Kotlicka-Antczak M., Trafalska E., Pawelczyk A. (2016) A randomized controlled study of the efficacy of six-month supplementation with concentrated fish oil rich in omega-3 polyunsaturated fatty acids in first episode schizophrenia 73:34-44.
- Pawelczyk T., Grancow-Grabka M., Trafalska E., Szemraj J., Zurner N., Pawelczyk A. (2019) An increase in plasma brain derived neurotrophic factor levels is related to n-3 polyunsaturated fatty acid efficacy in first episode schizophrenia: secondary outcome analysis of the OFFER randomized clinical trial 236:2811-2822.
- Pourmasoumi M., Vosoughi N., Derakhshandeh-Rishehri S.M., Assarroudi M., Heidari-Beni M. (2018) Association of omega-3 fatty acid and epileptic seizure in epileptic patients: A systematic review. International Journal of Preventive Medicine 9. DOI: http://dx.doi.org/10.4103/ijpvm.IJPVM_281_16.

- PubChem. (2021a) Docosahexaenoic acid, NIH; National Library of Medicine, National Center for Biotechnology Information, <https://pubchem.ncbi.nlm.nih.gov/compound/445580>.
- PubChem. (2021b) Eicosapentaenoic acid, NIH; National Library of Medicine, National Center for Biotechnology Information, <https://pubchem.ncbi.nlm.nih.gov/compound/Eicosapentaenoic-acid>.
- Quin C., Erland B.M., Loeppky J.L., Gibson D.L. (2016) Omega-3 polyunsaturated fatty acid supplementation during the pre and post-natal period: A meta-analysis and systematic review of randomized and semi-randomized controlled trials. *Journal of Nutrition and Intermediary Metabolism* 5. DOI: <http://dx.doi.org/10.1016/j.jnim.2016.04.005>.
- Regulation (EC) No 1925/2006 of the European Parliament and of the Council. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:404:0026:0038:en:PDF>.
- Regulation (EC) No 1925/2006 of the European Parliament and of the Council. of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods.
- Ren X., Vilhjalmsdottir B.L., Rohde J.F., Walker K.C., Runstedt S.E., Lauritzen L., Heitmann B.L., Specht I.O. (2021) Systematic Literature Review and Meta-Analysis of the Relationship Between Polyunsaturated and Trans Fatty Acids During Pregnancy and Offspring Weight Development. *Frontiers in Nutrition* 8:625596. DOI: <https://dx.doi.org/10.3389/fnut.2021.625596>.
- Ries A., Trottenberg P., Elsner F., Stiel S., Haugen D., Kaasa S., Radbruch L. (2012) A systematic review on the role of fish oil for the treatment of cachexia in advanced cancer: An EPCRC cachexia guidelines project. *Palliative Medicine* 26:294-304. DOI: <http://dx.doi.org/10.1177/0269216311418709>.
- Rizos E.C., Markozannes G., Tsapas A., Mantzoros C.S., Ntzani E.E. (2021) Omega-3 supplementation and cardiovascular disease: Formulation-based systematic review and meta-analysis with trial sequential analysis. *Heart* 107:150-158. DOI: <http://dx.doi.org/10.1136/heartjnl-2020-316780>.
- Rodriguez-Cruz M., Atilano-Miguel S., Barbosa-Cortes L., Bernabe-Garcia M., Almeida-Becerril T., Cardenas-Conejo A., Del Rocio Cruz-Guzman O., Maldonado-Hernandez J. (2019) Evidence of muscle loss delay and improvement of hyperinsulinemia and insulin resistance in Duchenne muscular dystrophy supplemented with omega-3 fatty acids: A randomized study 38:2087-2097.
- Rodriguez-Cruz M., Cruz-Guzman O.D.R., Almeida-Becerril T., Solis-Serna A.D., Atilano-Miguel S., Sanchez-Gonzalez J.R., Barbosa-Cortes L., Ruiz-Cruz E.D., Huicochea J.C., Cardenas-Conejo A., Escobar-Cedillo R.E., Yam-Ontiveros C.A., Ricardez-Marcial E.F. (2018) Potential therapeutic impact of omega-3 long chain-polyunsaturated fatty acids on inflammation markers in Duchenne muscular dystrophy: A double-blind, controlled randomized trial 37:1840-1851.
- Rodriguez C., Garcia T., Areces D., Fernandez E., Garcia-Noriega M., Domingo J.C. (2019) Supplementation with high-content docosahexaenoic acid triglyceride in

attention deficit hyperactivity disorder: A randomized double-blind placebo-controlled trial 15.

- Rogers P.J., Appleton K.M., Kessler D., Peters T.J., Gunnell D., Hayward R.C., Heatherley S.V., Christian L.M., McNaughton S.A., Ness A.R. (2008) No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. *British Journal of Nutrition* 99:421-431. DOI: <http://dx.doi.org/10.1017/S0007114507801097>.
- Romieu I., Lee H., Barraza A., Biessy C., Duarte-Salles T., Sly P., Ramakrishnan U., Rivera Dommarco J., Herceg Z. (2014) Dietary supplementation with polyunsaturated fatty acid during pregnancy modulates DNA methylation at IGF2/H19 imprinted genes and growth of infants: Experimental Biology 2014, EB. San Diego, CA United States. Conference Publication: (var.pagings). 28 (1 SUPPL. 1) (no pagination).
- Rondanelli M., Perna S., Riva A., Petrangolini G., Di Paolo E., Gasparri C. (2021) Effects of n-3 EPA and DHA supplementation on fat free mass and physical performance in elderly. A systematic review and meta-analysis of randomized clinical trial. *Mechanisms of Ageing and Development* 196. DOI: <http://dx.doi.org/10.1016/j.mad.2021.111476>.
- Ross B.M., Seguin J., Sieswerda L.E. (2007) Omega-3 fatty acids as treatments for mental illness: Which disorder and which fatty acid? *Lipids in Health and Disease* 6. DOI: <http://dx.doi.org/10.1186/1476-511X-6-21>.
- Sabour H., Norouzi Javidan A., Latifi S., Shidfar F., Heshmat R., Emami Razavi S.H., Vafa M.R., Larijani B. (2015) Omega-3 fatty acids' effect on leptin and adiponectin concentrations in patients with spinal cord injury: A double-blinded randomized clinical trial 38:599-606.
- Sacchetti M., Mantelli F., Merlo D., Lambiase A. (2015) Systematic Review of Randomized Clinical Trials on Safety and Efficacy of Pharmacological and Nonpharmacological Treatments for Retinitis Pigmentosa. *Journal of ophthalmology* 2015:737053.
- Sadeghi A., Djafarian K., Mohammadi H., Shab-Bidar S. (2017) Effect of omega-3 fatty acids supplementation on insulin resistance in women with polycystic ovary syndrome: Meta-analysis of randomized controlled trials. *Diabetes & Metabolic Syndrome* 11:157-162. DOI: <https://dx.doi.org/10.1016/j.dsx.2016.06.025>.
- Sahebkar A., Simental-Mendia L.E., Mikhailidis D.P., Pirro M., Banach M., Sirtori C.R., Reiner Z. (2018) Effect of omega-3 supplements on plasma apolipoprotein C-III concentrations: a systematic review and meta-analysis of randomized controlled trials. *Annals of Medicine* 50:565-575. DOI: <http://dx.doi.org/10.1080/07853890.2018.1511919>.
- Saller R., Romer-Luthi C., Muller M., Brignoli R., Noll G., Meier R. (2006) Docosahexaenoic acid (DHA) and long chain omega-3 fatty acids: Clinical relevance for the cardiovascular system. *Schweizerische Zeitschrift fur GanzheitsMedizin* 18:272-280. DOI: <http://dx.doi.org/10.1159/000282057>.

- SanGiovanni J.P., Berkey C.S., Dwyer J.T., Colditz G.A. (2000a) Dietary essential fatty acids, long-chain polyunsaturated fatty acids, and visual resolution acuity in healthy fullterm infants: A systematic review. *Early Human Development* 57:165-188. DOI: <http://dx.doi.org/10.1016/S0378-3782%2800%2900050-5>.
- SanGiovanni J.P., Parra-Cabrera S., Colditz G.A., Berkey C.S., Dwyer J.T. (2000b) Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. *Pediatrics* 105:1292-8.
- Saravanan P., Davidson N.C., Schmidt E.B., Calder P.C. (2010) Cardiovascular effects of marine omega-3 fatty acids. *Lancet* 376:540-50. DOI: [https://dx.doi.org/10.1016/S0140-6736\(10\)60445-X](https://dx.doi.org/10.1016/S0140-6736(10)60445-X).
- Sarmiento Vasconcelos V., Macedo C.R., de Souza Pedrosa A., Pereira Gomes Morais E., Porfirio G.J., Torloni M.R. (2016) Polyunsaturated fatty acid supplementation for drug-resistant epilepsy. *Cochrane Database of Systematic Reviews*:CD011014. DOI: <https://dx.doi.org/10.1002/14651858.CD011014.pub2>.
- Sarris J. (2017) Clinical use of nutraceuticals in the adjunctive treatment of depression in mood disorders. *Australasian Psychiatry* 25:369-372. DOI: <http://dx.doi.org/10.1177/1039856216689533>.
- Sarris J., Murphy J., Mischoulon D., Papakostas G.I., Fava M., Berk M., Ng C.H. (2016) Adjunctive nutraceuticals for depression: A systematic review and meta-analyses. *American Journal of Psychiatry* 173:575-587. DOI: <http://dx.doi.org/10.1176/appi.ajp.2016.15091228>.
- Sattar Y., Arshad J., Ahmad B., Suleiman A.R., Ullah W., Alhatemi G., Alhajri N., Alraies M.C. (2021) Meta Analysis of Cardiovascular Outcomes of Ethyl Eicosapentaenoic Acid in Diabetes Mellitus and Coronary Artery Disease. *Journal of the American College of Cardiology Conference:ACC.21*. Virtual, Online. 77 (18 Supplement 1) (pp 1188). DOI: <http://dx.doi.org/10.1016/S0735-1097%2821%2902547-X>.
- Saunders E.F.H., Ramsden C.E., Sherazy M.S., Gelenberg A.J., Davis J.M., Rapoport S.I. (2016) Omega-3 and omega-6 polyunsaturated fatty acids in bipolar disorder: A review of biomarker and treatment studies. *Journal of Clinical Psychiatry* 77:e1301-e1308. DOI: <http://dx.doi.org/10.4088/JCP.15r09925>.
- Scaioli E., Sartini A., Bellanova M., Campieri M., Festi D., Bazzoli F., Belluzzi A. (2018) Eicosapentaenoic Acid Reduces Fecal Levels of Calprotectin and Prevents Relapse in Patients With Ulcerative Colitis 16:1268-1275.e2.
- See V.H.L., Mas E., Prescott S.L., Beilin L.J., Burrows S., Barden A.E., Huang R.C., Mori T.A. (2017) Effects of postnatal omega-3 fatty acid supplementation on offspring pro-resolving mediators of inflammation at 6 months and 5 years of age: A double blind, randomized controlled clinical trial 126:126-132.
- Sekikawa A., Cui C., Sugiyama D., Fabio A., Harris W.S., Zhang X. (2019) Effect of high-dose marine omega-3 fatty acids on atherosclerosis: A systematic review and meta-

analysis of randomized clinical trials. *Nutrients* 11. DOI: <http://dx.doi.org/10.3390/nu11112599>.

- Shulkin M., Pimpin L., Bellinger D., Kranz S., Fawzi W., Duggan C., Mozaffarian D. (2018) N-3 fatty acid supplementation in mothers, preterm infants, and term infants and childhood psychomotor and visual development: A systematic review and meta-analysis. *Journal of Nutrition* 148:409-418. DOI: <http://dx.doi.org/10.1093/jn/nxx031>.
- Signorini C., De Felice C., Leoncini S., Durand T., Galano J.M., Cortelazzo A., Zollo G., Guerranti R., Gonnelli S., Caffarelli C., Rossi M., Pecorelli A., Valacchi G., Ciccoli L., Hayek J. (2014) Altered erythrocyte membrane fatty acid profile in typical Rett syndrome: Effects of omega-3 polyunsaturated fatty acid supplementation 91:183-193.
- Siscovick D.S., Barringer T.A., Fretts A.M., Wu J.H.Y., Lichtenstein A.H., Costello R.B., Kris-Etherton P.M., Jacobson T.A., Engler M.B., Alger H.M., Appel L.J., Mozaffarian D. (2017) Omega-3 Polyunsaturated Fatty Acid (Fish Oil) Supplementation and the Prevention of Clinical Cardiovascular Disease: A Science Advisory from the American Heart Association. *Circulation* 135:e867-e884. DOI: <http://dx.doi.org/10.1161/CIR.0000000000000482>.
- Skae M., Avatapalle H.B., Banerjee I., Rigby L., Vail A., Foster P., Charalambous C., Bowden L., Padidela R., Patel L., Ehtisham S., Cosgrove K.E., Dunne M.J., Clayton P.E. (2014) Reduced glycemic variability in diazoxide-responsive children with congenital hyperinsulinism using supplemental omega-3-polyunsaturated fatty acids; A pilot trial with MaxEPAR 5.
- Skouroliaou M., Konstantinou D., Agakidis C., Kaliora A., Kalogeropoulos N., Massara P., Antoniadou M., Panagiotakos D., Karagiozoglou-Lampoudi T. (2016) Parenteral MCT/omega-3 Polyunsaturated Fatty Acid-Enriched Intravenous Fat Emulsion Is Associated With Cytokine and Fatty Acid Profiles Consistent With Attenuated Inflammatory Response in Preterm Neonates: A Randomized, Double-Blind Clinical Trial 31:235-44.
- Smuts C.M., Greeff J., Kvalsvig J., Zimmermann M.B., Baumgartner J. (2015) Long-chain n-3 PUFA supplementation decreases physical activity during class time in iron-deficient South African school children 113:212-24.
- Spahis S., Alvarez F., Delvin E., Dubois J., Peretti N., Ahmad N., Moreau A., Garofalo C., Tang A., Seidman E.G., Levy E. (2016) N-3 fatty acid supplementation improves hepatic-and cardiometabolic-related biomarkers in pediatric patients with non-alcoholic fatty liver disease: A randomized controlled intervention: *Digestive Disease Week 2016, DDW 2016*. San Diego, CA United States. Conference Publication: (var.pagings). 150 (4 SUPPL. 1) (pp S602).
- Su M.I., Cheng Y.C., Huang Y.C., Liu C.W. (2021) Omega-3 Polyunsaturated Fatty Acid Supplementation in Patients with Lower Extremity Arterial Disease. *Journal of the American College of Nutrition*:1-9. DOI: <https://dx.doi.org/10.1080/07315724.2021.1891155>.

- Sublette M.E., Ellis S.P., Geant A.L., Mann J.J. (2011) Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. *Journal of Clinical Psychiatry* 72:1577-84. DOI: <https://dx.doi.org/10.4088/JCP.10m06634>.
- Sun H., Como P.G., Downey L.C., Murphy D., Ariagno R.L., Rodriguez W. (2015) Infant formula and neurocognitive outcomes: Impact of study end-point selection. *Journal of Perinatology* 35:867-874. DOI: <http://dx.doi.org/10.1038/jp.2015.87>.
- Suradom C., Suttajit S., Oon-Arom A., Maneeton B., Srisurapanont M. (2021) Omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation for prevention and treatment of perinatal depression: a systematic review and meta-analysis of randomized-controlled trials. *Nordic Journal of Psychiatry* 75:239-246. DOI: <https://dx.doi.org/10.1080/08039488.2020.1843710>.
- Susai S.R., Sabherwal S., Mongan D., Focking M., Cotter D.R. (2021) Omega-3 fatty acid in ultra-high-risk psychosis: A systematic review based on functional outcome. *Early intervention in psychiatry* 02:02. DOI: <https://dx.doi.org/10.1111/eip.13133>.
- Szajewska H., Makrides M. (2011) Is early nutrition related to short-term health and long-term outcome? *Annals of Nutrition and Metabolism* 58:38-48. DOI: <http://dx.doi.org/10.1159/000323465>.
- Tanaka K., Tanaka S., Shah N., Ota E., Namba F. (2020) Docosahexaenoic acid and bronchopulmonary dysplasia in preterm infants: a systematic review and meta-analysis. *Journal of Maternal Fetal and Neonatal Medicine*. DOI: <http://dx.doi.org/10.1080/14767058.2020.1769590>.
- Todorovic M., Hodson L. (2016) The effect of marine derived n-3 fatty acids on adipose tissue metabolism and function. *Journal of Clinical Medicine* 5. DOI: <http://dx.doi.org/10.3390/jcm5010003>.
- Totland T.H., Melnæs B.K., Lundberg-Hallén N., Helland-Kigen K.M., Lund-Blix N.A., Myhre J.B., Johansen A.M.W., Løken E.B., Andersen L.F. (2012) Norkost 3 En landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18-70 år, 2010-11, https://www.helsedirektoratet.no/rapporter/norkost-3-en-landsomfattende-kostholdsundersokelse-blant-menn-og-kvinner-i-norge-i-alderen-18-70-ar-2010-11/Norkost%203%20en%20landsomfattende%20kostholdsundersokelse%20blant%20menn%20og%20kvinner%20i%20Norge%20i%20alderen-18-70%20%C3%A5r%202010-11.pdf/_attachment/inline/b7bafaab-6059-4450-8d76-c3ed9f3eaf3f:be251cd1153cf1ae8e4c46eedddc13b36da3d11d/Norkost%203%20en%20landsomfattende%20kostholdsundersokelse%20blant%20menn%20og%20kvinner%20i%20Norge%20i%20alderen-18-70%20%C3%A5r%202010-11.pdf.
- Tsiami A., Sookhoo D., Tingle A., Loveday H., Golsorkhi M. (2012) A systematic review of the effectiveness of polyunsaturated fatty acids in reducing the clinical symptoms of paediatric bipolar disorder in children and adolescents. *JBIC Library of Systematic Reviews* 10.
- Tu C.H., Chen C.M., Yang C.C., Galecki P., Su K.P. (2020) Brain responses to emotional stimuli after eicosapentaenoic acid and docosahexaenoic acid treatments in major

- depressive disorder: Toward personalized medicine with anti-inflammatory nutraceuticals. *Journal of Personalized Medicine* 10:1-14. DOI: <http://dx.doi.org/10.3390/jpm10040283>.
- Turnbull T., Cullen-Drill M., Smaldone A. (2008) Efficacy of omega-3 fatty acid supplementation on improvement of bipolar symptoms: a systematic review. *Archives of Psychiatric Nursing* 22:305-11. DOI: <https://dx.doi.org/10.1016/j.apnu.2008.02.011>.
- Uauy R., Hoffman D.R., Mena P., Llanos A., Birch E.E. (2003) Term infant studies of DHA and ARA supplementation on neurodevelopment: Results of randomized controlled trials. *Journal of Pediatrics* 143:17-25.
- Ueno Y., Miyamoto N., Yamashiro K., Tanaka R., Hattori N. (2019) Omega-3 polyunsaturated fatty acids and stroke burden. *International Journal of Molecular Sciences* 20. DOI: <http://dx.doi.org/10.3390/ijms20225549>.
- Vahdaninia M., Mackenzie H., Dean T., Helps S. (2019) The effectiveness of omega-3 polyunsaturated fatty acid interventions during pregnancy on obesity measures in the offspring: an up-to-date systematic review and meta-analysis. *European Journal of Nutrition* 58:2597-2613. DOI: <http://dx.doi.org/10.1007/s00394-018-1824-9>.
- Van Dael P. (2021) Role of n-3 long-chain polyunsaturated fatty acids in human nutrition and health: review of recent studies and recommendations. *Nutrition Research & Practice* 15:137-159. DOI: <https://dx.doi.org/10.4162/nrp.2021.15.2.137>.
- van der Burg K.P., Cribb L., Firth J., Karmacoska D., Sarris J. (2021) Nutrient and genetic biomarkers of nutraceutical treatment response in mood and psychotic disorders: a systematic review. *Nutritional Neuroscience* 24:279-295. DOI: <http://dx.doi.org/10.1080/1028415X.2019.1625222>.
- van der Wurff I.S.M., Meyer B.J., de Groot R.H.M. (2020) Effect of omega-3 long chain polyunsaturated fatty acids (N-3 LCPUFA) supplementation on cognition in children and adolescents: A systematic literature review with a focus on n-3 LCPUFA blood values and dose of DHA and EPA. *Nutrients* 12:1-28. DOI: <http://dx.doi.org/10.3390/nu12103115>.
- van der Wurff I.S.M., von Schacky C., Bergeland T., Leontjevas R., Zeegers M.P., Jolles J., Kirschner P.A., de Groot R.H.M. (2019) Effect of 1 Year Krill Oil Supplementation on Cognitive Achievement of Dutch Adolescents: A Double-Blind Randomized Controlled Trial 11:30.
- Van Der Wurff I.S.M., Von Schacky C., Bergeland T., Leontjevas R., Zeegers M.P., Kirschner P.A., De Groot R.H.M. (2018) Randomized controlled trial investigating the effect of krill oil supplementation on mental well-being in adolescents of a lower educational level with a low Omega-3 Index: *International Society for Nutritional Psychiatry Research, ISNPR 2017*. Bethesda, MD United States. 21 (Supplement 1) (pp S26).
- Verduci E., Agostoni C., Radaelli G., Banderali G., Riva E., Giovannini M. (2014) Blood lipids profile in hyperlipidemic children undergoing different dietary long chain polyunsaturated supplementations: a preliminary clinical trial 65:375-9.

- Vericel E., Mazur S., Colas R., Delaup V., Calzada C., Reix P., Durieu I., Lagarde M., Bellon G. (2016) Moderate intake of docosahexaenoic acid raises plasma and platelet vitamin E levels in cystic fibrosis patients 115:41-47.
- Vieira A.D.S., Silveira G. (2017) Effectiveness of n-3 fatty acids in the treatment of hypertriglyceridemia in HIV/AIDS patients: a meta-analysis. *Ciencia & Saude Coletiva* 22:2659-2669. DOI: <https://dx.doi.org/10.1590/1413-81232017228.21752015>.
- Villani A.M., Crotty M., Cleland L.G., James M.J., Fraser R.J., Cobiac L., Miller M.D. (2013) Fish oil administration in older adults: is there potential for adverse events? A systematic review of the literature. *BMC Geriatrics* 13:41. DOI: <https://dx.doi.org/10.1186/1471-2318-13-41>.
- VKM. (2011) Evaluation of negative and positive health effects of n-3 fatty acids as constituents of food supplements and fortified foods. Opinion of the Steering Committee of the Norwegian Scientific Committee for Food Safety, Norwegian Scientific Committee for Food Safety, Oslo, Norway, Norwegian Scientific Committee for Food Safety, <https://vkm.no/download/18.a665c1015c865cc85bab93e/1501509143166/c7a41adb79.pdf>.
- VKM. (2015) Risk assessment of "other substances" –eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid. Opinion of the Panel on Nutrition, Dietetic Products, Novel Food and Allergy of the Norwegian Scientific Committee for Food Safety, Norwegian Scientific Committee for Food Safety, Oslo, Norway, Norwegian Scientific Committee for Food Safety, <https://vkm.no/download/18.645b840415d03a2fe8f26067/1502708618203/3ef862160c.pdf>.
- Voigt R.G., Mellon M.W., Katusic S.K., Weaver A.L., Matern D., Mellon B., Jensen C.L., Barbaresi W.J. (2014) Dietary docosahexaenoic acid supplementation in children with autism 58:715-722.
- Vors C., Allaire J., Mejia S.B., Khan T.A., Sievenpiper J.L., Lamarche B. (2021) Comparing the Effects of Docosahexaenoic and Eicosapentaenoic Acids on Inflammation Markers Using Pairwise and Network Meta-Analyses of Randomized Controlled Trials. *Advances in Nutrition* 12:128-140. DOI: <https://dx.doi.org/10.1093/advances/nmaa086>.
- Wang C., Harris W.S., Chung M., Lichtenstein A.H., Balk E.M., Kupelnick B., Jordan H.S., Lau J. (2006) n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: A systematic review. *American Journal of Clinical Nutrition* 84:5-17.
- Wang H., Chen J., Zhao L. (2018) N-3 polyunsaturated fatty acids for prevention of postoperative atrial fibrillation: updated meta-analysis and systematic review. *Journal of Interventional Cardiac Electrophysiology* 51:105-115. DOI: <http://dx.doi.org/10.1007/s10840-018-0315-5>.
- Wang Q., Cui Q., Yan C. (2016) The Effect of Supplementation of Long-Chain Polyunsaturated Fatty Acids during Lactation on Neurodevelopmental Outcomes of

- Preterm Infant from Infancy to School Age: A Systematic Review and Meta-analysis. *Pediatric Neurology* 59. DOI: <http://dx.doi.org/10.1016/j.pediatrneurol.2016.02.017>.
- Wang Q., Zhou B., Cui Q., Chen C. (2019) Omega-3 Long-chain Polyunsaturated Fatty Acids for Bronchopulmonary Dysplasia: A Meta-analysis. *Pediatrics* 144:07. DOI: <https://dx.doi.org/10.1542/peds.2019-0181>.
- Watson H., Stackhouse C. (2020) Omega-3 fatty acid supplementation for cystic fibrosis. *Cochrane Database of Systematic Reviews*. DOI: <http://dx.doi.org/10.1002/14651858.CD002201.pub6>.
- Wei-Hong L., Cheng-Gui Z., Peng-Fei G., Heng L., Jian-Fang Y. (2017) Omega-3 fatty acids as monotherapy in treating depression in pregnant women: A Meta-Analysis of randomized controlled trials. *Iranian Journal of Pharmaceutical Research* 16:1593-1599.
- Wei M.Y., Jacobson T.A. (2011) Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: A systematic review and meta-analysis. *Current Atherosclerosis Reports* 13:474-483. DOI: <http://dx.doi.org/10.1007/s11883-011-0210-3>.
- Whiting P., Savovic J., Higgins J.P.T., Caldwell D.M., Reeves B.C., Shea B., Davies P., Kleijnen J., Churchill R., Grp R. (2016) ROBIS: A new tool to assess risk of bias in systematic reviews was developed. *Journal of Clinical Epidemiology* 69:225-234. DOI: 10.1016/j.jclinepi.2015.06.005.
- WHO. (1994) Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits, World Health Organization, <http://www.inchem.org/documents/ehc/ehc/ehc170.htm>.
- Widenhorn-Muller K., Schwanda S., Scholz E., Spitzer M., Bode H. (2014) Effect of supplementation with long-chain omega-3 polyunsaturated fatty acids on behavior and cognition in children with attention deficit/hyperactivity disorder (ADHD): a randomized placebo-controlled intervention trial 91:49-60.
- Wojcicki J.M., Heyman M.B. (2011) Maternal omega-3 fatty acid supplementation and risk for perinatal maternal depression. *Journal of Maternal Fetal and Neonatal Medicine* 24:680-686. DOI: <http://dx.doi.org/10.3109/14767058.2010.521873>.
- Wolters M., von der Haar A., Baalman A.K., Wellbrock M., Heise T.L., Rach S. (2021) Effects of n-3 polyunsaturated fatty acid supplementation in the prevention and treatment of depressive disorders- a systematic review and meta-analysis. *Nutrients* 13. DOI: <http://dx.doi.org/10.3390/nu13041070>.
- Wu H., Xu L., Ballantyne C.M. (2020) Dietary and Pharmacological Fatty Acids and Cardiovascular Health. *Journal of Clinical Endocrinology and Metabolism* 105. DOI: <http://dx.doi.org/10.1210/clinem/dgz174>.
- Wu J.H.Y., Micha R., Imamura F., Pan A., Biggs M.L., Ajaz O., Djousse L., Hu F.B., Mozaffarian D. (2012) Omega-3 fatty acids and incident type 2 diabetes: A systematic review and meta-analysis. *British Journal of Nutrition* 107:S214-S227. DOI: <http://dx.doi.org/10.1017/S0007114512001602>.

- Xin W., Li X. (2012) Short-term effects of fish oil supplementation on heart rate variability in humans: A meta-analysis of randomized controlled trials. *Cardiology (Switzerland) Conference:International Heart Forum 2012. Beijing China. Conference Publication: (var.pagings). 123 (SUPPL. 1) (no pagination). DOI: <http://dx.doi.org/10.1159/000341270>.*
- Xin W., Wei W., Lin Z., Zhang X., Yang H., Zhang T., Li B., Mi S. (2013) Fish Oil and Atrial Fibrillation after Cardiac Surgery: A Meta-Analysis of Randomized Controlled Trials. *PLoS ONE 8. DOI: <http://dx.doi.org/10.1371/journal.pone.0072913>.*
- Xu J., Bartz T.M., Eiriksdottir G., Frazier-Wood A.C., Gudnason V., Lahousse L., Manichaikul A., Rohde R.R., Sun F., Terzikhan N., Zhou X., Barr R.G., Brusselle G.G., Dupuis J., Gharib S.A., London S.J., North K.E., Psaty B.M., Smith A.V., Steffen L.M., Hancock D.B., Cassano P.A. (2017) Meta-analysis of the association of omega-3 fatty acids biomarkers with pulmonary function. *FASEB Journal Conference:Experimental Biology 2017, EB. Chicago, IL United States. 31 (1 Supplement 1) (no pagination).*
- Xu J., Gaddis N.C., Bartz T.M., Hou R., Manichaikul A.W., Pankratz N., Smith A.V., Sun F., Terzikhan N., Markunas C.A., Patchen B.K., Schu M., Beydoun M.A., Brusselle G.G., Eiriksdottir G., Zhou X., Wood A.C., Graff M., Harris T.B., Arfan Ikram M., Jacobs D.R., Launer L.J., Lemaitre R.N., O'Connor G.T., Oelsner E.C., Psaty B.M., Vasani R.S., Rohde R.R., Rich S.S., Rotter J.I., Seshadri S., Smith L.J., Tiemeier H., Tsai M.Y., Uitterlinden A.G., Saroja Voruganti V., Xu H., Zilhao N.R., Fornage M., Carola Zillikens M., London S.J., Graham Barr R., Dupuis J., Gharib S.A., Gudnason V., Lahousse L., North K.E., Steffen L.M., Cassano P.A., Hancock D.B. (2019) Omega-3 fatty acids and genome-wide interaction analyses reveal DPP10-pulmonary function association. *American Journal of Respiratory and Critical Care Medicine 199:631-642. DOI: <http://dx.doi.org/10.1164/rccm.201802-0304OC>.*
- Yang B., Shi M.Q., Li Z.H., Yang J.J., Li D. (2016) Fish, long-chain n-3 PUFA and incidence of elevated blood pressure: A meta-analysis of prospective cohort studies. *Nutrients 8. DOI: <http://dx.doi.org/10.3390/nu8010058>.*
- Yang G.Y., Wu T., Huang S.Y., Huang B.X., Wang H.L., Lan Q.Y., Li C.L., Zhu H.L., Fang A.P. (2021) No effect of 6-month supplementation with 300 mg/d docosahexaenoic acid on executive functions among healthy school-aged children: a randomized, double-blind, placebo-controlled trial *60:1985-1997.*
- Yang J., Fernandez-Galilea M., Martinez-Fernandez L., Gonzalez-Muniesa P., Perez-Chavez A., Martinez J.A., Moreno-Aliaga M.J. (2019) Oxidative stress and non-alcoholic fatty liver disease: Effects of omega-3 fatty acid supplementation. *Nutrients 11. DOI: <http://dx.doi.org/10.3390/nu11040872>.*
- Yang J.R., Han D., Qiao Z.X., Tian X., Qi D., Qiu X.H. (2015) Combined application of eicosapentaenoic acid and docosahexaenoic acid on depression in women: A meta-analysis of double-blind randomized controlled trials. *Neuropsychiatric Disease and Treatment 11. DOI: <http://dx.doi.org/10.2147/NDT.S86581>.*
- Yurko-Mauro K., Alexander D.D., Van E. (2015) Docosahexaenoic acid and adult memory: A systematic review and meta-analysis. *PLoS ONE 10. DOI: <http://dx.doi.org/10.1371/journal.pone.0120391>.*

- Zhang B., Zhen Y., Tao A., Bao Z., Zhang G. (2014) Polyunsaturated fatty acids for the prevention of atrial fibrillation after cardiac surgery: An updated meta-analysis of randomized controlled trials. *Journal of Cardiology* 63:53-59. DOI: <http://dx.doi.org/10.1016/j.ijcc.2013.06.014>.
- Zhang M.M., Zou Y., Li S.M., Wang L., Sun Y.H., Shi L., Lu L., Bao Y.P., Li S.X. (2020) The efficacy and safety of omega-3 fatty acids on depressive symptoms in perinatal women: a meta-analysis of randomized placebo-controlled trials. *Translational Psychiatry* 10. DOI: <http://dx.doi.org/10.1038/s41398-020-00886-3>.
- Zhang X.W., Hou W.S., Li M., Tang Z.Y. (2016a) Omega-3 fatty acids and risk of cognitive decline in the elderly: a meta-analysis of randomized controlled trials. *Aging Clinical and Experimental Research* 28:165-166. DOI: <http://dx.doi.org/10.1007/s40520-015-0381-9>.
- Zhang Y., Chen J., Qiu J., Li Y., Wang J., Jiao J. (2016b) Intakes of fish and polyunsaturated fatty acids and mild-to-severe cognitive impairment risks: a dose-response meta-analysis of 21 cohort studies. *American Journal of Clinical Nutrition* 103:330-40. DOI: <https://dx.doi.org/10.3945/ajcn.115.124081>.
- Zhao Y., Wu Y., Pei J., Chen Z., Wang Q., Xiang B. (2015) Safety and efficacy of parenteral fish oil-containing lipid emulsions in premature neonates. *Journal of Pediatric Gastroenterology and Nutrition* 60:708-716. DOI: <http://dx.doi.org/10.1097/MPG.0000000000000665>.
- Zhong Y., Wang K., Jiang L., Wang J., Zhang X., Xu J., Yao K. (2021) Dietary fatty acid intake, plasma fatty acid levels, and the risk of age-related macular degeneration (AMD): a dose-response meta-analysis of prospective cohort studies. *European Journal of Nutrition*. DOI: <http://dx.doi.org/10.1007/s00394-020-02445-4>.
- Zulyniak M.A., Roke K., Gerling C., Logan S.L., Spriet L.L., Mutch D.M. (2016) Fish oil regulates blood fatty acid composition and oxylipin levels in healthy humans: A comparison of young and older men 60:631-641.

10 Appendix Adverse effects

10.1 Literature search for systematic reviews

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to date of the search>

Date of search: May 26, 2021

Result: 609

1	("Eicosapentaenoic acid" or Icosapent or Timnodonic acid or Icosapentaenoic acid or EPA or docosahexaenoic acid or DHA*).tw.	41680
2	Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	386821
3	1 and 2	609

Database: Embase 1974 to date of the search

Date of search: May 26, 2021

Result: 792

1	("Eicosapentaenoic acid" or Icosapent or Timnodonic acid or Icosapentaenoic acid or EPA or docosahexaenoic acid or DHA*).tw.	52840
2	Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kw.	560051
	1 and 2	881
3	Elsevier.cr.	27162799
	3 and 4	792

10.1.1 Studies excluded after full-text evaluation

An overview of the publications considered not to fulfil the eligibility criteria is given in Table 10.1.1-1.

Table 10.1.1-1. Publications considered not eligible.

Reference	Reason for exclusion
(Abdullah et al., 2019)	Outcome
(Abdulrazaq et al., 2017)	Outcome

Reference	Reason for exclusion
(Abeywardena and Patten, 2011)	Publication type
(Akter et al., 2012)	Publication type
(Alexander et al., 2017)	Outcome
(Anthony et al., 2021)	Outcome
(Asfaw et al., 2021)	Publication type
(Astill Wright et al., 2019)	Outcome
(Astorg, 2004)	Publication type
(Balachandar et al., 2020)	Outcome
(Bellino et al., 2012)	Publication type
(Benedetto et al., 2013)	Publication type
(Bernasconi et al., 2021)	Outcome
(Bloch and Hannestad, 2012)	Outcome
(Bloch and Qawasmi, 2011)	Outcome
(Bourre, 2007)	Publication type
(Breslow, 2006)	Publication type
(Brouwer, 2008)	Publication type
(Brownawell et al., 2009)	Publication type
(Buhling et al., 2019)	Outcome
(Calder, 2013)	Publication type
(Campoy et al., 2012)	Outcome
(Carlson, 2009)	Publication type
(Carr, 2018)	Publication type
(Cederholm, 2017)	Publication type
(Chang et al., 2018b)	Publication type
(Chen et al., 2016)	Outcome
(Chen et al., 2020)	Intervention
(Chen et al., 2013)	Language
(Chowdhury et al., 2020)	Publication type
(Ciappolino et al., 2017)	Publication type
(Clayton et al., 2007)	Publication type
(Cohen et al., 2005)	Publication type
(Couce et al., 2019)	Outcome
(Crawford et al., 1997)	Publication type
(de Souza Fernandes et al., 2015)	Outcome
(Delarue et al., 2007)	Publication type
(Delgado-Lista et al., 2012)	Outcome
(DiNicolantonio and O'Keefe, 2020)	Publication type
(Elagizi et al., 2021)	Publication type
(Emery et al., 2020)	Outcome
(Fassett et al., 2010)	Publication type
(Firth et al., 2019)	Publication type
(Freeman et al., 2006)	Publication type
(Fusar-Poli and Berger, 2012)	Publication type
(Gawlik et al., 2020)	Publication type
(Gil-Campos and Sanjurjo Crespo, 2012)	Publication type

Reference	Reason for exclusion
(Glaser and Koletzko, 2009)	Publication type
(Gould et al., 2021)	Publication type
(Gow et al., 2015)	Publication type
(Grosso et al., 2016)	Outcome
(Grosso et al., 2014)	Publication type
(Grynberg, 2005)	Publication type
(Guo et al., 2017)	Publication type
(Guo et al., 2018)	Outcome
(Guo et al., 2014)	Outcome
(Guu et al., 2019)	Outcome
(Hadders-Algra et al., 2010)	Publication type
(Hammad et al., 2016)	Publication type
(Han et al., 2019)	Publication type
(Hansen and Harris, 2007)	Publication type
(Harper and Jacobson, 2005)	Outcome
(Harris et al., 2013)	Publication type
(Harris et al., 2007)	Publication type
(Harris and Zotor, 2019)	Publication type
(Hartweg et al., 2009)	Publication type
(Hegarty and Parker, 2013)	Publication type
(Hendrich, 2010)	Publication type
(Hibbeln and Gow, 2014)	Publication type
(Hidayat et al., 2018)	Outcome
(Hosseini et al., 2019)	Outcome
(Hou et al., 2017)	Outcome
(Hsu et al., 2018)	Outcome
(Hu et al., 2019)	Publication type
(Innes and Calder, 2020)	Publication type
(Jacobson, 2008)	Publication type
(Jans et al., 2010)	Outcome
(Jasani et al., 2017)	Outcome
(Jenkins et al., 2008)	Publication type
(Jho et al., 2004)	Publication type
(Jia et al., 2020)	Publication type
(Jiao et al., 2014)	Outcome
(Kalkman et al., 2021)	Publication type
(Keenan and Hipwell, 2015)	Publication type
(Kidd, 2007)	Publication type
(Kim and Kim, 2020)	Outcome
(Kimmig and Karalis, 2013)	Publication type
(Klosiewicz-Latoszek et al., 2020)	Publication type
(Koletzko et al., 2014)	Publication type
(Koletzko et al., 2018)	Publication type
(Koletzko et al., 2010)	Publication type
(Kromhout, 2012)	Publication type

Reference	Reason for exclusion
(Kromhout and De Goede, 2014)	Publication type
(Kuratko et al., 2013)	Publication type
(Lachance et al., 2016)	Outcome
(Langlois et al., 2019)	Outcome
(Lapillonne and Jensen, 2009)	Publication type
(Larque et al., 2012)	Publication type
(Lenighan et al., 2019)	Publication type
(Leslie et al., 2015)	Publication type
(Liao et al., 2019)	Outcome
(Lohner et al., 2013)	Publication type
(Lorente-Cebrian et al., 2013)	Publication type
(Lovegrove et al., 2015)	Publication type
(Marc et al., 2020)	Intervention
(Marti Del Moral and Fortique, 2019)	Publication type
(Miller et al., 2014)	Outcome
(Mischoulon, 2007)	Publication type
(Mischoulon, 2009)	Publication type
(Panahi et al., 2016)	Publication type
(Patel and Budoff, 2016)	Publication type
(Pourmasoumi et al., 2018)	Outcome
(Ries et al., 2012)	Publication type
(Rizos et al., 2021)	Outcome
(Rogers et al., 2008)	Publication type
(Rondanelli et al., 2021)	Outcome
(Ross et al., 2007)	Outcome
(Sacchetti et al., 2015)	Outcome
(Saller et al., 2006)	Publication type
(SanGiovanni et al., 2000a)	Publication type
(SanGiovanni et al., 2000b)	Publication type
(Saravanan et al., 2010)	Publication type
(Sarris et al., 2016)	Outcome
(Sarris, 2017)	Publication type
(Sattar et al., 2021)	Publication type
(Saunders et al., 2016)	Publication type
(Sekikawa et al., 2019)	Outcome
(Shulkin et al., 2018)	Outcome
(Siscovick et al., 2017)	Publication type
(Sublette et al., 2011)	Publication type
(Sun et al., 2015)	Publication type
(Susai et al., 2021)	Outcome
(Szajewska and Makrides, 2011)	Publication type
(Tanaka et al., 2020)	Outcome
(Todorcevic and Hodson, 2016)	Publication type
(Tsiami et al., 2012)	Publication type
(Tu et al., 2020)	Publication type

Reference	Reason for exclusion
(Turnbull et al., 2008)	Outcome
(Uauy et al., 2003)	Publication type
(Ueno et al., 2019)	Publication type
(Van Dael, 2021)	Publication type
(van der Burg et al., 2021)	Outcome
(van der Wurff et al., 2020)	Publication type
(Vieira and Silveira, 2017)	Outcome
(Vors et al., 2021)	Intervention
(Wang et al., 2018)	Outcome
(Wang et al., 2016)	Publication type
(Wang et al., 2019)	Outcome
(Wei-Hong et al., 2017)	Outcome
(Wei and Jacobson, 2011)	Publication type
(Wojcicki and Heyman, 2011)	Publication type
(Wolters et al., 2021)	Outcome
(Wu et al., 2012)	Publication type
(Wu et al., 2020)	Publication type
(Xin and Li, 2012)	Outcome
(Xu et al., 2017)	Publication type
(Xu et al., 2019)	Publication type
(Yang et al., 2015)	Outcome
(Yang et al., 2016)	Publication type
(Yurko-Mauro et al., 2015)	Outcome
(Zhang et al., 2014)	Outcome
(Zhang et al., 2016a)	Outcome
(Zhang et al., 2016b)	Outcome
(Zhao et al., 2015)	Outcome
(Zhong et al., 2021)	Outcome

10.1.2 Relevance

10.1.2.1 Evaluation of relevance

For the eligible systematic reviews, relevance for the present risk assessment was evaluated using the ROBIS RoB tool (Phase 1). An overview of the result is shown in Table 10.3.1-1.

Table 10.3.1-1. Results of the evaluation of relevance.

Reference	Relevance
Abdelhamid et al. (2020)	Partly relevant
(AbuMweis et al., 2021)	Partly relevant
(AbuMweis et al., 2018)	Partly relevant
(AlAmmar et al., 2019)	Partly relevant

Reference	Relevance
Atlantis and Cochrane (2016)	Not relevant
(Aung et al., 2018)	Not relevant
(Azzi et al., 2018)	Partly relevant
Bai et al. (2018)	Not relevant
Bakouei et al. (2020)	Not relevant
Balk et al. (2016)	Partly relevant
Bath-Hextall et al. (2012)	Not relevant
(Becic and Studenik, 2018)	Partly relevant
(Bernstein et al., 2012)	Partly relevant
Brito-Garcia et al. (2017)	Not relevant
Burgess et al. (2018)	Updated in Dushianthan et al. (2019)
(Casula et al., 2020)	Partly relevant
Chang et al. (2018a)	Partly relevant
(Chen et al., 2015)	Partly relevant
Chen et al. (2010)	Not relevant
Colomer et al. (2007)	Not relevant
(Chua et al., 2012)	Partly relevant
(Chua et al., 2013)	Not relevant
(Craddock et al., 2017)	Partly relevant
(de Aguiar Pastore Silva et al., 2015)	Not relevant
Delgado-Noguera et al. (2015)	Not relevant
Dewey et al. (2007)	Not relevant
Downie et al. (2019)	Partly relevant
Dushianthan et al. (2020)	Not relevant
Dushianthan et al. (2019)	Not relevant
(Elia et al., 2006)	Not relevant
Eslick et al. (2009)	Not relevant
Fogacci et al. (2020)	Partly relevant
Fu et al. (2015)	Partly relevant
Goh et al. (2021)	Partly relevant
(Gonzalez-Becerra et al., 2019)	Not relevant
(Guo et al., 2019)	Partly relevant
(Hu et al., 2018)	Partly relevant
(Innes and Calder, 2018)	Partly relevant
Irving et al. (2006)	Partly relevant
(Kar et al., 2016)	Partly relevant
Koekkoek et al. (2018)	Not relevant
Kwak et al. (2012)	Partly relevant
(Lehner et al., 2021)	Partly relevant
Leon et al. (2008)	Not relevant
Lopez-Huertas (2012)	Partly relevant
Maki et al. (2017)	Not relevant
Marik and Varon (2009)	Not relevant
(Miles and Calder, 2012)	Not relevant
Mocellin et al. (2016)	Partly relevant

Reference	Relevance
Morsy et al. (2019)	Not relevant
Newberry et al. (2016)	Partly relevant
Ostadrahimi et al. (2016)	Partly relevant
(Quin et al., 2016)	Partly relevant
(Ren et al., 2021)	Partly relevant
Sadeghi et al. (2017)	Partly relevant
Sahebkar et al. (2018)	Not relevant
Sarmento Vasconcelos et al. (2016)	Partly relevant
Su et al. (2021)	Partly relevant
Suradom et al. (2021)	Not relevant
Vahdaninia et al. (2019)	Not relevant
Villani et al. (2013)	Partly relevant
Wang et al. (2006)	Partly relevant
Watson and Stackhouse (2020)	Partly relevant
Yang et al. (2019)	Not relevant
Xin et al. (2013)	Partly relevant
Zhang et al. (2020)	Not relevant

10.1.2.2 Systematic reviews considered relevant/partly relevant

In total, 38 systematic reviews were considered to be partly relevant. The evaluations of relevance were as follows:

Abdelhamid et al. (2020)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease).
Intervention(s)	DHA and EPA, in combination and single exposure.	Omega-3 fats from fish sources including eicosapentaenoic acid, docosahexaenoic acid and docosapentaenoic acid.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Usual diet, no advice, no supplementation, placebo or lower-dose omega-3.
Outcome(s):	Adverse health effects.	Adverse effects was a tertiary outcome.

Relevance assessment	Reasoning
Partly relevant	The population is adults are not necessarily representative for the population addressed in our assessment.

AbuMweis et al. (2021)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Healthy adults and adults with different conditions (diabetes, hypertension, hypertriglyceridaemia, hypercholesterolaemia, impaired glucose tolerance.
Intervention(s)	DHA and EPA, in combination and single exposure	Supplemental dose of EPA and DHA
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo controlled
Outcome(s):	Adverse health effects	Lipid profile and inflammation

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

AbuMweis et al. (2018)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Healthy adults and adults with different conditions (diabetes, hypertension, hypertriglyceridaemia, hypercholesterolaemia, impaired glucose tolerance.
Intervention(s)	DHA and EPA, in combination and single exposure	Supplemental dose of EPA and DHA
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo controlled
Outcome(s):	Adverse health effects	Lipid profile and inflammation

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

AlAmmar et al. (2019)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Adults (aged 18 years at minimum) diagnosed with multiple sclerosis according to McDonald 2010 criteria.
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFA supplements.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Poorly described
Outcome(s):	Adverse health effects	Inflammatory markers

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Azzi et al. (2018)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Patients (>18 years) with periodontal disease.
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	No intervention or placebo
Outcome(s):	Adverse health effects	Gingival bleeding

Relevance assessment	Reasoning
Partly relevant	The review has assessed gingival bleeding and bleeding have been hypothesized to be a concern with high intake of EPA and DHA. The population is not necessarily representative for the population addressed in our assessment.

Balk et al. (2016)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Healthy adults (≥ 18 years) without cardiovascular diseases (CVD) or with low to intermediate risk for CVD, adults at high risk for CVD, and adults with clinical CVD.
Intervention(s)	DHA and EPA, in combination and single exposure.	N-3 fatty acids in supplements, in supplemented foods (e.g., eggs), or in the diet.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	No, lower, or other n-3 fatty acid intake (placebo or no n-3 FA intervention).
Outcome(s):	Adverse health effects.	Adverse events (e.g., bleeding, major gastrointestinal disturbance), only from intervention studies of supplements.

Relevance assessment	Reasoning
Partly relevant	The population is adult patients and are not necessarily representative for the population addressed in our assessment.

Becic and Studenik (2018)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Adults (≥ 18 years), diagnosed with insulin resistance, impaired glucose tolerance, impaired fasting glucose, or T2DM
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not reported
Outcome(s):	Adverse health effects	Cytokines

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Bernstein et al. (2012)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Adults >18 years
Intervention(s)	DHA and EPA, in combination and single exposure	Algae-derived DHA and EPA
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not reported
Outcome(s):	Adverse health effects	Serum lipoproteins (HDL, LDL)

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Casula et al. (2020)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Subjects with previous cardiovascular events, control group could be healthy (RCTs conducted only on subjects free from CVD at baseline were not eligible).
Intervention(s)	DHA and EPA, in combination and single exposure	Omega-3fatty acid supplements at least 1 g per day dosage and for at least 1 year
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo
Outcome(s):	Adverse health effects	Bleeding

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Chang et al. (2018a)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	All age groups.
Intervention(s)	DHA and EPA, in combination and single exposure.	RCTs, prescription Omega-3 fatty acids products.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo, other medication, or no treatment.
Outcome(s):	Adverse health effects.	Treatment related adverse effect.

Relevance assessment	Reasoning
Partly relevant	The population is predominantly adult patients and therefore not necessarily representative for the population addressed in our assessment. The main focus of the review is all adverse effects, and the effects assessed is judged to be of importance for the target population of our assessment.

Chen et al. (2015)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	People with type 2 diabetes
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not reported
Outcome(s):	Adverse health effects	Blood glucose, fasting insulin, HbA1c, total cholesterol

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Chua et al. (2012)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Men >40 years
Intervention(s)	DHA and EPA, in combination and single exposure	N-PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not reported
Outcome(s):	Adverse health effects	Risk of prostate cancer

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Craddock et al. (2017)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Vegetarian (vegan, ovo-lacto-, ovo-, lacto-) populations aged 18 years or over.
Intervention(s)	DHA and EPA, in combination and single exposure	Algal DHA
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not reported
Outcome(s):	Adverse health effects	Serum LDL

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Downie et al. (2019)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Study populations with dry eye diagnosis. No restrictions on age, gender, severity of disease, or classification of dry eye.
Intervention(s)	DHA and EPA, in combination and single exposure.	Omega-3 and/or omega-6 PUFA interventions.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Other forms of dry eye treatment, such as artificial tears, placebo, or no treatment.
Outcome(s):	Adverse health effects.	Adverse effects in studies on persons diagnosed with dry eye disease.

Relevance assessment	Reasoning
Partly relevant	The population is adults with a disease and are therefore not necessarily representative for the population addressed in our assessment.

Fogacci et al. (2020)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Patients with HIV.
Intervention(s)	DHA and EPA, in combination and single exposure.	Omega-3 PUFAs.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Controlled design.
Outcome(s):	Adverse health effects.	Plasma lipids and safety profile.

Relevance assessment	Reasoning
Partly relevant	The population included are adults with HIV, and are therefore not necessarily representative for the population addressed in our assessment.

Fu et al. (2015)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Males
Intervention(s)	DHA and EPA, in combination and single exposure.	Dietary n-3 PUFAs or blood n-3 PUFAs concentrations.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Not reported
Outcome(s):	Adverse health effects.	Prostate cancer

Relevance assessment	Reasoning
Partly relevant	The population included are adults, and are therefore not necessarily representative for the population addressed in our assessment.

Goh et al. (2021)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Patients with schizophrenia spectrum disorder, including schizophrenia, schizoaffective disorder, and other psychotic disorders.
Intervention(s)	DHA and EPA, in combination and single exposure.	All types of n3-PUFA supplements.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo groups were defined as those who were treated with a placebo mixture or capsule that was identical to the active treatment. The content of the placebo preparation could be any type of oil except for n3-PUFAs.
Outcome(s):	Adverse health effects.	Safety of the intervention was a secondary outcome.

Relevance assessment	Reasoning
Partly relevant	The population included are patients with schizophrenia spectrum disorder and are therefore not necessarily representative for the population addressed in our assessment.

Guo et al. (2019)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Adults with different health status; healthy, coronary artery disease, dyslipidemia, diabetes type 2, chronic heart failure and metabolic syndrome.
Intervention(s)	DHA and EPA, in combination and single exposure	EPA or DHA
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo
Outcome(s):	Adverse health effects	Inflammatory mediators (CRP, cytokines)

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Hu et al. (2018)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Adults with chronic kidney disease
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Other supplement or placebo
Outcome(s):	Adverse health effects	Inflammation (CRP, cytokines)

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Innes and Calder (2018)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Adults with different conditions; dyslipidemic subjects, treated hypertensive type 2 diabetics, mildly hyperlipidemic men, subjects with abdominal obesity and low-grade inflammation, and healthy subjects.
Intervention(s)	DHA and EPA, in combination and single exposure	EPA and DHA
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo
Outcome(s):	Adverse health effects	HDL, LDL, platelet activity, inflammatory markers, glycaemic control, oxidative stress.

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Irving et al. (2006)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	People with schizophrenia or similar chronic mental illnesses, diagnosed by any criteria, irrespective of gender, age or race.
Intervention(s)	DHA and EPA, in combination and single exposure.	Any type of polyunsaturated fatty acid supplementation of a standard neuroleptic care: any dose.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo
Outcome(s):	Adverse health effects.	Adverse effects were included as secondary outcomes.

Relevance assessment	Reasoning
Partly relevant	The population included are patients with schizophrenia or similar chronic mental illnesses and are therefore not necessarily representative for the population addressed in our assessment.

Kar et al. (2016)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Pregnant women.
Intervention(s)	DHA and EPA, in combination and single exposure	Supplements, DHA and EPA, in combination or alone during pregnancy.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not reported.
Outcome(s):	Adverse health effects	Early (< 34 weeks) and any (< 37 weeks) preterm delivery, and birthweight.

Relevance assessment	Reasoning
Partly relevant	The population receiving the intervention is not necessarily representative for the population addressed in our assessment.

Kwak et al. (2012)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Adult patients (male or female aged 18 years and up) with a history of cardiovascular disease.
Intervention(s)	DHA and EPA, in combination and single exposure.	Omega-3 fatty acid supplements.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Not reported.
Outcome(s):	Adverse health effects.	Secondary prevention of cardiovascular disease. Included trials should report on outcome measures like angina, unstable angina, CVD or events, sudden cardiac death, cardiovascular death, all-cause mortality, congestive heart failure, transient ischemic attack and stroke, or fatal or nonfatal myocardial infarction. Adverse events

		(gastrointestinal troubles and gastrointestinal bleeding) in the included studies were summarised.
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Relevance assessment	Reasoning
Partly relevant	The population included are adult patients with a history of cardiovascular disease, and are therefore not necessarily representative for the population addressed in our assessment.

Lehner et al. (2021)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Pregnant and lactating mothers.
Intervention(s)	DHA and EPA, in combination and single exposure	LC-PUFA supplementation.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo.
Outcome(s):	Adverse health effects	Cognitive outcomes in children and birth weight.

Relevance assessment	Reasoning
Partly relevant	The population receiving the intervention is not necessarily representative for the population addressed in our assessment.

Lopez-Huertas et al. (2012)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Subjects diagnosed with metabolic syndrome who had three, four or five characteristics of the metabolic syndrome, without history of cardiovascular disease.
Intervention(s)	DHA and EPA, in combination and single exposure	EPA + DHA (as either supplements or dietary components).
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not reported
Outcome(s):	Adverse health effects	Cytokines, CRP and LDL

Relevance assessment	Reasoning
Partly relevant	Inflammation and lipids that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not representative for our target population. The population is not necessarily representative for the population addressed in our assessment.

Mocellin et al. (2016)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Colorectal cancer patients, age >50 years.
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not n-3 PUFAs as intervention.
Outcome(s):	Adverse health effects	Cytokines and acute phase proteins. Adverse effects are described shortly in the text, no quantitative data were reported.

Relevance assessment	Reasoning
Partly relevant	Inflammation, which has been hypothesized to be a concern with high intake of EPA and DHA, was assessed. The population is not necessarily representative for the population addressed in our assessment.

Newberry et al. (2016)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Pregnant women and infants 0-12 months.
Intervention(s)	DHA and EPA, in combination and single exposure.	Maternal intake of omega-3 fatty acids. <ul style="list-style-type: none"> • Route of delivery: supplementation and/or diet • Type (EPA, DHA, ALA), source (e.g., marine, plant) and amount (e.g.,

		dose, serving size) of omega-3 fatty acid content
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo, non-fortified infant formula.
Outcome(s):	Adverse health effects.	<p>Duration of gestation</p> <ul style="list-style-type: none"> • Infants born small for gestational age • Gestational hypertension, preeclampsia, or eclampsia • Adverse effects (Incidence of specific adverse events reported in trials by study arm) • Ante- and postnatal depression

Relevance assessment	Reasoning
Partly relevant	The population is not necessarily representative for the population addressed in our assessment.

Ostadrhimi et al. (2016)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Pregnant women of any gestational age and parity with singleton pregnancies, normal and high risk group for gestational diabetes, were included.
Intervention(s)	DHA and EPA, in combination and single exposure	Fish oil supplementation
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo or no intervention
Outcome(s):	Adverse health effects	Incidence of gestational diabetes mellitus in pregnant women, blood glucose, insulin resistance, lipid profiles, side-effects (gastrointestinal and non-gastrointestinal).

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Quin et al. (2016)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Breastfeeding women and infants
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFA supplementation taken maternally during gestation, gestation and lactation, or lactation only, and long chain n-3 PUFA supplemented milk-based formula or capsule
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo or no supplementation
Outcome(s):	Adverse health effects	Immune and inflammatory markers

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Ren et al. (2021)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Humans, all age groups	Healthy pregnant women and children.
Intervention(s)	EPA and/or DHA supplementation	DHA and/or EPA as supplements during pregnancy.
Comparator(s):	Without EPA and/or DHA supplementation	No treatment or oil with no EPA/DHA.
Outcome(s):	Adverse effects (side effects)	Birthweight and weight development in offspring.

Relevance assessment	Reasoning
Partly relevant	The population receiving the intervention is not necessarily representative for the population addressed in our assessment.

Sadeghi et al. (2017)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Women with polycystic ovary syndrome (aged >18 years).
Intervention(s)	DHA and EPA, in combination and single exposure	Oral omega-3 supplementation
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo
Outcome(s):	Adverse health effects	Insulin resistance

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Sarmiento et al. (2016)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	All individuals (adults and children) with a diagnosis of drug-resistant epilepsy, irrespective of their seizure type or epilepsy syndrome.
Intervention(s)	DHA and EPA, in combination and single exposure.	Supplementation with PUFAs belonging to the omega-3 series (eicosapentaenoic and docosahexaenoic acid) at any dosage and over any period of time, combined with antiepileptic drugs.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	No supplementation, placebo treatment or other treatment.

Outcome(s):	Adverse health effects	Adverse effects were included as secondary outcomes.
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Relevance assessment	Reasoning
Partly relevant	The population, individuals with a diagnosis of drug-resistant epilepsy, is not necessarily representative for the population addressed in our assessment.

Su et al. (2021)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Patients with lower extremity arterial disease
Intervention(s)	DHA and EPA, in combination and single exposure	Omega-3 PUFA supplementation
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo
Outcome(s):	Adverse health effects	Relevant outcomes were addressed as secondary outcomes: CRP, LDL, HDL, total cholesterol.

Relevance assessment	Reasoning
Partly relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment.

Villani et al. (2013)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Elderly persons with a reported mean age ≥ 60 years.
Intervention(s)	DHA and EPA, in combination and single exposure.	Ingestion of liquid fish oil or fish oil capsules.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Not reported

Category (PICO equivalents)	Target question	Review being assessed
Outcome(s):	Adverse health effects.	Adverse events

Relevance assessment	Reasoning
Partly relevant	The population, elderly persons with a reported mean age ≥ 60 years, is not necessarily representative for the population addressed in our assessment.

Wang et al. (2006)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Human adults
Intervention(s)	DHA and EPA, in combination and single exposure.	Any type of n-3 FA intake.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Not given
Outcome(s):	Adverse health effects.	All-cause mortality and the following clinical CVD outcomes: cardiac death, sudden death, myocardial infarction (MI), and stroke. Adverse events reported in the included trials were described, and for some events, rates and doses were included.

Relevance assessment	Reasoning
Partly relevant	The population is adults and are therefore not necessarily representative for the population addressed in our assessment.

Watson and Stackhouse (2020)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	People with cystic fibrosis, of any age and severity.
Intervention(s)	DHA and EPA, in combination and single exposure.	Dietary supplementation of omega-3 essential fatty acids.

Category (PICO equivalents)	Target question	Review being assessed
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo
Outcome(s):	Adverse health effects.	Adverse events were a primary outcome.

Relevance assessment	Reasoning
Partly relevant	The population, people with cystic fibrosis, of any age and severity, is not necessarily representative for the population addressed in our assessment.

Xin et al. (2013)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Adult human subjects who underwent a cardiac surgery.
Intervention(s)	DHA and EPA, in combination and single exposure.	Perioperative fish oil supplementation (orally or intravenously).
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Not given
Outcome(s):	Adverse health effects.	Prevention of postoperative atrial fibrillation.

Relevance assessment	Reasoning
Partly relevant	The population is not necessarily relevant for the population addressed in our assessment. Data on adverse events present (bleeding).

10.1.2.3 Systematic reviews considered not relevant

In total, 28 systematic reviews were considered to be not relevant for the present assessment. The evaluation of relevance was as follows:

Atlantis and Cochrane (2016)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	People with chronic lung disease within the spectrum of chronic obstructive pulmonary disease-related diagnoses.
Intervention(s)	DHA and EPA, in combination and single exposure.	Observational (dietary intake) and intervention (supplements) with omega-3 and/or omega-6 fatty acids.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Low dietary intake, placebo or no treatment.
Outcome(s):	Adverse health effects.	Airway and systemic inflammatory markers and functional capacity outcomes. Functional capacity outcomes including exercise-based physical performance measures (e.g. muscle strength and endurance), and health status or health-related quality of life measures.

Relevance assessment	Reasoning
Not relevant	The participants had chronic lung disease. Mainly beneficial effects. Adverse effects were not systematically addressed. The population and outcomes assessed are not relevant to our assessment.

Aung et al. (2018)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Adults, mean age at entry was 64 years.
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo or open-label control
Outcome(s):	Adverse health effects	LDL, HDL

Relevance assessment	Reasoning
Not relevant	Effects that have been hypothesized to be a concern with high intake of EPA and DHA were assessed. The population is not necessarily representative for the population addressed in our assessment. The review contains no usable data on adverse effects for our review.

Bai et al. (2018)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Participants' age ranged from 60 and up.
Intervention(s)	DHA and EPA, in combination and single exposure.	N-3 PUFAs without other type of depression treatment.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo
Outcome(s):	Adverse health effects.	Main outcome: depression

Relevance assessment	Reasoning
Not relevant	The population and outcomes assessed are not relevant to our assessment.

Bakouei et al. (2020)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Pregnant women with both high and low risk of pregnancies (any age, any gestational age, any gravidity and parity, singleton and in multiple gestations).
Intervention(s)	DHA and EPA, in combination and single exposure.	Clinical trials, daily supplements of either n-3 fatty acids (DHA and/or EPA and/or ALA) orally alone or together with other supplements at least twice a week.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Any control groups (i.e. placebo or other supplementation or no treatment).
Outcome(s):	Adverse health effects.	Efficacy of n-3 fatty acids supplementation on the prevention of pregnancy-induced hypertension or preeclampsia.

Relevance assessment	Reasoning
Not relevant	The population and outcomes assessed are not relevant to our assessment.

Bath-Hextall et al. (2012)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	People with atopic eczema, all age groups.
Intervention(s)	DHA and EPA, in combination and single exposure.	RCTs, dietary supplements (fish oil, zinc, selenium, vitamin D, vitamin E, pyridoxine (vitamin B6), sea buckthorn oil, hempseed oil, and sunflower oil).
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	The comparators were no treatment, placebo, or any other active intervention.
Outcome(s):	Adverse health effects.	Improvement in the symptoms of atopic eczema, e.g. itching and loss of sleep and evidence of reduced need for treatment of the eczema or a reduction in the number of flares. Adverse (harmful) effects are only mentioned in a sentence: "We found no evidence of adverse (harmful) effects in those who took part in the trials".

Relevance assessment	Reasoning
Not relevant	Adverse effects are not addressed in the studies on fish oil, thus, the outcome is not relevant.

Brito-Garcia et al. (2017)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	People diagnosed with hereditary retinal dystrophies, all age groups.
Intervention(s)	DHA and EPA, in combination and single exposure.	Human studies (RCTs, non-RCTs, observational studies). Safety of nutritional supplements.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Standard of care, placebo, no treatment or alternative treatments.
Outcome(s):	Adverse health effects.	The search words include adverse effects and it is described in the results. However, the type of adverse effect or events that have been assessed are mainly not described.

Relevance assessment	Reasoning
Not relevant	The description on type of adverse effect are not described, thus, the outcome addressed cannot be used.

Chen et al. (2010)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Patients undergoing major abdominal surgery. Age of the subjects in not reported in the review.
Intervention(s)	DHA and EPA, in combination and single exposure.	RCTs, fish oil-supplemented parenteral (intravenous) nutrition (supplemented with PUFAs, including EPA and DHA).
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Fish oil without supplementation.
Outcome(s):	Adverse health effects.	Clinical safety variables: the incidence rate of cardiac complications and serum levels of liver enzymes, bilirubin, and triglycerides. The efficacy variables: mortality, postoperative infection rate, length of hospital and intensive care unit stays, and PUFA levels in plasma phospholipids. All measured on postoperative day 6.

Relevance assessment	Reasoning
Not relevant	<p>The population is patients undergoing major abdominal surgery and the age of the patients is not specified. The administration route is intravenous. It is not clearly specified whether the supplemented fish-oil is only supplemented with only DHA and EPA or whether they contain other supplementations as well. The review has assessed adverse effects.</p> <p>Since the population is of little relevance to our target population and the administration route is different, the review is considered not relevant for our assessment.</p>

Chua et al. (2013)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Men >40 years
Intervention(s)	DHA and EPA, in combination and single exposure	No intervention
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	-
Outcome(s):	Adverse health effects	Risk of prostate cancer and blood level of n-3 PUFAs.

Relevance assessment	Reasoning
Not relevant	No intervention

Colomer et al. (2007)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Enteral-fed patients with cancer, adults (18 and older).
Intervention(s)	DHA and EPA, in combination and single exposure.	Clinical trials and prospective observational studies. Oral supplements with omega-3 fatty acids (EPA and DHA or EPA alone).
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo or no supplements (not specified for all studies).
Outcome(s):	Adverse health effects.	Clinical outcomes, functional parameters and laboratory parameters.

Relevance assessment	Reasoning
Not relevant	Subjects included consists of enteral-fed adult cancer patients, although some of the primary studies included healthy controls. Some of the outcomes are relevant, e.g. inflammatory markers and gastrointestinal parameters.

De Aguiar Pastore Silva et al. (2015)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Oncologic patients undergoing treatment; radiotherapy, chemotherapy or chemoradiotherapy
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo or no intervention
Outcome(s):	Adverse health effects	Cytokines, CRP

Relevance assessment	Reasoning
Not relevant	The participants were oncologic patients undergoing radiotherapy, chemotherapy or chemoradiotherapy, and are considered not relevant.

Delgado-Noguera et al. (2015)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Breastfeeding mothers.
Intervention(s)	DHA and EPA, in combination and single exposure.	Supplementation with long chain polyunsaturated fatty acids.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo or no supplementation.
Outcome(s):	Adverse health effects.	Improvement of child growth and development. Adverse effects are only mentioned in one sentence in the abstract "No adverse effects were reported."

Relevance assessment	Reasoning
Not relevant	The review looks at effects in children, although exposed through breastfeeding. The review contains no usable data on adverse effects for our review.

Dewey et al. (2007)

Category (PICO equivalents)	Target question	Review being assessed
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Patients/Population(s):	Children and adolescents (3-18 years).	Patients with advanced cancer and cachexia (muscle loss).
Intervention(s)	DHA and EPA, in combination and single exposure.	Oral EPA.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo or other control.
Outcome(s):	Adverse health effects.	Primary outcomes: weight gain, body composition, median survival. Secondary outcomes: functional or performance status, improvement in quality of life, energy expenditure, reduction in fatigue, nutritional status, compliance rates, side effects, adverse events.

Relevance assessment	Reasoning
Not relevant	Adverse events and side effects have been assessed. However, the subjects included in the review is critically ill and of little relevance to our target population.

Dushianthan et al. (2019)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Mechanically ventilated adults (aged 18 years or older) with acute respiratory distress syndrome.
Intervention(s)	DHA and EPA, in combination and single exposure.	Immunonutrition (supplemented with e.g. omega-3 fatty acids, antioxidants).
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Control or placebo.
Outcome(s):	Adverse health effects.	All-cause mortality and adverse events (cardiac, gastrointestinal and total adverse events).

Relevance assessment	Reasoning
Not relevant	The population is critically ill and not relevant.

Dushianthan et al. (2020)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Mechanically ventilated adults (aged 18 years or older) with acute respiratory distress syndrome.
Intervention(s)	DHA and EPA, in combination and single exposure.	RCTs, quasi-RCTs. Immunonutrition (supplemented with e.g. omega-3 fatty acids, antioxidants).
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Control or placebo.
Outcome(s):	Adverse health effects.	All-cause mortality and adverse events (cardiac, gastrointestinal and total adverse events).

Relevance assessment	Reasoning
Not relevant	The population is critically ill and not relevant.

Elia et al. (2006)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Enteral tube fed patients, adults
Intervention(s)	DHA and EPA, in combination and single exposure	EPA or fish oil
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	
Outcome(s):	Adverse health effects	Inflammatory markers (cytokines)

Relevance assessment	Reasoning
Not relevant	The participants were enteral tube fed patients, and are considered not relevant.

Eslick et al. (2009)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Adult males and/or females with cardiovascular risk factors.

Category (PICO equivalents)	Target question	Review being assessed
Intervention(s)	DHA and EPA, in combination and single exposure.	Omega-3 (DHA and/or EPA) supplementation.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo
Outcome(s):	Adverse health effects.	Lipid biomarkers (total cholesterol, HDL, LDL, TG). Adverse events reported by the participants.

Relevance assessment	Reasoning
Not relevant	The population included are adults with CVD risk factors and are therefore not necessarily representative for the population addressed in our assessment. The review contains no usable data on adverse effects for our review.

Gonzalez-Becerra et al. (2019)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Age not given.
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not given
Outcome(s):	Adverse health effects	Metabolic alterations through epigenetic mechanisms.

Relevance assessment	Reasoning
Not relevant	Age and comparator were not described.

Koekkoek et al. (2018)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Critically ill patients.
Intervention(s)	DHA and EPA, in combination and single exposure.	Enteral omega-3 fatty acid supplementation.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo.

Category (PICO equivalents)	Target question	Review being assessed
Outcome(s):	Adverse health effects.	Tolerability and adverse events.

Relevance assessment	Reasoning
Not relevant	The population are critically ill patients.

Leon et al. (2008)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Patients with implantable cardiac defibrillator. Subgroup analyses in patients with coronary artery disease or myocardial infarction.
Intervention(s)	DHA and EPA, in combination and single exposure.	Omega-3 fatty acid supplements.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo.
Outcome(s):	Adverse health effects.	The primary outcomes were arrhythmic end points of appropriate implantable cardiac defibrillator intervention and sudden cardiac death. The secondary outcomes were all cause mortality and death from cardiac causes. Non-cardiovascular adverse effects in included studies were summarised.

Relevance assessment	Reasoning
Not relevant.	The population included are patients with a history of cardiovascular disease, and are therefore not necessarily representative for the population addressed in our assessment. The review contains no usable data on adverse effects for our review.

Maki et al. (2017)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Humans

Category (PICO equivalents)	Target question	Review being assessed
Intervention(s)	DHA and EPA, in combination and single exposure.	Mainly EPA and/or DHA dietary supplement or pharmaceutical concentrate.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Not reported
Outcome(s):	Adverse health effects.	Myocardial infarct (fatal), sudden cardiac death, coronary death, cardiac death, ischemic heart disease death, sudden cardiac mortality, coronary mortality, cardiac mortality, or ischemic heart disease mortality.

Relevance assessment	Reasoning
Not relevant	No adverse effects reported, thus, the outcome reported is not relevant.

Marik and Varon (2009)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Patients
Intervention(s)	DHA and EPA, in combination and single exposure.	Dietary supplements of EPA/DHA.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo
Outcome(s):	Adverse health effects.	Incidence of cardiovascular death, sudden death, all-cause mortality, and nonfatal cardiovascular events.

Relevance assessment	Reasoning
Not relevant	No adverse effects reported, thus, the outcome reported is not relevant.

Miles and Calder (2012)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Adults with rheumatoid arthritis.
Intervention(s)	DHA and EPA, in combination and single exposure	N-3 PUFAs
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Placebo, other supplementation
Outcome(s):	Adverse health effects	Immune function, reported as joint swelling and pain, duration of morning stiffness, global assessments of pain and disease activity.

Relevance assessment	Reasoning
Not relevant	The effects reported are not considered as relevant for the present assessment.

Morsy et al. (2019)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years)	Participants had Huntington disease clinical features and a confirmatory genetic diagnosis or a compatible family history.
Intervention(s)	DHA and EPA, in combination and single exposure	Ethyl-EPA
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration)	Not given
Outcome(s):	Adverse health effects	Safety and efficacy. Quantitative data on adverse effects reported.

Relevance assessment	Reasoning
Not relevant	The population is not necessarily representative for the population addressed in our assessment. The data on adverse effects cannot be used.

Sahebkar et al. (2018)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Clinical trials
Intervention(s)	DHA and EPA, in combination and single exposure.	Omega-3 products
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Control
Outcome(s):	Adverse health effects.	Total circulating concentrations of apo C-III (a marker/predictor of cardiovascular disease risk).

Relevance assessment	Reasoning
Not relevant	No adverse effects reported, thus, the outcome reported is not relevant.

Suradom et al. (2021)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Perinatal women without depression, with subthreshold depression, and with depression.
Intervention(s)	DHA and EPA, in combination and single exposure.	N-3 PUFA supplementation
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo
Outcome(s):	Adverse health effects.	Efficacy as measured by the change of depression severity scores. In addition, adverse effects reported in the included studies were described. These effects are described shortly in the text, no quantitative data were reported.

Relevance assessment	Reasoning
Not relevant	The data on adverse effects cannot be used.

Vahdaninia et al. (2019)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Pregnant women and their offspring.
Intervention(s)	DHA and EPA, in combination and single exposure.	Long chain n-3 PUFA supplementation during pregnancy. Trials were also included if the intervention(s) had been extended after pregnancy either during breast-feeding or directly to the infants or both.
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Not given
Outcome(s):	Adverse health effects.	Obesity or growth in the offspring, either as a primary or secondary endpoint.

Relevance assessment	Reasoning
Not relevant	No adverse effects reported, thus, the outcome reported is not relevant.

Yang et al. (2019)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Adults with cardiometabolic disorders, chronic inflammatory diseases, or healthy adults.
Intervention(s)	DHA and EPA, in combination and single exposure.	N-3 PUFA
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Not given
Outcome(s):	Adverse health effects.	Oxidative stress

Relevance assessment	Reasoning
Not relevant	No adverse effects reported, thus, the outcome reported is not relevant.

Zhang et al. (2020)

Category (PICO equivalents)	Target question	Review being assessed
Patients/Population(s):	Children and adolescents (3-18 years).	Pregnant or postpartum women with major depressive disorder.
Intervention(s)	DHA and EPA, in combination and single exposure.	Omega-3 FA including DHA and EPA as monotherapy
Comparator(s):	Placebo, no treatment, other supplementation (e.g. less concentration).	Placebo
Outcome(s):	Adverse health effects.	Efficacy and safety. Adverse effects are described shortly in the text, no quantitative data were reported.

Relevance assessment	Reasoning
Not relevant	The population is not necessarily representative for the population addressed in our assessment. The data on adverse effects cannot be used.

10.1.3 Evaluation of risk of bias

To identify concerns with the review process, four domains were included (study eligibility, identification and selection of studies, data collection and study appraisal, and synthesis and findings), before the risk of bias were judged. The detailed RoB evaluations for the systematic reviews are shown below. The response options used for the rating were “low” “unclear” and “high”.

Abdelhamid et al. (2020)

Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Yes	An update of a previous systematic review. A protocol was prepared for the original review.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	Eligibility criteria seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed with regard to study design (including follow up), participants, intervention and comparator.

Signalling question	Rating	Reasoning
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions based on types of study design, participants (age, health condition), and intervention (multiple risk factor interventions on lifestyle factors such as e.g. smoking and weight reduction) were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were rated "yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Searches were performed in several electronic databases. Also, trial registers were searched.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Handsearching of included trials in systematic reviews up to April 2017 (not 2019) was performed.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	The search terms included was likely to receive as many eligible studies as possible.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably yes	No language restrictions. Date limits to the searches from the original strategies were applied so that the search included only new records. A filter for RCT was applied.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Both selection steps were performed independently by two reviewers.
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were rated "yes" or "probably yes".

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	The data extraction form was tested by the review authors on a common training trial. Two review authors independently extracted data.

Signalling question	Rating	Reasoning
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available. Authors of large trials were contacted for further trial data.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were extracted and was well described.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	Cochrane criteria were used for the RoB assessment, including in the domains of sequence generation; allocation concealment; blinding of participants and personnel, blinding of outcome assessors; incomplete outcome data; and selective outcome reporting. Additional review-specific criteria included similarity of type and intensity of intervention in both arms (attention) and evidence of appropriate moderate to high compliance (to establish that the intervention group were receiving a different intake of omega-3 fats than the control group).
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two review authors independently assessed risk of bias.
Concerns regarding methods used to collect data and appraise studies	Low	All signaling questions were rated "yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	The number of studies of which data was extracted for each outcome is given in a table. These numbers match the number of studies included in the forest plot.
4.2 Were all predefined analyses followed or departures explained?	Yes	Deviations from the protocol were described and explained.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	The authors assessed heterogeneity using the I^2 test and assumed it to be important when I^2 test value was more than 60%.

Signalling question	Rating	Reasoning
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	Funnel plot or sensitivity analyses were not performed for adverse effects. However, an assessment of the certainty in evidence was conducted.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	Biases was addressed in the grading of the certainty in evidence.
Concerns regarding methods used to synthesize results	Low	No concerns were identified. Five of six questions were rated "yes".

Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were rated "yes".
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were rated "yes" or "probably yes".
Concerns regarding methods used to collect data and appraise studies	Low	All signaling questions were rated "yes".
Concerns regarding the synthesis and findings	Low	No concerns were identified. Five of six questions were rated "yes".

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	Yes	No concerns identified.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results were presented.
Risk of bias	Low	

AbuMweis et al. (2018)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria were appropriate.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably no	Some criteria were not described in detail. No specific information was provided on which criteria that were used to determine whether trials included outcomes of interest.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	The restriction on language were considered not to be a major issue. The restrictions based on reporting of outcome (incomplete data) were clearly described and appeared to be appropriate.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. Concerns regarding restrictions in the eligibility criteria was identified.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in electronic databases. Search for unpublished reports were not performed.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Bibliographies of review articles were hand-searched.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	The search terms used in the search and the search strings used were reported. The full search strategy or the combination of the terms, were not reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably yes	The search was restricted to human studies, and this restriction seemed appropriate.
2.5 Were efforts made to minimise errors in selection of studies?	No	One person screened the titles and abstracts.

Signalling question	Rating	Reasoning
Concerns regarding methods used to identify and/or select studies	High	Concerns regarding the lack of efforts to minimise errors in selection of studies and the lack of information on the search strategy were identified.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	One reviewer checked the extracted data for accuracy, two other investigators controlled the data extraction.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	No	Information from the included studies were presented in tables. However, it was not indicated in which studies the different outcomes were included. Also, study sample size was extracted in the data extraction, but not reported.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	The Jadad score method was used to evaluate the methodological quality. Allocation concealment was not included in the assessment.
3.5 Were efforts made to minimise error in risk of bias assessment?	Probably yes	Quality assessment was performed by a research assistant and the main investigator. There was no information on how discrepancies were solved.
Concerns regarding methods used to collect data and appraise studies	Unclear	Concerns regarding the insufficient study characteristics and the method used for the RoB evaluation were identified.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably no	Studies with incomplete data were excluded.
4.2 Were all predefined analyses followed or departures explained?	Probably no	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably no	A random effects meta-analysis was performed. There was no information on whether sample sizes affected the results.

Signalling question	Rating	Reasoning
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably no	There was considerable heterogeneity among the included studies. Random effects models were used to calculate pooled mean difference for each outcome, but no details were given on how the heterogeneity were addressed or impacted this analysis.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	Publication bias was investigated using funnel plots for some of the outcomes (including triglycerides), but not for LDL.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably yes	Studies with high risk of bias according to the Jadad score were excluded from the analysis.
Concerns regarding methods used to synthesize results	High	The synthesis probably did not include all studies that it should due to exclusion of studies not reporting mean outcome values. Publication bias were not described for all outcomes, and considerable heterogeneity was found.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	Eligibility criteria seemed appropriate to answer the objectives, but were not pre-defined or described in detail.
Concerns regarding methods used to identify and/or select studies	High	The full search strategy was not reported. Only one investigator screened titles and abstracts.
Concerns regarding methods used to collect data and appraise studies	Unclear	Bias due to allocation concealment was not evaluated, and it was unclear how study sample was handled.
Concerns regarding the synthesis and findings	High	The synthesis probably do not include all studies that it should due to exclusion of studies not reporting mean outcome values. Publication bias were not described, and considerable heterogeneity was found.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	One or more of the concerns raised during the Phase 2 assessment was not addressed in the review conclusions.
B. Was the relevance of identified studies to the review's research question appropriately considered	No	Relevant studies were included according to the eligibility criteria. However, the relevance or limitations of included studies, and the impact of excluded studies were not discussed.

Signalling question	Rating	Reasoning
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All studies with available outcome values were included in the review.
Risk of bias	High	Some parts of the review did not include methods approved for systematic review, resulting in a high risk of bias of the review.

AbuMweis et al. (2021)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria were appropriate.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably no	Some criteria were not described in detail. No specific information was provided on which criteria that were used to determine whether trials included outcomes of interest.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	The restriction on language were considered not to be a major issue. The restrictions based on reporting of outcome (incomplete data) were clearly described and appeared to be appropriate.
Concerns regarding specification of study eligibility criteria	Unclear	Concerns regarding the lack of information on whether the objectives and eligibility criteria were predefined and restrictions in the eligibility criteria were identified.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in electronic databases. Search for unpublished reports were not performed.

Signalling question	Rating	Reasoning
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Bibliographies of review articles were hand-searched.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	The search terms used in the search and the search strings used were reported. The full search strategy or the combination of the terms, were not reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably yes	The search was restricted to human studies, and this restriction seemed appropriate.
2.5 Were efforts made to minimise errors in selection of studies?	No	One person screened the titles and abstracts.
Concerns regarding methods used to identify and/or select studies	High	Concerns regarding the lack of efforts to minimise errors in selection of studies and the lack of information on the search strategy were identified.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	One reviewer checked the extracted data for accuracy, two other investigators controlled the data extraction.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected. Many studies from the initial literature search did not include data on the EPA:DHA ratio and were not included, but this exclusion is not likely to have introduced bias.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	The Jadad score method was used to evaluate the methodological quality. Allocation concealment was not included in the assessment.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	RoB was assessed independently by two reviewers.
Concerns regarding methods used to collect data and appraise studies	Unclear	Risk of bias from allocation concealment was not evaluated.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Relevant data for assessment of possible adverse effects (lipoproteins and inflammation markers) were included.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably no	Heterogeneity for studies included in this study was not addressed.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	There was no funnel plot or sensitivity analyses.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	Studies with a Jadad score below 3 were not included in the meta-analyses.
Concerns regarding methods used to synthesize results	Unclear	There is insufficient information reported to make a judgement on risk of bias.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	Concerns regarding the lack of information on whether the objectives and eligibility criteria were predefined and restrictions in the eligibility criteria were identified.
Concerns regarding methods used to identify and/or select studies	High	Concerns regarding the lack of efforts to minimise errors in selection of studies and the lack of information on the search strategy were identified.
Concerns regarding methods used to collect data and appraise studies	Unclear	Risk of bias from allocation concealment was not evaluated.
Concerns regarding the synthesis and findings	Unclear	There is insufficient information reported to make a judgement on risk of bias.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	One or more of the concerns raised during the Phase 2 assessment was not addressed in the review conclusions.
B. Was the relevance of identified studies to the review's research question appropriately considered	No	Relevant studies were included according to the eligibility criteria. However, the relevance or limitations of included studies, and the impact of excluded studies, were not discussed.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All studies with available outcome values were included in the review.
Risk of bias	High	The phase 2 assessment identified a number of areas of concern with the review process which were not addressed by the authors. Concerns regarding the lack of efforts to minimise errors in selection of studies and the lack of information on the search strategy were identified.

AlAmmar et al. (2019)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	Restrictions on intervention and population seemed appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	The restriction on language were considered not to be a major issue.

Signalling question	Rating	Reasoning
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Searches were performed in PubMed, Oxford, Cochrane, Embase, International pharmaceutical abstract, PsychINFO, and clinical trials government.
2.2 Were methods additional to database searching used to identify relevant reports?	No information	
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	Key words and specific terms used were reported. The full search strategy, or the combination of the search terms, were not reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably no	Filters for study design and type (randomized controlled trial, cohort study, follow-up study, and controlled clinical trial) were used and seemed appropriate. The search was restricted to cover literature from 2009 up to 2018.
2.5 Were efforts made to minimise errors in selection of studies?	No information	
Concerns regarding methods used to identify and/or select studies	High	The full search strategy was not available. Information on searches additional to database searching and efforts made to minimise errors in full text selection were lacking.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.

Signalling question	Rating	Reasoning
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	Cochrane collaboration's tool for assessing risk of bias in RCTs were used.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	High	There was no information on efforts made to minimise error in data collection or RoB evaluation.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	There was no report of exclusion of relevant data.
4.2 Were all predefined analyses followed or departures explained?	Probably no	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably no	No effect sizes were reported, only p-values for each study. Direction of association was reported for studies with low p-values only.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	No information	
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No information	
Concerns regarding methods used to synthesize results	High	The synthesis is likely to produce biased results, because findings are incompletely reported in a way that raises concern. No information was available about between-studies variation, robustness of findings, or biases in primary studies.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined.

Domain	Concern	Rationale for concern
Concerns regarding methods used to identify and/or select studies	High	The full search strategy was not available. Information on searches additional to database searching and efforts made to minimise errors in full text selection were lacking.
Concerns regarding methods used to collect data and appraise studies	High	There was no information on efforts made to minimise error in data collection or RoB evaluation.
Concerns regarding the synthesis and findings	High	The synthesis is likely to produce biased results, because findings are incompletely reported.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several of the concerns raised during the Phase 2 assessment was not addressed in the review conclusions.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	The scarcity of data and limitations of included studies were discussed.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	No	Extraction of results were based on p-values.
Risk of bias	High	One or more of the concerns raised during the Phase 2 assessment was not addressed in the review conclusions.

Azzi et al. (2018)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Probably yes	No study protocol was published. Objective and eligibility criteria were described and was reported to be predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	No	Exclusion criteria were described in a flowchart, but the numbers of excluded articles (1357) did not match the number of articles listed with a reason for exclusion (1346). Also, the criterion "related to periodontal disease" were not described in detail.

Signalling question	Rating	Reasoning
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	The restrictions based on population (humans with periodontal disease) were clearly described and appeared to be appropriate. The restrictions based on types of study design (no animal studies or literature reviews) were described. In addition, 134 of 1357 hits were excluded as "non-articles", but this criterion was not specified.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in electronic databases. Search for unpublished reports were not performed.
2.2 Were methods additional to database searching used to identify relevant reports?	No	No additional searches were performed.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably yes	The search terms were reported. The full search strategy was not reported, however how the search words were combined was reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No restrictions were applied in the search.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two persons selected the studies independently.
Concerns regarding methods used to identify and/or select studies	High	Searches was limited to published articles in databases and no additional searching was done. Some eligible studies are likely to be missing from the review.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Probably no	Effect sizes were not quantified.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably yes	The quality criteria adopted were the following: randomization, blind assessment, anamnesis questionnaire, separation by gender, oral supplementation of n-3, calibration of examiners, use of a diet questionnaire, measure of n-3 plasma concentrations, and longitudinal periodontal probing (before and after n-3 treatment).
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	High	Concerns regarding lack of information on efforts to minimise error in data synthesis, the collection of study results, and the lack of information on efforts made to minimise error in risk of bias assessment were identified.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably yes	Results were reported from all studies included. However, some studies were excluded with no reported reason. Availability of data in excluded studies was not reported.
4.2 Were all predefined analyses followed or departures explained?	Probably no	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably yes	A narrative synthesis was appropriate given the low number of included studies, but effect sizes were not quantified.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably yes	Heterogeneity was not reported, however, data were not combined.

Signalling question	Rating	Reasoning
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably no	Only 3 studies were included with supplements as intervention. Main effects were not significant. No funnel plot or sensitivity analysis was performed.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably no	Quality evaluation score was provided in a table (scores 10-30), and all studies were scored <20.
Concerns regarding methods used to synthesize results	Unclear	Concerns regarding lack of information on predefinition of the analyses, the robustness of the data, and the addressing of biases in primary studies in the synthesis were identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	High	Searches was limited to published articles in databases and no additional searching was done. Some eligible studies are likely to be missing from the review.
Concerns regarding methods used to collect data and appraise studies	High	Concerns regarding lack of information on efforts to minimise error in data synthesis, the collection of study results, and the lack of information on efforts made to minimise error in risk of bias assessment were identified.
Concerns regarding the synthesis and findings	Unclear	Concerns regarding lack of information on predefinition of the analyses, the robustness of the data, and the addressing of biases in primary studies in the synthesis were identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns were identified and not addressed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	No	The relevance or limitations of included studies, and the impact of excluded studies were not discussed.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	

Signalling question	Rating	Reasoning
Risk of bias	High	Several of the concerns raised during the Phase 2 assessment was not addressed in the review conclusions.

Balk et al. (2016)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Yes	A protocol was prepared and registered in PROSPERO (PROSPERO registry number CRD42014015602).
1.2 Were the eligibility criteria appropriate for the review question?	Yes	9 RCTs ² observational studies
1.3 Were eligibility criteria unambiguous?	No	Properly described criteria addressing study type, population, exposure and outcome. However, the criteria for restriction of the total literature were insufficiently described.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	No	There were restrictions on sample size. It was stated this was due to the need to limit the number of included studies. The criteria for sample size varied for the different outcomes and this is not probably justified.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	Restrictions on language and peer-review were applied.
Concerns regarding specification of study eligibility criteria	High	Concerns regarding eligibility criteria were identified.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Searches were performed in several electronic databases.
2.2 Were methods additional to database	Yes	All studies from the original reviews that met current eligibility criteria were rescreened. Reference lists of

Signalling question	Rating	Reasoning
searching used to identify relevant reports?		related systematic reviews were reviewed for other potentially eligible studies.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	The search term included some key words for adverse event, but some were lacking, e.g. adverse events or side effects such as gastrointestinal disturbances was not included.
2.4 Were restrictions based on date, publication format, or language appropriate?	No	Restrictions on language (only English language), format and study type were applied to the search strategy without any justification.
2.5 Were efforts made to minimise errors in selection of studies?	Probably no	<p>It is stated that all citations found by literature searches or through other sources were independently screened by two researchers. Upon the start of citation screening, a training session were implemented.</p> <p>All potentially eligible abstracts were entered into an "evidence map", and a single researcher extracted data on the study sample size, study design, study duration, the population category, the specific n-3 FA analysed, whether biomarkers were reported, whether subgroup or factorial analyses were reported, and the outcomes mentioned in the abstract. Based on the study descriptions in the evidence map, only the largest studies and those with subgroup or factorial analyses were selected for full text review. It is not stated that this extraction, which was the basis of which publications that should be reviewed in full-text was checked by another reviewer.</p>
Concerns regarding methods used to identify and/or select studies	High	Some eligible studies are likely to be missing from the review as only the largest studies and those with subgroup or factorial analyses were selected for full text review.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	One methodologist extracted data, another methodologist double-checked for errors.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Detailed information from the included studies were presented.
3.3 Were all relevant study results collected for use in the synthesis?	No information	This information is not described in the Methods section.

Signalling question	Rating	Reasoning
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	The Cochrane risk of RoB tool was used for RCTs, which includes evaluation of selection bias, performance bias, detection bias, attrition bias, reporting bias, and other potential biases. The Newcastle Ottawa Scale was used for observational studies. Nutrition study specific RoB questions were also included.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	High	Concerns regarding collection of relevant study results and efforts made to minimize error in risk of bias assessment were identified.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	No	Outcomes with too small sample size were extracted if another outcome fulfilled the criteria.
4.2 Were all predefined analyses followed or departures explained?	Probably yes	Deviations from the protocol was not reported.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate and well described.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably yes	Data synthesis for adverse events was narrative.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No information	
Concerns regarding methods used to synthesize results	High	Concerns were identified regarding inclusion of studies in the synthesis, the robustness of the findings, and the inclusion of biases in primary studies in the synthesis.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	High	There is insufficient information reported to make a judgement on risk of bias.
Concerns regarding methods used to identify and/or select studies	High	Some eligible studies are likely to be missing from the review as only the largest studies and those with subgroup or factorial analyses were selected for full text review.
Concerns regarding methods used to collect data and appraise studies	High	Some bias may have been introduced through the RoB assessment processes.
Concerns regarding the synthesis and findings	High	There is insufficient information reported to make a judgement on risk of bias.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	It is likely that not all relevant studies were included.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	Relevant studies were included according to the eligibility criteria and relevance is part of the GRADE assessment
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	Both studies that reported adverse events and no adverse events related to n-3 PUFAs were included in the review. All adverse events were included, and these results were presented narratively.
Risk of bias	High	High concern for all four domains included in the identification of concerns with the review process.

Becic and Studenik (2018)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population and intervention.

Signalling question	Rating	Reasoning
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Restrictions based on study design (RCTs) seemed appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Searches were performed in several electronic databases.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Retrieved articles, systematic reviews and meta-analyses were searched manually.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	The keywords used in the search was reported. No information on the combination of the key words or the full search strategy was available.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No such restrictions were applied.
2.5 Were efforts made to minimise errors in selection of studies?	No information	
Concerns regarding methods used to identify and/or select studies	High	Information on efforts made to minimise errors in study selection were lacking. The search strategy was not available.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Two authors independently extracted the data.

Signalling question	Rating	Reasoning
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	Cochrane Collaboration's tool for assessing the risk of bias was applied.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	Unclear	Concerns regarding lack of information on concerns regarding efforts made to minimise error in risk of bias assessment were identified.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Relevant data for assessment of possible adverse effects were included.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Heterogeneity was addressed, and as the heterogeneity was low. Meta-analyses were performed.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably yes	Funnel plots for adiponectin, leptin, IL-6, and TNF- α revealed a moderate asymmetry in all of the outcomes.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No information	
Concerns regarding methods used to synthesize results	High	Concerns regarding lack of information on predefinition of the analyses and on how biases were assessed in the synthesis were identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes".
Concerns regarding methods used to identify and/or select studies	High	Information on efforts made to minimise errors in study selection were lacking. The search strategy was not available.
Concerns regarding methods used to collect data and appraise studies	Unclear	Concerns regarding lack of information on concerns regarding efforts made to minimise error in risk of bias assessment were identified.
Concerns regarding the synthesis and findings	High	Concerns regarding lack of information on predefinition of the analyses and on how biases were assessed in the synthesis were identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns were identified and not addressed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	No	
Risk of bias	High	High and unclear concern for all four domains included in the identification of concerns with the review process.

Bernstein et al. (2012)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.

Signalling question	Rating	Reasoning
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Studies of animals and children and of persons with diseases that may affect absorption or metabolism of algal oil were excluded. These restrictions seemed appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably Yes	No language restrictions were imposed. There was some restriction; studies of children were excluded, and a clear justification was not provided.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Searches were performed in several electronic databases, and searches for unpublished reports were performed.
2.2 Were methods additional to database searching used to identify relevant reports?	Probably yes	Bibliographies of articles by elected authors were screened.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	The terms and structure of the search strategy were likely to retrieve as many eligible studies as possible.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably No	The search included no such restrictions. There were language restrictions in the search terms (online Supporting Material).
2.5 Were efforts made to minimise errors in selection of studies?	No information	
Concerns regarding methods used to identify and/or select studies	Unclear	Information on efforts made to minimise errors in full text selection were lacking. Language restrictions in the search.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	

Signalling question	Rating	Reasoning
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably yes	The quality of each study, defined as the certainty that its design, conduct, analysis, and presentation limited bias in its results, was reported to be assessed by a validated quality scoring system.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	High	Concerns were identified with regard to lacking information on methods used to minimise errors in data collection and risk of bias assessment.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Relevant data for assessment of possible adverse effects were included.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably Yes	The synthesis seemed appropriate. For meta-analysis, a fixed-effects model was used and study weights were determined by the inverse variance method.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Yes	The funnel plots were symmetrical.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No information	

Signalling question	Rating	Reasoning
Concerns regarding methods used to synthesize results	High	Concerns regarding lack of information on predefinition of the analyses and how the biases in primary studies were addressed in the synthesis were identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	Unclear	Information on efforts made to minimise errors in full text selection were lacking. Language restrictions in the search.
Concerns regarding methods used to collect data and appraise studies	High	Concerns were identified with regard to lacking information on methods used to minimise errors in data collection and risk of bias assessment.
Concerns regarding the synthesis and findings	High	Concerns regarding lack of information on predefinition of the analyses and how the biases in primary studies were addressed in the synthesis were identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns were identified and not addressed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	
Risk of bias	High	High and unclear concern for all four domains included in the identification of concerns with the review process.

Casula et al. (2020)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Restrictions based on study characteristics seemed appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	Restrictions based on sources of information seemed appropriate.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in several electronic databases. No search for unpublished studies.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Previously published reviews were examined for additional relevant studies.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	The terms and structure of the search strategy were likely to retrieve as many eligible studies as possible.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably no	Language restriction.
2.5 Were efforts made to minimise errors in selection of studies?	Probably Yes	All selected articles were screened by two reviewers, and minor differences resolved by discussion and consultation with a third reviewer. However, it was not stated explicitly whether the reviewers assessed independently.

Signalling question	Rating	Reasoning
Concerns regarding methods used to identify and/or select studies	Unclear	One signalling question was answered as "Probably No", so there is some potential concerns about identification and selection of studies.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	There was no information how the data extraction was performed.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Probably yes	The data for bleeding were described in the text. For CRP, no quantitative data were included.
3.3 Were all relevant study results collected for use in the synthesis?	Probably no	No all relevant results from the included studies were collected. No useful data on bleeding or CRP, only a text in the discussion.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	Quality assessment of the included RCTs was evaluated using the Jadad scale. Allocation concealment was not included.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	There was no information how the quality of included study was assessed.
Concerns regarding methods used to collect data and appraise studies	High	Two signalling questions were answered as "No information" and another two signalling questions were answered as "Probably No", so there is potential concerns about data collection and study appraisal.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably no	A study characteristic table with the included 16 studies was provided. No useful or relevant data were collected to perform synthesis.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably No	No useful or relevant data were collected to perform synthesis. Only a text in the discussion was provided about bleeding and CRP.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	No information	A heterogeneity (between-studies variation) was assessed, however, no useful data were collected.

Signalling question	Rating	Reasoning
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	The studies were evaluated for quality using Jaded scale. No GRADE and no synthesis of useful or relevant data.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No	No synthesis of relevant data.
Concerns regarding methods used to synthesize results	High	All signalling questions were answered as "No information" or "Probably No", so there is potential concerns about synthesis and findings.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	Most of signalling questions were answered as "YES" or "Probably Yes", however, no information on pre-defined protocol about objectives and eligibility criteria.
Concerns regarding methods used to identify and/or select studies	Unclear	One signalling question was answered as "Probably No", so there is some potential concerns about identification and selection of studies.
Concerns regarding methods used to collect data and appraise studies	High	Two signalling questions were answered as "No information" and another two signalling questions were answered as "Probably No", so there is potential concerns about data collection and study appraisal.
Concerns regarding the synthesis and findings	High	All signalling questions were answered as "No information" or "Probably No", so there is potential concerns about synthesis and findings.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	There were some concerns identified during the phase 2 assessment.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably yes	No useful or relevant data were collected to perform synthesis. Only a text in the discussion was provided about bleeding and CRP.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Probably Yes	The review reflects both the statistically significant and non-significant review findings.
Risk of bias	High	The phase 2 assessment identified concerns with the review process.

Chang et al. (2018)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	The objectives and eligibility criteria were specified in the background and Methods. The study followed the PRISMA guidelines. However, there was no evidence of pre-specification of objectives and eligibility criteria.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria seemed appropriate to the review question. The details of studies eligible for inclusion provided in the article and appeared appropriate to the review question.
1.3 Were eligibility criteria unambiguous?	Yes	The eligibility criteria were sufficient to answer the review question.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Restrictions were provided in the Methods section and seemed to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably Yes	The only restrictions were that only marketed PFUA were included. No observational studies were included and no justification for this restriction was provided.
Concerns regarding specification of study eligibility criteria	Unclear	Most of signalling questions were answered as "Yes" or "Probably Yes", however, no information on pre-defined protocol about objectives and eligibility criteria. Therefore, there are potential concerns about the specification of eligibility criteria were identified.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably Yes	Search were performed in PubMed, EMBASE, ProQuest, ScienceDirect, Cochrane Central Register of Controlled Trials, and ClinicalTrials.gov. Material published as conference reports were not searched for.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	A manual search for references found in relevant articles and package inserts were performed.

Signalling question	Rating	Reasoning
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably Yes	A search strategy was provided in the Methods section, however; an appendix displaying the complete search strategy was not provided.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	The search included no such restrictions.
2.5 Were efforts made to minimise errors in selection of studies?	Probably Yes	The titles and abstracts were screened by two reviewers and disagreements were solved through consensus. However, it was not stated explicitly whether the reviewers assessed independently.
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered as "Yes" or "Probably Yes", so no potential concerns about the identification and selection of studies were identified.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Data extraction was performed independently by two reviewers using a predetermined data extraction form. Disagreements were resolved by discussion among the team, with the team leader, or between extractors.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	A study characteristic table of the 21 included articles was presented.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant results from the included studies were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	The quality of each study was assessed using the Jadad scale. Allocation concealment was not evaluated.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	There were no details on risk of bias assessment.
Concerns regarding methods used to collect data and appraise studies	High	Some bias may have been introduced through the data collection or risk of bias assessment processes.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should	Yes	Flow chart reported the included 21 studies. A study characteristic table with the included 21 studies was provided. Four studies used EPA-only products and the other 17 studies involved EPA/DHA combinations.
4.2 Were all predefined analyses followed or departures explained?	Yes	Analysis stated in the Methods were performed.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seems appropriate and well described. A random-effects model was used for meta-analysis.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Statistical heterogeneity test was performed Q statistics and the corresponding p-values. Meta-regression and subgroup analyses were performed to explore the sources of potential heterogeneity.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably Yes	Funnel plot was used to investigate publication bias, and sensitivity analysis was conducted. No information about the assessment of the robustness. No GRADE.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably No	The level of publication bias was adjusted for in the meta-analysis. Other types of bias were not included.
Concerns regarding methods used to synthesize results	Unclear	Two signalling questions were answered as "Probably No". There are concerns regarding the methods used/ not used to synthesize results.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	Most of signalling questions were answered as "YES" or "Probably Yes", however, no information on pre-defined protocol about objectives and eligibility criteria.
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered as "YES" or "Probably Yes", so no potential concerns
Concerns regarding methods used to collect data and appraise studies	High	Some bias may have been introduced through the data collection or risk of bias assessment processes.
Concerns regarding the synthesis and findings	Unclear	Some signalling questions were answered as "Probably No", so there are potential concerns.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	Probably yes	There were no concerns identified during the phase 2 assessment, except assessment of risk of bias. There were no details on risk of bias assessment.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	Relevant studies were included and shown in the synthesis of the results from the included studies.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	The review conclusions reflect both the statistically significant and non-significant review findings.
Risk of bias	High	The phase 2 assessment identified concerns with the review process which are likely to have introduced bias.

Chen et al. (2015)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Restrictions based on trial length and available data seemed appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	The restriction on language were considered not to be a major issue.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Several electronic databases were searched for published and unpublished studies.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	The bibliographic sections of all publications of included and excluded trials were searched for additional trials.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	The terms and structure of the search strategy were likely to retrieve as many eligible studies as possible.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No such restrictions were applied in the search.
2.5 Were efforts made to minimise errors in selection of studies?	No information	Two separate investigators reviewed the titles, abstracts and keywords to determine the relevance of studies. No information about the full text selection.
Concerns regarding methods used to identify and/or select studies	Unclear	Most of signalling questions were answered as "Yes", however, no information on bias on full text assessment.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	All data were extracted independently from the studies by two investigators.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	The Jadad score system was applied. Allocation concealment was not included in the assessment.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two investigators assessed quality scores of studies independently.
Concerns regarding methods used to collect data and appraise studies	Unclear	One signalling question was answered as "Probably No", so there is some concerns about data collection and study appraisal.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably No	There is a discrepancy between the 5 included studies presented in the flow chart reported and the study characteristic table with the included 20 studies presented in an online Supporting Information and Result section.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Statistical heterogeneity was assessed using Chi ² test and the I ² statistic.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Yes	The combined results for clinical outcomes were stable though the specified variables changed in sensitivity analyses.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	
Concerns regarding methods used to synthesize results	High	One signalling question was answered as "Probably No", and another question was answered as "No information", so there is some concerns about data collection and study appraisal.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	Unclear	Most of signalling questions were answered as "YES", however, no information on bias on full text assessment.
Concerns regarding methods used to collect data and appraise studies	Unclear	One signalling question was answered as "Probably No", so there is some concerns about data collection and study appraisal.
Concerns regarding the synthesis and findings	High	One signalling question was answered as "Probably No", and another question was answered as "No information", so there is some concerns about data collection and study appraisal.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	Probably no	Concerns from the phase 2 assessment were not addressed in the interpretation of findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably Yes	Relevant studies were included and shown in the synthesis of the results from the included studies.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	The review conclusions reflect both the statistically significant and non-significant review findings.
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Chua et al. (2013)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Restrictions on study design and publication type seemed appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Several electronic databases were searched for published and unpublished studies. In addition, inquiry from industry/nutrition experts was done to obtain unpublished data.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference lists of studies that met the inclusion criteria and review articles or textbooks of related topics were searched for potentially relevant studies.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably yes	The search terms and the combination of the terms were reported and seemed appropriate.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No such restrictions were applied in the search.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two reviewers performed the study selection independently.
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered "Yes" or "Probably yes"

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Probably Yes	One reviewer tabulated data from each study, which was counterchecked by another reviewer. However, it was not stated explicitly whether the reviewers assessed independently. There was no information pre-defined data extraction protocol.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	RoB was scored according to the recommendation of the National Health Service, UK, on critical appraisal and evaluation of descriptive (Cohort) studies. Each included study was then graded by the same reviewers using Newcastle-Ottawa Quality Assessment Scale from Cochrane Collaboration for cohort studies.

Signalling question	Rating	Reasoning
3.5 Were efforts made to minimise error in risk of bias assessment?	Probably Yes	The same reviewers using (NOQAS) independently graded each included study. If any discrepancy of the rating was found, reviewers discussed the differences until both reviewers reached a mutual agreement of the score.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions answered "Yes" or "Probably yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Relevant data for assessment of possible adverse effects were included.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Heterogeneity between studies were assessed and addressed.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Yes	The findings were robust as demonstrated through subgroup analyses.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No information	
Concerns regarding methods used to synthesize results	Unclear	Two signalling questions were answered as "NO information".

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered as "YES" or "Probably Yes", so no potential concerns

Domain	Concern	Rationale for concern
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered as "YES" or "Probably Yes", so no potential concerns
Concerns regarding the synthesis and findings	Unclear	Two signalling questions were answered as "NO information".

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Concerns from the phase 2 assessment were not addressed in the interpretation of findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	Relevant studies were included and shown in the synthesis of the results from the included studies.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	The review reflects both the statistically significant and non-significant review findings.
Risk of bias	Unclear	The phase 2 assessment identified some concerns with the review process that were not addressed.

Craddock et al. (2017)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Probably yes	A protocol for the review was registered a priori. Potential discrepancies from the protocol are not described.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions based on publication type, population and intervention were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	The restriction was considered not to be a major issue.

Signalling question	Rating	Reasoning
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Three electronic databases were used for the search. Search for unpublished reports were not performed.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference lists of all included publications were screened.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably yes	The search terms and combination of the terms were reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably yes	No such restrictions to the search were reported, however, the full search strategy was not reported.
2.5 Were efforts made to minimise errors in selection of studies?	No information	
Concerns regarding methods used to identify and/or select studies	Unclear	Concerns regarding lack of information on efforts made to minimise errors in selection of studies were identified.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	No information	The type of study results that were extracted is not reported in the Method section and it is not possible to decipher this from the Result section.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	The quality criteria checklist of the Evidence Analysis Library of the Academy of Nutrition and Dietetics were used. This assessment tool does not include allocation concealment.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	

Signalling question	Rating	Reasoning
Concerns regarding methods used to collect data and appraise studies	High	Concerns were identified with regard to lacking information on methods used to minimise errors in data collection and risk of bias assessment.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Relevant data for assessment of possible adverse effects were included.
4.2 Were all predefined analyses followed or departures explained?	Yes	No deviations from the protocol were reported.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	A narrative synthesis was conducted, which seems appropriate considering differences in doses, study duration and few studies assessing each outcome.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Heterogeneity was addressed by not combining data.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably no	It is stated that all included studies reported increases for the different blood parameters. However, only one study assessed effects of DHA on LDL-cholesterol, and therefore this finding cannot be considered robust.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably no	It is stated that all included studies received positive scores on the study quality. However, the assessment of study quality had shortcomings. In addition, publication bias was not assessed.
Concerns regarding methods used to synthesize results	High	Only one study assessed LDL-cholesterol and biases were not addressed in the synthesis.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	Unclear	Concerns regarding lack of information on efforts made to minimise errors in selection of studies were identified.

Domain	Concern	Rationale for concern
Concerns regarding methods used to collect data and appraise studies	High	Concerns were identified with regard to lacking information on methods used to minimise errors in data collection and risk of bias assessment.
Concerns regarding the synthesis and findings	High	Only one study assessed LDL-cholesterol and biases were not addressed in the synthesis.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Concerns from the phase 2 assessment were not addressed in the interpretation of findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	No	The relevance of included studies was not discussed.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Downie et al. (2019)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Yes	A protocol was prepared. The objectives and eligibility criteria were specified clearly in the background and Methods.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	Eligibility criteria seemed appropriate to answer the review question.
1.3 Were eligibility criteria unambiguous?	Probably yes	Eligibility criteria were sufficiently to answer the review question.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions based on types of study design were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.

Signalling question	Rating	Reasoning
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered as "Yes", so no potential concerns about the specification of eligibility criteria were identified.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Several electronic databases were searched (Cochrane Central Register of Controlled Trials, MEDLINE, Embase, PubMed, LILACS (Latin American and Caribbean Health Sciences Literature Database), metaRegister of Controlled Trials (mRCT), ClinicalTrials.gov and World Health Organization (WHO)-International Clinical Trials Registry Platform ICTRP).
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference lists of included studies were searched to identify other potentially relevant studies.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably yes	Search terms for participants and intervention and RCTs were applied. No specific terms for outcome (adverse effects) were included.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	The search included no such restrictions.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two reviewers performed both selection steps independently, and consensus were reached by discussion.
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered as "Yes" or "Probably Yes", so no potential concerns about the identification and selection of studies were identified.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Two reviewers independently extracted data using forms developed by Cochrane Eyes and Vision.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Detailed information from the included studies were presented. The data were presented in both overview tables and in tables with more details regarding the outcome of the studies.

Signalling question	Rating	Reasoning
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant results from the included studies were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	RoB was evaluated according to the Cochrane guidance, and included selection bias (sequence generation and allocation concealment), performance and detection bias (masking of participants, study personnel, and outcome assessors), attrition bias (incomplete outcome data), reporting bias (selective outcome reporting), and other sources of bias.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two persons independently evaluated the RoB. Discrepancies were resolved through discussion between the two reviewers' authors and a third reviewer author, when necessary.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered as "Yes", so no potential concerns about data collection and study appraisal were identified.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	For adverse effects, all relevant data from the included studies were extracted and presented in a summary table.
4.2 Were all predefined analyses followed or departures explained?	Yes	Departures from the protocol were identified and explained.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate and well described. Data were presented narratively due to substantial heterogeneity.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Clinical and methodological heterogeneity was assessed using the I^2 statistic. $I^2 > 60\%$ was interpreted as indicative of substantial statistical heterogeneity.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably yes	A sensitivity analysis was not performed, as no individual meta-analyses included a sufficient number of studies. However, selective outcome reporting was assessed as part of the risk of bias assessment for each included study.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	Bias was addressed in the evaluation of the certainty in evidence.
Concerns regarding methods used to synthesize results	Low	All signaling questions answered yes or probably yes

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered as "Yes" or "Probably Yes".
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered as "Yes" or "Probably Yes".
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered as "Yes".
Concerns regarding the synthesis and findings	Low	All signalling questions were answered as "Yes" or "Probably Yes".

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	Yes	There were no concerns identified during the phase 2 assessment.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	Relevant studies were included and shown in the synthesis of the results from the included studies.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	The review conclusions reflect both the statistically significant and non-significant review findings.
Risk of bias	Low	The phase 2 assessment identified no concerns with the review process. The potential limitations of the systematic review was the evidence for gastrointestinal adverse effects was graded as low certainty evidence and only three studies was included. These three studies had relatively small sample sizes with substantial heterogeneity between the studies.

Fogacci et al. (2020)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There is no mention of a protocol or whether the objectives and eligibility criteria were predefined.

Signalling question	Rating	Reasoning
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed criteria addressing study type, population, exposure and outcome.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions based on study design and information required about plasma lipids at baseline or follow-up were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Several electronic databases were searched. No search for unpublished reports.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference lists of identified papers, references of review articles on the issue, and abstracts from relevant congresses were checked for additional relevant studies.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably Yes	The search included terms for study design, intervention, outcome and population, and no inappropriate restrictions were included. Search terms were adapted for each database. The full search strategy was not reported, however how the search words were combined was reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	The search included no restrictions on language or date.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	All paper abstracts were screened by two reviewers in an initial process. The remaining articles were obtained in full-text and assessed again by the same two reviewers.

Signalling question	Rating	Reasoning
Concerns regarding methods used to identify and/or select studies	Low	All signaling questions were answered "Yes" or "Probably yes".

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Data extraction was performed by two persons. An additional person double-checked for errors.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Detailed information from the included studies were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant results on adverse effects were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	A systematic assessment of the risk of bias in the included studies was performed using the Cochrane criteria addressing the following: adequacy of sequence generation, allocation concealment, blinding, dropouts/incomplete outcome data, selective outcome reporting and other probable sources of bias.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two reviewers performed the risk-of-bias assessment independently.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered "Yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably no	Only adverse events that occurred in at least two clinical trials were included and studies with zero events in both arms were excluded. Analysis indicated publication bias for some of the outcomes, however, this was adjusted for (see 4.6).
4.2 Were all predefined analyses followed or departures explained?	Probably no	There is no mention of a protocol or whether the data analysis was predefined.

Signalling question	Rating	Reasoning
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	Meta-analysis with random-effect model and fixed-effect model were performed. Overall, the synthesis seemed appropriate. However, for the outcome "LDL-cholesterol", there was high heterogeneity, and a qualitative synthesis would be more appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably Yes	I ² statistic were performed. For the outcome LDL-cholesterol, there was high heterogeneity (I ² =>60%). For the other outcomes there was low to moderate heterogeneity.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Yes	Sensitivity analysis using the leave-one-out method were performed and it is stated that the findings on adverse effects were consistent across studies and robust.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably no	Potential publication biases were explored using visual inspection of Begg's funnel plot asymmetry, Begg's rank correlation test, and Egger's weighted regression test. The Duval and Tweedie "trim and fill" method was used to adjust the analysis for the effects of publication biases. Risk of bias was not addressed in the synthesis.
Concerns regarding methods used to synthesize results	High	The methods used in the synthesis of the results had several shortcomings. Studies with zero events in both arms were excluded, however, if an outcome was assessed in a study and there were no cases, this would also be an important finding. It is not possible to know whether the methods for synthesis were predefined or not. In addition, risk of bias was not addressed in the synthesis.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes".
Concerns regarding methods used to identify and/or select studies	Low	All signaling questions were answered "Yes" or "Probably yes".

Domain	Concern	Rationale for concern
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered "Yes".
Concerns regarding the synthesis and findings	High	The methods used in the synthesis of the results had several shortcomings. Studies with zero events in both arms were excluded, however, if an outcome was assessed in a study and there were no cases, this would also be an important finding. It is not possible to know whether the methods for synthesis were predefined or not. In addition, risk of bias was not addressed in the synthesis.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	No, the concerns identified in the phase 2 evaluation were not addressed in the interpretation.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably Yes	The review did not consider the relevance of the included studies to the review question. However, based on the information given in the publication, it is likely that the identified studies are relevant for the review question.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results are equally represented.
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Fu et al. (2015)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There is no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	Eligibility criteria were clearly described and seemed appropriate.

Signalling question	Rating	Reasoning
1.3 Were eligibility criteria unambiguous?	Yes	Inclusion criteria were sufficiently detailed and included study design (prospective cohort, nested case-control, and case-cohort studies); intervention (dietary n-3 PUFAs or blood n-3 PUFAs concentrations); outcome (incidence of prostate cancer), and reporting of risk estimate (relative risk, odd ratio, or hazard ratio) with corresponding 95% confidence intervals for individual n-3 PUFA exposure.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	Retrospective or cross-sectional studies, animal or cell culture studies, reviews, editorials, and commentaries were all excluded from the present study. These restrictions seemed appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Probably Yes" or "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in two electronic databases. Search for unpublished reports were not performed.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference lists of relevant studies were screened to identify additional studies.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	Some search terms for the intervention and the outcome were applied, and the full search strategy was reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably no	Only articles in English were included.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two persons independently performed the study selection.
Concerns regarding methods used to identify and/or select studies	Unclear	The search was limited to publications in English. It is unclear to what extent this affect the results.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Two persons independently performed the data extraction.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Detailed data relating to participants (study population and region, age of participants, number of cases and non-cases), characteristics of study (first author's name, study design, follow-up period, method of n-3 PUFA measurement, adjusted confounding factors), and risk estimates with the g 95% CIs were extracted. Authors of included studies were contacted for detailed information when needed.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant results from the included studies were collected. When missing, data were obtained by contacting the authors.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	Study quality was assessed using the Newcastle-Ottawa criteria for evaluation of selection, comparability, and outcomes (for cohort studies) or exposures (for case-control studies).
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	It is not described whether one or two reviewers performed the quality assessment.
Concerns regarding methods used to collect data and appraise studies	Unclear	Data collection was well performed. However, for the study appraisal there is no information on whether two reviewers performed the assessment.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably yes	For studies evaluating the association between dietary n-3PUFAs and prostate cancer risk, dose-response analysis was only conducted for studies with exposure units reported as or transformable to g/day. Dose-response analysis was not performed for studies reporting units as % energy/day due to the limited number of studies.
4.2 Were all predefined analyses followed or departures explained?	No information	There is no information on whether the analyses were predefined or not.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.

Signalling question	Rating	Reasoning
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Study heterogeneity was assessed using I ² statistic. Subgroup analyses were conducted to examine sources of study heterogeneity.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Yes	Subgroup analyses were conducted to examine Funnel plot and sensitivity analysis using the leave-one-out method were performed. Sensitivity analysis for EPA og DHA intakes showed that the results were robust.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably Yes	Publication bias was assessed. The scores for the quality assessment was not considered in the synthesis. However, all studies were of moderate or high quality.
Concerns regarding methods used to synthesize results	Unclear	It is not possible to know whether the methods for synthesis were predefined or not. All other signalling questions were scored as "Probably Yes" or "Yes".

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Probably Yes" or "Yes".
Concerns regarding methods used to identify and/or select studies	Unclear	The search was limited to publications in English. It is unclear to what extent this affect the results.
Concerns regarding methods used to collect data and appraise studies	Unclear	Data collection was well performed. However, for the study appraisal there is no information on whether two reviewers performed the assessment.
Concerns regarding the synthesis and findings	Unclear	It is not possible to know whether the methods for synthesis were predefined or not. All other signalling questions were scored as "Probably Yes" or "Yes".

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	All the concerns were not addressed in the interpretation. However, some biases, such as restriction to only publications in English were addressed.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably Yes	The review did not consider the relevance of the included studies to the review question. However, based on the information given in the publication, it is likely that the identified studies are relevant for the review question.
C. Did the reviewers avoid emphasizing results based	Yes	All results are represented.

on their statistical significance?		
Risk of bias	Unclear	The review has shortcomings in all four domains, with the most severe being the lack of information on predefined eligibility criteria and for the synthesis of results. Only publications in English were included, which could possibly lead to publication bias. However, analysis did not identify any publication bias.

Goh et al. (2021)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Probably Yes	A protocol for the review was registered a priori. Potential discrepancies from the protocol are not described.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The selection criteria were chosen in accordance with the participants, interventions, comparisons, and outcomes (PICO) reporting structure.
1.3 Were eligibility criteria unambiguous?	Yes	The eligibility criteria were sufficiently detailed.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions based on types of study design were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably No	Studies for which data were unavailable were excluded. No other restrictions on sources of information were described.
Concerns regarding specification of study eligibility criteria	Unclear	Studies for which data were unavailable were excluded. It is not stated how many studies this applied to.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably Yes	Several major electronic databases were searched. There was no search for unpublished reports, however, unpublished results were provided from one study.
2.2 Were methods additional to database searching used to identify relevant reports?	No	

Signalling question	Rating	Reasoning
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	A limited number of search terms for intervention and population were applied. The full search strategy was not reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No such restrictions were applied.
2.5 Were efforts made to minimise errors in selection of studies?	No information	There was no information on how the selection process was performed.
Concerns regarding methods used to identify and/or select studies	High	The methods and information on how the identification and selection of studies were performed had several shortcomings, and therefore relevant publications could have been missed.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Two reviewers working independently extracted the data for all studies.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Detailed information from the included studies were included.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant results from the included studies were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	RoB was assessed by using the Cochrane Risk of Bias Assessment Tool. Sequence generation, allocation concealment, blinding of participants, personal, and outcome assessors, incomplete outcome data, selective outcome reporting, and other potential sources of bias, were appraised
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two reviewers working independently evaluated the RoB.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were rated "Yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	All studies were included.
4.2 Were all predefined analyses followed or departures explained?	Yes	No deviations from the protocol were reported.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably No	Adverse events was examined by calculating the risk ratios (RRs) for the discontinuation rate and adverse events in the included studies. Meta-analysis was performed for blood parameters. Fixed-effects models with inverse variance weighting were used to summarize the effects across studies and estimate the SMDs and their corresponding 95% confidence intervals (CIs) for continuous outcomes. Mantel-Haenszel fixed-effects models were used to analyze the pooled RRs and 95% CIs for binary outcomes. Two-sided <i>p</i> -values were calculated for each outcome. The heterogeneity for some of the blood parameters were high (>70%) and meta-analysis with a fixed-effect model is not appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably no	Heterogeneity was addressed in the synthesis for the blood parameters by sensitivity analysis. Only summary data are reported for the adverse events, and there is no information about consistency across studies.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably No	Multiple sensitivity analysis based on study quality, alternative statistical approach, study design, population, and sample size were conducted to examine the robustness of the study outcomes for some of the parameters. For the adverse outcomes, robustness was not assessed. In addition, there were only a few studies for each outcome (1-4 studies), indicating that the findings are not robust.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably no	Risk of bias was not addressed in the meta-analysis or on the synthesis of the different adverse events. However, the quality of evidence was appraised using the GRADE criteria for discontinuation due to adverse events (all combined).
Concerns regarding methods used to synthesize results	High	A fixed-effect model was applied without justification. Heterogeneity, robustness and biases were not sufficiently addressed.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	Studies for which data were unavailable were excluded. It is not stated how many studies this applied to.
Concerns regarding methods used to identify and/or select studies	High	The methods and information on how the identification and selection of studies were performed had several shortcomings, and therefore relevant publications could have been missed.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were rated "Yes".
Concerns regarding the synthesis and findings	High	A fixed-effect model was applied without justification. Heterogeneity, robustness and biases were not sufficiently addressed.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	All concerns were not addressed in the interpretation.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably yes	The review did not consider the relevance of the included studies to the review question. However, based on the information given in the publication, it is likely that the identified studies are relevant for the review question.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results are presented.
Risk of bias	High	There were several shortcomings in the identification and selection of studies, and this could have led to relevant studies being missed. There were also several shortcomings in the synthesis.

Guo et al. (2019)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.

Signalling question	Rating	Reasoning
1.3 Were eligibility criteria unambiguous?	Probably yes	Both RCT with parallel and crossover design was included, however, acceptable wash-out period for studies with crossover design was not described.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions based on study design, intervention and available data were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably no	Studies with insufficient data for quantitative analysis data were excluded.
Concerns regarding specification of study eligibility criteria	High	There is no information on whether the objectives and eligibility criteria were predefined. Studies with insufficient data were excluded. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably no	Searches were performed in two electronic databases. Search for unpublished reports were not performed.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	The reference lists of original studies, reviews and meta-analyses were scrutinized.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	A limited amount of search terms were applied. The full search strategy or the combination of the terms were not available.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably yes	No such restrictions were described, however, the full search strategy was not reported.
2.5 Were efforts made to minimise errors in selection of studies?	No information	
Concerns regarding methods used to identify and/or select studies	High	The methods for identification and selection of studies had several shortcomings.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Data extraction was independently conducted by two investigators.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.

Signalling question	Rating	Reasoning
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	The Jada score criteria were used, and allocation concealment was included.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	Unclear	There is no information on the process of the risk of bias assessment.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably no	Twelve studies were excluded due to insufficient data for quantitative analysis. Twenty studies were included in the analysis.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Heterogeneity for the outcome CRP was overall low. Heterogeneity was addressed by subgroup analyses.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably no	In a sensitivity analysis, the pooled estimates of EPA and DHA supplementation on SBP, DBP, CRP, IL-6 and TNF- α levels were not substantially driven with deleting one trial at a time. However, the forest plot shows that there are inconsistencies across studies.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably no	Study quality was not addressed in the synthesis.
Concerns regarding methods used to synthesize results	High	One-third of the studies were excluded on the basis of lack of available data. In addition, several other shortcomings were identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	High	There is no information on whether the objectives and eligibility criteria were predefined. Studies with insufficient data were excluded. All other signalling questions were scored as "Yes" or "Probably yes".

Domain	Concern	Rationale for concern
Concerns regarding methods used to identify and/or select studies	High	The methods for identification and selection of studies had several shortcomings.
Concerns regarding methods used to collect data and appraise studies	Unclear	There is no information on the process of the risk of bias assessment.
Concerns regarding the synthesis and findings	High	One-third of the studies were excluded on the basis of lack of available data. In addition, several other shortcomings were identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	All concerns were not addressed in the interpretation.
B. Was the relevance of identified studies to the review's research question appropriately considered	No	The relevance of included studies were not discussed.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results are presented.
Risk of bias	High	There were several shortcomings in the identification and selection of studies, and this could have led to relevant studies being missed. There were also several shortcomings in the synthesis.

Hu et al. (2018)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions were clearly described and appeared to be appropriate.

Signalling question	Rating	Reasoning
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably no	Studies were excluded if data were incorrect or incomplete.
Concerns regarding specification of study eligibility criteria	High	There is no information on whether the eligibility criteria and studies were excluded if data were incorrect or incomplete with no effort to obtain correct/sufficient data.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Electronic databases were searched for published and unpublished studies.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference lists were screened.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	The search terms were reported. No other information was given.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably yes	No such restrictions were described; however, the full search strategy was not reported.
2.5 Were efforts made to minimise errors in selection of studies?	No information	
Concerns regarding methods used to identify and/or select studies	High	Concerns were identified regarding the search strategy, and the lack of information on efforts made to minimise errors in selection of studies.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	The data extraction was collected independently by two investigators.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	No	A table of study characteristics is provided. However, age of the subjects in the included primary studies are not reported, either in the table or in the text.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	The Cochrane risk of bias guidelines were used.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	High	Concerns regarding the available study characteristics and the lack of information on efforts made to minimise error in RoB assessment.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably no	Studies with no available data were excluded without effort to obtain these data. It is not reported how many studies this applied to, so it is uncertain how this affected the findings.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably yes	Fixed-effect model was used where there was no heterogeneity. Otherwise, random-effect model was used. However, from the forest plot it seems that a fixed-effect model was also used for IL-6 which had moderate heterogeneity.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Between-studies variation was assessed and addressed.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably yes	Funnel plot demonstrated that publication bias did not affect the result.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No	Both studies with low and high risk of bias was included in the meta-analysis without further consideration. Publication bias was assessed and no indication of this type of bias was found.
Concerns regarding methods used to synthesize results	High	Concerns regarding lack of information on inclusion of studies in the synthesis and on predefinition of analyses were identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	High	There is no information on whether the eligibility criteria and studies were excluded if data were incorrect or incomplete with no effort to obtain correct/sufficient data.
Concerns regarding methods used to identify and/or select studies	High	Concerns were identified regarding the search strategy, and the lack of information on efforts made to minimise errors in selection of studies.
Concerns regarding methods used to collect data and appraise studies	High	Concerns regarding the available study characteristics and the lack of information on efforts made to minimise error in RoB assessment.
Concerns regarding the synthesis and findings	High	Concerns regarding lack of information on inclusion of studies in the synthesis and on predefinition of analyses were identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns identified in phase 2 were not assessed in the interpretation of the findings.

Signalling question	Rating	Reasoning
B. Was the relevance of identified studies to the review's research question appropriately considered	No	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Irving et al. (2006) (an update published 2011)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Yes	A protocol for the review was registered a priori. Discrepancies between the protocol and review were described.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria seemed appropriate.
1.3 Were eligibility criteria unambiguous?	Yes	Properly described eligibility criteria addressing study design, population, and intervention.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	The restrictions based on types of study design were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in several electronic databases. No search for unpublished reports.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Citing references of the included studies were screened, and authors of all studies initially selected for inclusion were contacted in order to identify further relevant trials.

Signalling question	Rating	Reasoning
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	Search terms for study design, population and intervention were applied.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	This was an update, and the date restriction was therefore appropriate. No other restrictions were described.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two reviewers performed both selection steps independently.
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered "Yes" or "Probably yes".

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Two reviewers extracted data independently
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Probably yes	Continuous data from rating scales were included only if the measuring instrument had been described in a peer-reviewed journal and the instrument is either a self-report or completed by an independent rater or relative (not the therapist). If more than 30% of data was unaccounted for in a study by the first follow up, the data was not included in the analyses.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	The Cochrane tool for RoB evaluation was used, including consideration of how the sequence was generated, how allocation was concealed, the integrity of blinding at outcome, the completeness of outcome data, selective reporting and other biases.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two reviewers evaluated RoB independently.
Concerns regarding methods used to collect data and appraise studies	Low	All signaling questions were rated "Probably Yes" or "Yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	The synthesis seemed to include all studies that it should.
4.2 Were all predefined analyses followed or departures explained?	Yes	No deviations from the protocol with regard to the data analyses were described.

Signalling question	Rating	Reasoning
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Heterogeneity was addressed.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably no	Both funnel plot and sensitivity analysis were included. For each adverse outcome, only a few studies (for the majority only one study) had assessed the outcome.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	Studies where sequence generation was at high risk of bias or where allocation was clearly not concealed were not included.
Concerns regarding methods used to synthesize results	Unclear	For the majority of the adverse outcomes, the findings were only provided from one study, indicating that the findings are not robust. All other questions were answered "Yes" or "Probably yes".

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered "Yes" or "Probably yes".
Concerns regarding methods used to collect data and appraise studies	Low	All signaling questions were rated "Probably Yes" or "Yes".
Concerns regarding the synthesis and findings	Unclear	For the majority of the adverse outcomes, the findings were only provided from one study, indicating that the findings are not robust. All other questions were answered "Yes" or "Probably yes".

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	Yes	No major shortcomings were identified. Few studies addressing the different outcomes were addressed in the conclusions.
B. Was the relevance of identified studies to the review's research question appropriately considered?	Probably Yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results were presented equally.
Risk of bias	Low	Minor concerns with the review process were identified.

Kar et al. (2016)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria were appropriate.
1.3 Were eligibility criteria unambiguous?	Probably yes	It was stated that studies that included women on any other treatment that could prevent preterm delivery were excluded. No further explanation was given.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Only randomized controlled studies were included. The restriction based on study design was clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in electronic databases. Search for unpublished reports were not performed.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference list of the primary articles were searched for additional studies.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	The search terms used in the search and the search strings used were reported. The full search strategy or the combination of the terms, were not reported. It was stated that the searches were undertaken with input from a clinical librarian.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No such restrictions were described.

Signalling question	Rating	Reasoning
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two reviewers independently screened abstracts and full-texts.
Concerns regarding methods used to identify and/or select studies	Unclear	Concerns regarding the lack of information on the search strategy were identified. Since the full search strategy is not given, there is not enough information to assess whether it is likely that all relevant studies were identified. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Data were extracted in duplicate by two independent reviewers using predesigned forms. Any disagreements were resolved by discussion, and if needed by involving a third reviewer.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	No	Exact doses of EPA and DHA is not given. In addition, the participants age was not reported, and it was not specified which of the RCTs that reported on birth weight.
3.3 Were all relevant study results collected for use in the synthesis?	Probably yes	Dichotomous data were entered in 2x2 tables and continuous data in 2x1 tables. It was not stated how data were transformed if they were not reported in the appropriate format.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	The Cochrane RoB tool was applied.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two reviewers independently assessed risk of bias.
Concerns regarding methods used to collect data and appraise studies	Unclear	Concerns regarding the available study characteristics were identified. Exact doses of EPA and DHA for the different studies was not reported. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	Weighted meta-analysis and random effects model were performed.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	No	Heterogeneity was large and not addressed in the synthesis.

Signalling question	Rating	Reasoning
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably no	No small-group effect was detected. Summary risk of bias is not reported and potential high risk of bias is not adjusted for in the synthesis.
Concerns regarding methods used to synthesize results	High	It is not reported whether the analysis was predefined or not.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	Unclear	Concerns regarding the lack of information on the search strategy were identified. Since the full search strategy is not given, there is not enough information to assess whether it is likely that all relevant studies were identified. All other signalling questions were scored as "Yes" or "Probably yes".
Concerns regarding methods used to collect data and appraise studies	Unclear	Concerns regarding the available study characteristics were identified. Exact doses of EPA and DHA for the different studies was not reported. All other signalling questions were scored as "Yes" or "Probably yes".
Concerns regarding the synthesis and findings	High	It is not reported whether the analysis was predefined or not.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns identified in phase 2 were not assessed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	No	Relevance of the included studies was not assessed or discussed.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results were presented equally.
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Kwak et al. (2012)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There is not mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria included the population, intervention, study design and outcome.
1.3 Were eligibility criteria unambiguous?	Yes	Eligibility criteria were sufficiently detailed.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	The restrictions based on types of study design were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No other restrictions than language, and this was considered not to be an important issue.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Probably yes" or "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in three electronic databases. No search for unpublished reports.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Bibliographies of relevant articles were reviewed for additional publications.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	The full search strategy is not given; only the search words without information on how they were combined are provided.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably no	Language restrictions were included in the search strategy.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two persons performed the study selection independently.
Concerns regarding methods used to identify and/or select studies	High	There were several shortcomings regarding the identification and selection of studies; the full search strategy is not given and only publications in English were included.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Probably yes	Detailed information from the included studies were presented.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	It is assumed that all relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	The Jadad scale was used. The scale measures the following characteristics of RCTs: randomization, double-blind design, and follow-up reporting. Allocation concealment was not included.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	High	There were several shortcomings regarding the data collection and study appraisal; there was no information on efforts made to minimise errors in data collection and RoB assessment, and allocation concealment was not included in the evaluation of RoB.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	All studies were included.
4.2 Were all predefined analyses followed or departures explained?	No information	There is not mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably Yes	Relative risk with 95% confidence intervals was used for adverse events.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably Yes	For adverse events the heterogeneity (I^2 value) was less than 35%.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	For gastrointestinal bleeding there was only one study included, and the findings on this outcome are therefore not robust. There is not enough information to judge whether the findings on gastrointestinal troubles, which of eight studies had addressed, were robust.

Signalling question	Rating	Reasoning
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably no	Publication bias was assessed. Risk of bias of the included studies was low to moderate. However, the risk of bias assessment was incomplete since allocation concealment was not considered.
Concerns regarding methods used to synthesize results	High	Concerns regarding lack of information on predefinition of analyses and the robustness of the data were identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Probably yes" or "Yes".
Concerns regarding methods used to identify and/or select studies	High	There were several shortcomings regarding the identification and selection of studies; the full search strategy is not given and only publications in English were included.
Concerns regarding methods used to collect data and appraise studies	High	There were several shortcomings regarding the data collection and study appraisal; there was no information on efforts made to minimise errors in data collection and RoB assessment, and allocation concealment was not included in the evaluation of RoB.
Concerns regarding the synthesis and findings	High	Concerns regarding lack of information on predefinition of analyses and the robustness of the data were identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns identified in phase 2 were not assessed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	Relevance of the included studies was addressed in the discussion.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Lehner et al. (2021)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria were appropriate.
1.3 Were eligibility criteria unambiguous?	Probably yes	It was stated only women who chose to primarily breastfeed their infant were included, however, "primarily" was not further explained.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were described.
Concerns regarding specification of study eligibility criteria	Unclear	Concerns regarding the lack of information on whether the objectives and eligibility criteria were predefined were identified. All other signalling questions were scored as "Probably yes" or "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Searches were performed in several electronic databases. Also, trial registers were searched.,
2.2 Were methods additional to database searching used to identify relevant reports?	No	
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably yes	The full search strategy was reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No such restrictions were applied.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two reviewers independently screened abstracts and full-texts.
Concerns regarding methods used to identify and/or select studies	Unclear	No additional methods than database searches were performed, thus, potentially relevant publications could have been missed. All other signalling questions were scored as "Yes".

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Data were extracted by two independent reviewers using a standard data extraction sheet.

Signalling question	Rating	Reasoning
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were extracted and described.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	Assessment of study quality were performed and included assessment of randomization, concealment of allocation, and implementation of blinding and intention-to-treat analyses. Bias in the exposure characterization and outcome assessment, and other potential biases, were not included in this evaluation.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	High	Concerns regarding lack of information on efforts made to minimize errors in RoB assessment, and concerns regarding the RoB method, were identified.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably no	A meta-analysis was performed, and data were combined using fixed-effect models. The use of a fixed-effect model was justified by no statistically significant heterogeneity. However, the synthesis was not weighted by the size of the studies.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Heterogeneity between studies for the outcome "birth weight" was small.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Yes	Sensitivity analyses indicate that the findings are robust.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably yes	Publication bias was checked, and study quality is addressed in the discussion of the findings, but not in the synthesis.
Concerns regarding methods used to synthesize results	Unclear	Concerns regarding the synthesis was identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	Concerns regarding the lack of information on whether the objectives and eligibility criteria were predefined were identified. All other signalling questions were scored as "Probably yes" or "Yes".
Concerns regarding methods used to identify and/or select studies	Unclear	No additional methods than database searches were performed, thus, potentially relevant publications could have been missed. All other signalling questions were scored as "Yes".
Concerns regarding methods used to collect data and appraise studies	High	Concerns regarding lack of information on efforts made to minimize errors in RoB assessment, and concerns regarding the RoB method, were identified.
Concerns regarding the synthesis and findings	Unclear	Concerns regarding the synthesis was identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns identified in phase 2 were not assessed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	No	Relevance of included studies was not assessed or discussed.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results were presented as equally.
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Lopez-Huertas et al. (2012)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.

Signalling question	Rating	Reasoning
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions based on study design, population (subjects diagnosed with MS) and effect (chronic), were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	The restriction on language were considered not to be a major issue.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in two electronic databases. Search for unpublished reports were not performed.
2.2 Were methods additional to database searching used to identify relevant reports?	Probably yes	The references sections of the 11 included studies revealed 6 additional RCT with MS patients that met the search criteria, and were also included
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably yes	The MeSH terms used in the search and the search strings used were reported. The full search strategy was not reported, however how the search words were combined was reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably yes	The search was limited to RCTs.
2.5 Were efforts made to minimise errors in selection of studies?	No information	Titles and abstracts were analysed by two independent scientists of the field. No information about the full text selection.
Concerns regarding methods used to identify and/or select studies	Unclear	Information on efforts made to minimise errors in full text selection were lacking.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Probably no	Data tables on number, exposure time and doses available, but no information about age in the study population.
3.3 Were all relevant study results collected for use in the synthesis?	Probably yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	No information on concealment and blinding of assessors.

Signalling question	Rating	Reasoning
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	High	Concerns were identified with regard to lacking information on methods used to minimise errors in data collection and risk of bias assessment.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Relevant data for assessment of possible adverse effects were included.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably yes	Narrative synthesis of data on LDL and HDL.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	No information	
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably yes	No studies had significant bias.
Concerns regarding methods used to synthesize results	High	Many parameters without information, total score high concerns

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	No information on the criteria was predefined
Concerns regarding methods used to identify and/or select studies	Unclear	Information on efforts made to minimise errors in full text selection were lacking.
Concerns regarding methods used to collect data and appraise studies	High	No information on efforts made to minimise errors in data collection and bias assessment
Concerns regarding the synthesis and findings	H High	No information on predefined analysis, heterogeneity or robust data

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns identified in phase 2 were not assessed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results were presented as equally.
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Mocellin et al. (2016)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	The restrictions based on study design (controlled or randomized clinical trials performed in humans) were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	The restriction on language were considered not to be a major issue.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in electronic databases. Search for unpublished reports were not performed.

Signalling question	Rating	Reasoning
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference lists of all identified studies and important reviews about the theme were hand-searched for relevant trials.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	The search terms and search strategy were clearly described.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes/probably yes	No such restrictions were applied in the search.
2.5 Were efforts made to minimise errors in selection of studies?	No information	
Concerns regarding methods used to identify and/or select studies	Unclear	No information on efforts to minimise errors in study selection was identified. All other signalling questions were answered "Yes" or "Probably yes".

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Data were extracted independently by two reviewers and cross-checked.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	Quality assessment was conducted according to Cochrane Collaboration's tool for assessing quality and risk of bias and CONSORT-based checklist.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Quality assessment was conducted by two independent reviewers.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered "Yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Relevant data for assessment of possible adverse effects were included.
4.2 Were all predefined analyses followed or departures explained?	Probably no	There was no mention of a protocol or whether the data analyses were predefined.

Signalling question	Rating	Reasoning
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably yes	Despite of possible heterogeneity among them, the exclusion of the trials that were heterogeneity sources did not modify the significance of the analyses.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably yes	Heterogeneity was high. For minimizing heterogeneity among the trials, sub-analyses by clinical situation (surgery or chemotherapy) and route of administration (oral or parenteral) were performed, and the heterogeneity decreased considerably or ceased to exist.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably yes	There was significant heterogeneity in many of the reported outcomes, especially in pooled analyses, indicating variation between the studies in the estimates of the effect of n-3 PUFA on the measured outcomes. Despite of possible heterogeneity among them, the exclusion of the trials that were heterogeneity sources did not modify the significance of the analyses.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No information	
Concerns regarding methods used to synthesize results	Unclear	Concerns regarding the lack of information on bias in the data synthesis and predefined analyses.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	Unclear	No information on efforts to minimise errors in study selection was identified. All other signalling questions were answered "Yes" or "Probably yes".
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered "Yes".
Concerns regarding the synthesis and findings	Unclear	Concerns regarding the lack of information on bias in the data synthesis and predefined analyses.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Not all concerns were addressed.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably yes	

Signalling question	Rating	Reasoning
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results were presented as equally.
Risk of bias	Unclear	The phase 2 assessment identified some concerns with the review process that were not addressed.

Newberry et al. (2016)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Yes	Objectives and eligibility criteria was predefined in a protocol. The protocol was registered in PROSPERO (registry number CRD42015020638).
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The inclusion and exclusion criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Properly described criteria addressing study type, population, intervention/exposure, comparators, and outcomes.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	The restrictions based on types of study design were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	Only published and peer-reviewed studies, written in English, were included.
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered "Yes" or "Probably Yes", so no potential concerns regarding the eligibility criteria were identified.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Literature searches were performed in several electronic databases. There was no search for unpublished literature; however, a notice was published in the Federal Register requesting unpublished data from manufacturers of omega-3 fatty acid-fortified infant formulae and dietary supplements.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference lists of existing recent systematic reviews on outcomes of interest were reviewed.

Signalling question	Rating	Reasoning
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	Lack of search terms for adverse events would retrieve several studies, however restrictions on some adverse effects would rule out others. Maternal or childhood adverse events were included as a key question, however, several hypothesized adverse effects were not included in the search terms.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably no	Restriction on date were justified as this is an update of a previous report. The search was limited to English publications.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	The DistillerSR software package was used. Title/abstract screening was conducted in duplicate (after a training session). Two reviewers conducted second-level screening of full text articles. Discussion with group leader if needed.
Concerns regarding methods used to identify and/or select studies	High	Some concern regarding the identification and selection of studies were identified as there was lack of specific search terms for adverse effects. The other signaling questions were answered "Yes" or "Probably yes".

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Accepted studies underwent single abstraction of study-level data and risk-of-bias assessment in Distiller, with audit by an experienced reviewer. Outcome data were abstracted by a biostatistician and audited by an experienced reviewer.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	All included studies were summarized narratively in tables, and detailed study characteristics were available in the appendix. For several of the studies, adverse events were not reported by study arm.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected. Data from studies included in the original report that were included in new pooled analyses, were re-extracted as needed.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	RCTs: The Cochrane Risk of Bias tool was used, which evaluates risk of selection bias, performance bias, detection bias, attrition bias, reporting bias, and other potential sources of bias. Observational studies: RoB was assessed using questions relevant for prospective studies from the Newcastle-Ottawa tools. Both tools were supplemented with nutrition-specific items.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	No information on how many reviewers appraised each study.

Signalling question	Rating	Reasoning
Concerns regarding methods used to collect data and appraise studies	Unclear	No information available regarding the bias of the methodological quality appraisal.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	No relevant studies were excluded.
4.2 Were all predefined analyses followed or departures explained?	Yes	No deviations from the protocol was reported.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	Studies of different design were analysed separately. When appropriate, they were combined. Metanalysis where performed only when appropriate (sufficient data were available and clinical heterogeneity was minimal), not appropriate for adverse effects.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Heterogeneity was addressed in the synthesis.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably yes	For meta-analysis of data with clear outliers, sensitivity analyses were conducted if appropriate to the question. Funnel plots were not made for the data on adverse effects, as minimal data were available. Few adverse effects were reported in 20 studies.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably yes	Biases were addressed in the synthesis
Concerns regarding methods used to synthesize results	Low	All signaling questions were answered "Yes" or "Probably yes".

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered "Yes" or "Probably Yes", so no potential concerns regarding the eligibility criteria were identified.
Concerns regarding methods used to identify and/or select studies	High	Some concern regarding the identification and selection of studies were identified as there was lack of specific search terms for adverse effects. The other signaling questions were answered "Yes" or "Probably yes".
Concerns regarding methods used to collect data and appraise studies	Unclear	No information available regarding the bias of the methodological quality appraisal.

Domain	Concern	Rationale for concern
Concerns regarding the synthesis and findings	Low	All signalling questions were answered "Yes" or "Probably yes".

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns identified in phase 2 were not assessed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	
Risk of bias	Unclear	The phase 2 assessment identified some concerns with the review process that were not addressed.

Ostadrahimi et al. (2016)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	Exclusion of trials with a single dose treatment and trials in which fish oil was investigated in combination with other nutrients or drugs that may affect gestational diabetes mellitus (GDM) seemed appropriate. Only singleton pregnancies were included, any gestational age.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	Restrictions on publication type seemed appropriate. In addition, only studies published in English or Persian were included.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	PubMed, Scopus, Google Scholar, the Cochrane Library, ProQuest, Science Direct, SID, Magiran, and IranMedex were used for the literature search.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	References in the reviewed articles were used as additional resources.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	Search terms were reported, however, the search strategy or the combination of the search terms, were not reported.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably no	The search were restricted to articles in English and Persian.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two authors independently assessed the eligibility of studies.
Concerns regarding methods used to identify and/or select studies	High	Concerns regarding the search strategy and restrictions to the search were identified.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Two review authors extracted the data independently.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	The results were described narratively in the text.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	Relevant results from studies of satisfactory quality were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	The criteria determined in the Cochrane handbook for systematic reviews of interventions were used.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two review authors evaluated the RoB independently.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered "Yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Data from the two included studies were described in the text narratively.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably yes	The synthesis seemed appropriate (description of the data in the text)
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	No information	

Signalling question	Rating	Reasoning
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	11 of 13 studies were not included in the synthesis due to not sufficient quality.
Concerns regarding methods used to synthesize results	High	Concerns regarding lack of information on several point were identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes".
Concerns regarding methods used to identify and/or select studies	High	Concerns regarding the search strategy and restrictions to the search were identified.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered "Yes".
Concerns regarding the synthesis and findings	High	Concerns regarding lack of information on several point were identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns identified in phase 2 were not assessed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Quin et al. (2016)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Probably yes	There was no mention of a protocol. The eligibility criteria were predefined. No information about predefinition of the objectives.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions were clearly described and appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably no	Unpublished were excluded.
Concerns regarding specification of study eligibility criteria	Unclear	Concerns regarding restriction in the eligibility criteria based on sources of information were identified. There is no information on whether the objectives were predefined. All other signalling questions were scored as "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Electronic databases were searched for published and unpublished studies.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Reference lists of published narrative and systematic reviews were screened.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	The search strategy was reported. The terms and structure of the search strategy were likely to retrieve as many eligible studies as possible.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No such restrictions were applied.
2.5 Were efforts made to minimise errors in selection of studies?	Probably yes	Titles and abstracts were analysed by two independent scientists of the field. No information about the full text selection.
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered "Yes" or "Probably yes".

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	

Signalling question	Rating	Reasoning
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	No information	No table with overview of study characteristics was provided and data were insufficiently described in the text.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	The methodological quality of the trials was assessed according to the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	The methodological quality of the trials was assessed independently by two reviewers.
Concerns regarding methods used to collect data and appraise studies	Unclear	Concerns regarding lack of information on efforts made to minimise errors in data selection and the availability of sufficient study characteristics were identified.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Relevant data for assessment of possible adverse effects were included.
4.2 Were all predefined analyses followed or departures explained?	No information	There was no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	No information	The heterogeneity for the outcome "immunity" was low. However, heterogeneity for inflammatory markers was not provided.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	No information	
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No	
Concerns regarding methods used to synthesize results	High	Concerns regarding lack of information on predefinition of analyses and between-studies variation were identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	Concerns regarding restriction in the eligibility criteria based on sources of information were identified. There is no information on whether the objectives were predefined. All other signalling questions were scored as "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered "Yes" or "Probably yes".
Concerns regarding methods used to collect data and appraise studies	Unclear	Concerns regarding lack of information on efforts made to minimise errors in data selection and the availability of sufficient study characteristics were identified.
Concerns regarding the synthesis and findings	High	Concerns regarding lack of information on predefinition of analyses and between-studies variation were identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns identified in phase 2 were not assessed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	No	Relevance of included studies was not assessed or discussed.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	No	Statistically significant results were emphasized.
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Ren et al. (2021)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Yes	A protocol for the review was registered a priori.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	Eligibility criteria seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	The criteria were sufficiently detailed.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	RCTs and observational studies were included. An inclusion criterion was measurement of fatty acid levels in blood samples.

Signalling question	Rating	Reasoning
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	Only publications in English or Chinese were included.
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were rated "Probably yes" or "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	PubMed and EMBASE were searched.
2.2 Were methods additional to database searching used to identify relevant reports?	No	
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	The full search strategy is published, and the strategy includes an appropriate range of terms.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No such restrictions were described.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two reviewers independently screened abstracts and full-texts.
Concerns regarding methods used to identify and/or select studies	Unclear	No additional methods than database searches were performed. Potentially relevant publications could have been missed.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Data were extracted by two independent reviewers.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Study characteristics are presented in a table.
3.3 Were all relevant study results collected for use in the synthesis?	Probably yes	Continuous data from RCTs was analysed by mean differences and 95% CI. For results from observational studies, adjusted b correlation and corresponding 95% CI between fatty acid levels and birth or child weight were used for all eligible studies. It is not stated how data were transformed if they were not reported in the appropriate format.

Signalling question	Rating	Reasoning
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	Cochrane risk of bias tool was used to assess the quality of the RCTs. Risk of Bias in Non-Randomized Studies of Intervention (ROBINS-I) tool was used for observational studies. A diacyclic graph was drafted to identify possible confounders. Grading of Recommendation Assessment, Development and Evaluation (GRADE) methodology was used to assess the confidence in the evidence.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two reviewers independently assessed risk of bias.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were rated "Probably yes" or "Yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	
4.2 Were all predefined analyses followed or departures explained?	Yes	A protocol was registered a priori.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	A meta-analysis was performed, and data were combined using random-effect models. Those studies that lacked data to be included in the meta-analysis, was synthesized narratively.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably yes	For the RCTs, the heterogeneity was addressed in subgroup analyses and were low. For observational studies, heterogeneity was high.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably yes	
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	Risk of bias was not addressed in the synthesis, but was addressed in the GRADE assessment.
Concerns regarding methods used to synthesize results	Low	All signalling questions were rated "Probably yes" or "Yes".

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were rated "Probably yes" or "Yes".
Concerns regarding methods used to identify and/or select studies	Unclear	No additional methods than database searches were performed. Potentially relevant publications could have been missed.

Domain	Concern	Rationale for concern
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were rated "Probably yes" or "Yes".
Concerns regarding the synthesis and findings	Low	All signalling questions were rated "Probably yes" or "Yes".

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	The minor concerns identified in phase 2 were not assessed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	
Risk of bias	Low	

Sadeghi et al. (2017)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There was no mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The criteria were clearly described and seemed appropriate to answer the research question.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions based on study design and publication type appeared to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No such restrictions were applied.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Searches were performed in three electronic databases. Search for unpublished reports were not performed.
2.2 Were methods additional to database searching used to identify relevant reports?	No information	
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably yes	The search terms and combination of the terms were described. Both medical subject headings and text words were used. The full search strategy was not included.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No restrictions were applied.
2.5 Were efforts made to minimise errors in selection of studies?	No information	
Concerns regarding methods used to identify and/or select studies	High	Information on searches additional to database searching and efforts made to minimise errors in full text selection were lacking.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficiently data were extracted.
3.3 Were all relevant study results collected for use in the synthesis?	No	De beskrives at kun en studie rapporterer på fettsyrer I plasma, men dataene er ikke oppgitt
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably no	The Jadad scale was applied. Allocation concealment was not included.
3.5 Were efforts made to minimise error in risk of bias assessment?	No information	
Concerns regarding methods used to collect data and appraise studies	High	Concerns regarding multiple points in the data collection and study appraisal were identified.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	No eligible studies were excluded from the synthesis.
4.2 Were all predefined analyses followed or departures explained?	No information	There is no information on whether the analyses were predefined or not.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	No information	Only one study, no data reported.

4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	No	Only one study.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	NA	Only one study.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	NA	Only one study.
Concerns regarding methods used to synthesize results	High	Only narrative results from one study, no synthesis of results possible.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. All other signalling questions were scored as "Yes".
Concerns regarding methods used to identify and/or select studies	High	Information on searches additional to database searching and efforts made to minimise errors in full text selection were lacking.
Concerns regarding methods used to collect data and appraise studies	High	Concerns regarding multiple points in the data collection and study appraisal were identified.
Concerns regarding the synthesis and findings	High	Only narrative results from one study, no synthesis of results possible.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Several concerns identified in phase 2 were not assessed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered?	Probably yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	
Risk of bias	High	The phase 2 assessment identified several concerns with the review process that were not addressed.

Su et al. (2021)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Yes	A review protocol was prepared and registered in PROSPERO.
1.2 Were the eligibility criteria appropriate for the review question?	Probably yes	The criteria were clearly described and seemed appropriate to answer the research question. Abstracts without full text were excluded.
1.3 Were eligibility criteria unambiguous?	Yes	Sufficiently detailed eligibility criteria addressing study design, population, intervention and effect.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Articles reporting individual cases or series of cases and conference abstracts for which no full text was available were excluded. These restrictions were considered appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	Restrictions on language (English) were considered not to be a major issue.
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered "Yes" or "Probably yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Searches were performed in several electronic databases, including ClinicalTrials.gov.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Additional available studies were sought by searching the reference lists of original articles and relevant reviews to try to uncover related publications not retrieved through the electronic search.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	Three sets of key terms were used in the literature search, and no restrictions were applied.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	No such restrictions were applied.
2.5 Were efforts made to minimise errors in selection of studies?	No information	
Concerns regarding methods used to identify and/or select studies	Unclear	Some concerns regarding the efforts made to minimise errors in selection of studies were identified.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Three investigators independently extracted relevant information from the included studies.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics were available.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant study results were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	Methodological quality of eligible trials was evaluated using the Cochrane Collaboration risk of bias tool.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions answered "Yes".

Doain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Yes	Relevant data for assessment of possible adverse effects were included. 12 studies examined the mean change in lipid profiles, four examined the mean change in C-reactive protein.
4.2 Were all predefined analyses followed or departures explained?	Yes	
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Heterogeneity was low.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Yes	For CRP, the GRADE quality was considered moderate, whereas the GRADE quality for LDL and HDL was considered to be low.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	Biases were addressed in the GRADE evaluation.
Concerns regarding methods used to synthesize results	Low	All signalling questions answered "Yes".

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered "Yes" or "Probably yes".
Concerns regarding methods used to identify and/or select studies	Unclear	Some concerns regarding the efforts made to minimise errors in selection of studies were identified.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions answered "Yes".
Concerns regarding the synthesis and findings	Low	All signalling questions answered "Yes".

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Minor concerns identified in the phase 2 assessment were not addressed in the interpretation of the findings.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	
Risk of bias	Low	

Villani et al. (2013)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There is not mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria addressed study design and intervention.
1.3 Were eligibility criteria unambiguous?	Yes	Eligibility criteria were sufficiently detailed.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	The restrictions based on types of study design, administration, intervention and population were clearly described and appeared to be appropriate.

Signalling question	Rating	Reasoning
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	The restriction on language were considered not to be a major issue.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. Non-English publications were excluded. All other signalling questions were scored as "Yes" or "Probably Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Several major electronic databases were searched, including Cochrane register of clinical trials, indicating that also unpublished reports were identified in the search.
2.2 Were methods additional to database searching used to identify relevant reports?	No	
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably no	The full search strategy is not described; only text-words without information on how these were combined.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably no	The search strategy was limited to age (≥ 60 years), human studies, restricted to the English language and RCTs.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two reviewers performed the study selection independently.
Concerns regarding methods used to identify and/or select studies	High	The methods used to identify and select relevant studies had several shortcomings.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Probably yes	A pre-designed data extraction form was used, and the form was piloted. Two reviewers extracted data; however, it is not stated whether they did so independently or not.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Data on the types of participants, the dose and duration of EPA and/or DHA supplementation, the method of measuring adherence to the EPA and/or DHA intervention as well as the aim/s and primary findings for each of the reviewed studies were extracted.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	2x2 data were extracted from each study and chi-square analysis using Yates` correction was used to check for differences between the intervention group and placebo.

Signalling question	Rating	Reasoning
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	Cochrane Collaboration's tool for assessing risk of bias and Jadad scoring were applied.
3.5 Were efforts made to minimise error in risk of bias assessment?	Probably yes	The assessment was done by two reviewers; however, it is not stated whether it was done independently.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were scored as "Probably Yes" or "Yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably Yes	<p>A request by email was sent to all corresponding authors of the included studies to provide documented adverse effects that were not reported in the full-text article. For each outcome, the number of studies investigating the outcome is given.</p> <p>However, some data were not available to the reviewers. For two studies the reviewers did not have enough information in which allocation arm the adverse effect occurred or at which frequency.</p>
4.2 Were all predefined analyses followed or departures explained?	No information	There is no mention of a protocol or whether the data analysis was predefined.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis seemed appropriate.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	No	All studies had similar study design and the population were similar. However, doses and duration of intervention varied greatly, and was not addressed for the adverse events.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably no	Five studies addressed gastrointestinal disturbances; however, the findings were not consistent across studies. For other adverse effects, there were only one or two studies that had addressed each adverse outcome. Therefore, these outcomes cannot be considered robust.

Signalling question	Rating	Reasoning
4.6 Were biases in primary studies minimal or addressed in the synthesis?	No	Biases in primary studies were not addressed in the synthesis of the data on adverse events.
Concerns regarding methods used to synthesize results	High	The methods used had several shortcomings.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined. Non-English publications were excluded. All other signalling questions were scored as "Yes" or "Probably Yes".
Concerns regarding methods used to identify and/or select studies	High	The methods used to identify and select relevant studies had several shortcomings.
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were scored as "Probably Yes" or "Yes".
Concerns regarding the synthesis and findings	High	The methods used had several shortcomings.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	Concerns identified in the phase 2 assessment were not addressed.
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	This was addressed in the discussion
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	All results were presented.
Risk of bias	High	Several concerns were identified in the phase 2 assessment, and these were not addressed in the interpretation of the findings.

Wang et al. (2006)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	The objectives and eligibility criteria were specified in the Background and Methods. No flowchart was included, no protocol.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria were open and seemed appropriate to the review question.
1.3 Were eligibility criteria unambiguous?	Probably yes	The eligibility criteria were probably sufficient to answer the review question. The different inclusion criteria were not described in detail.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Probably yes	Restrictions were provided in the Methods section. Studies of any duration or dosage were included when reviewing adverse events.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably yes	Only English language.
Concerns regarding specification of study eligibility criteria	Unclear	Insufficient information is reported to make a judgement about risk of bias. No protocol or flowchart of inclusion criteria were included.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably yes	Search were performed in MEDLINE, PreMEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Biological Abstracts, and Commonwealth Agricultural Bureau of Health. There was no information about inclusion of unpublished work, such as conference proceedings.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	The authors consulted domain experts and examined references of retrieved articles.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably yes	Search terms were provided in the Methods section. The search strategy was not provided. All possible studies were included in the search and filtered afterwards with eligibility criteria.
2.4 Were restrictions based on date, publication format, or language appropriate?	Probably yes	The authors report that the search was done from 1966 to July 2005.
2.5 Were efforts made to minimise errors in selection of studies?	No information	No information about the process of study selection (number of reviewers, etc.).
Concerns regarding methods used to identify and/or select studies	Unclear	There is insufficient information reported to make a judgement on risk of bias. The complete search strategy was not provided.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	No information	Six authors were involved in the data collection, but details of this process were not reported.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Probably yes	Study characteristics were presented in tables. Limited data on adverse effects are presented.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	Relevant results seem to be included, but data on adverse effects was sparse.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Probably yes	The methodologic quality of the studies was evaluated for CVD outcomes using a 3-category summary quality grade.
3.5 Were efforts made to minimise error in risk of bias assessment?	Probably yes	No details were provided on how the assessment was made.
Concerns regarding methods used to collect data and appraise studies	Unclear	There is insufficient information about the data collection and study appraisal to make a judgement on risk of bias.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably yes	Many articles provided no information on adverse events, indicating that this often was included as a secondary outcome. Relevant studies with available data seem to be included.
4.2 Were all predefined analyses followed or departures explained?	Probably no	No protocol or predefined analysis strategy.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Probably yes	Sparse data and therefore no meta-analyses were performed, narrative approach.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	No data regarding variation between studies in adverse effects. The categorization and reporting of adverse events varied greatly across studies. Heterogeneity was addressed by not combining data.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably no	No statistical approach to visualize exposure effects on adverse effects. Primary studies graded for quality. Narrative statement that increases in dose increased GI symptoms referring to one study only.

Signalling question	Rating	Reasoning
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Probably yes	Biases were addressed for primary outcome only. Data quality assessment for adverse effects not available.
Concerns regarding methods used to synthesize results	Unclear	There is insufficient information reported to make a judgement on risk of bias. Adverse events were likely reported as secondary outcomes in primary studies.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	Insufficient information is reported to make a judgement about risk of bias. No protocol or flowchart of inclusion criteria were included.
Concerns regarding methods used to identify and/or select studies	Unclear	There is insufficient information reported to make a judgement on risk of bias. The complete search strategy was not provided.
Concerns regarding methods used to collect data and appraise studies	Unclear	There is insufficient information about the data collection and study appraisal to make a judgement on risk of bias.
Concerns regarding the synthesis and findings	Unclear concern	There is insufficient information reported to make a judgement on risk of bias. Adverse events were likely reported as secondary outcomes in primary studies.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	The interpretation did not address lack of information on adverse events and no quality assessment or bias were considered for this outcome.
B. Was the relevance of identified studies to the review's research question appropriately considered	No/ no information	The relevance of identified studies was not evaluated for adverse events.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	No meta-analysis was performed due to sparse information. Adverse events were reported in a narrative summary.
Risk of bias	High	The phase 2 assessment identified a number of areas with insufficient information on the review process of adverse events. These include lack of clarity in inclusion

		criteria, search strategy and insufficient details on included studies. This was not addressed in the interpretation. There is therefore a high risk of bias in this review.
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Watson and Stackhouse (2020)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	Yes	A protocol for the review was registered a priori. Discrepancies between the protocol and review are described. The objectives and eligibility criteria were specified clearly in the Background and Methods.
1.2 Were the eligibility criteria appropriate for the review question?	Yes	The eligibility criteria seemed appropriate to the review question.
1.3 Were eligibility criteria unambiguous?	Yes	The eligibility criteria were sufficient to answer the review question.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Restrictions were provided in the Methods section and seemed to be appropriate.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Yes	No observational studies were included, only RCTs with cystic fibrosis subjects.
Concerns regarding specification of study eligibility criteria	Low	No potential concerns about the specification of eligibility criteria were identified.

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Yes	Search were performed in the Cochrane Cystic Fibrosis and Genetic Disorders Group's Trials Register, which is compiled from electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL), weekly searches of MEDLINE, and a search of Embase to 1995 and handsearching of two relevant journals. Electronic searches of CINAHL and EMBASE (from 1995) were also performed.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	A manual search of relevant journals and conference abstracts were performed. In addition, the first author of each paper was contacted and invited to identify any other published or unpublished studies that might be relevant.

Signalling question	Rating	Reasoning
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Yes	A search strategy was provided in the Methods section and the full search strategy was provided in the appendix.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	The search included no such restrictions.
2.5 Were efforts made to minimise errors in selection of studies?	Yes	The titles and abstracts were screened independently by two reviewers and disagreements were solved through consensus.
Concerns regarding methods used to identify and/or select studies	Low	No potential concerns about the identification and selection of studies were identified.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Data extraction was performed independently by two reviewers using a predetermined data extraction form. All stages of data extraction and interpretation were discussed.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	A study characteristic table of the five included articles was presented.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	All relevant results from the included studies, where data were available (see 4.1), were collected.
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	The quality of each study was assessed using the Cochrane Handbook for Systematic Reviews of Interventions.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Risk of bias was performed independently by two reviewers. Assessments were compared and inconsistencies were resolved by discussion.
Concerns regarding methods used to collect data and appraise studies	Low	A potential concern regarding missing data were identified.

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	No	Flow chart reported the five included studies. Four studies were excluded due to insufficient information and a lack of response from the studies' authors.

Signalling question	Rating	Reasoning
4.2 Were all predefined analyses followed or departures explained?	Yes	The analysis strategy was clearly stated in the Methods. Two of five studies did not include necessary data needed to combine data for a meta-analysis.
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	The synthesis was appropriate and well described. A qualitative synthesis was conducted, which seem appropriate considering the low number of studies for each outcome and potential for high heterogeneity.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Probably yes	Statistical heterogeneity test was not performed due to insufficient number of studies with available data. Heterogeneity was addressed by not combining data.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Probably yes	The quality of evidence was assessed by using GRADE and found to be very-low, which was appropriately handled. Sensitivity analysis was not performed due to lack of data.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	The level of bias was found to be unclear and was appropriately addressed.
Concerns regarding methods used to synthesize results	High	A potential concern regarding missing data in the synthesis were identified.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Low	All signalling questions were answered as "Yes" or "Probably yes", so no potential concerns
Concerns regarding methods used to identify and/or select studies	Low	All signalling questions were answered as "Yes" or "Probably yes", so no potential concerns
Concerns regarding methods used to collect data and appraise studies	Low	All signalling questions were answered as "Yes" or "Probably yes", so no potential concerns
Concerns regarding the synthesis and findings	High	A potential concern regarding missing data in the synthesis were identified.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	Probably Yes	Missing data were clearly described.

Signalling question	Rating	Reasoning
B. Was the relevance of identified studies to the review's research question appropriately considered	Yes	Relevant studies were included and shown in the synthesis of the results from the included studies.
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	The review conclusions reflect both the statistically significant and non-significant review findings.
Risk of bias	Low	The phase 2 assessment identified a concern regarding missing data in the synthesis. No other concerns were identified. The potential limitations of the studies included in the review in terms of risk of bias were discussed in detail in the discussion. The review conclusions appropriately reflect the results of the review.

Xin et al. (2013)

Phase 2: Identifying concerns with the review process

Domain 1 – study eligibility criteria

Signalling question	Rating	Reasoning
1.1 Did the review adhere to pre-defined objectives and eligibility criteria?	No information	There is not mention of a protocol or whether the objectives and eligibility criteria were predefined.
1.2 Were the eligibility criteria appropriate for the review question?	Probably Yes	The inclusion criteria seemed appropriate to answer the research question. Exclusion criteria were not described in detail.
1.3 Were eligibility criteria unambiguous?	Yes	Eligibility criteria described study type, population, intervention, comparators and outcomes.
1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	Yes	Prospective, randomized and controlled human trials with a parallel design (regardless of sample size), adults >18 years who underwent a cardiac surgery and assigned to perioperative fish oil supplementation.
1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	Probably Yes	Only studies available as full-length article was considered eligible. This would mean that unpublished results were not included.
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined and only studies available as full-length article was considered eligible. All other signalling questions were scored as "Yes".

Domain 2: Identification and selection of studies

Signalling question	Rating	Reasoning
2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	Probably Yes	PubMed, Embase and Cochrane library were searched. No search for unpublished reports.
2.2 Were methods additional to database searching used to identify relevant reports?	Yes	Handsearching of the reference lists of reviews and original articles were performed.
2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	Probably No	The full search strategy is not given; only the search words without information on how they were combined are provided.
2.4 Were restrictions based on date, publication format, or language appropriate?	Yes	
2.5 Were efforts made to minimise errors in selection of studies?	Yes	Two reviewers independently screened abstracts and full-texts.
Concerns regarding methods used to identify and/or select studies	Unclear	Since the full search strategy is not given, there is not enough information to assess whether it is likely that all relevant studies were identified.

Domain 3: Data collection and study appraisal

Signalling question	Rating	Reasoning
3.1 Were efforts made to minimise error in data collection?	Yes	Data was extracted independently by two reviewers and disagreements were resolved by consensus.
3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	Yes	Sufficient study characteristics are presented in a table.
3.3 Were all relevant study results collected for use in the synthesis?	Yes	
3.4 Was risk of bias (or methodological quality) formally assessed using appropriate criteria?	Yes	Both Jadad and Cochrane risk of bias tool were used to evaluate risk of bias.
3.5 Were efforts made to minimise error in risk of bias assessment?	Yes	Two reviewers independently assessed risk of bias.
Concerns regarding methods used to collect data and appraise studies	Low	All signaling questions were rated "Yes".

Domain 4: Synthesis and findings

Signalling question	Rating	Reasoning
4.1 Did the synthesis include all studies that it should?	Probably Yes	
4.2 Were all predefined analyses followed or departures explained?	Probably no	It is stated that subgroup analyses were performed, however no protocol was presented.

Signalling question	Rating	Reasoning
4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs and outcomes across included studies?	Yes	Meta-analysis was performed, which seems appropriate considering similar study design and low to moderate heterogeneity for the outcome "incidence of major bleeding" (<40%). Data was weighted according to study size.
4.4 Was between-studies variation (heterogeneity) minimal or addressed in the synthesis?	Yes	Heterogeneity for the outcome "incidence of major bleeding" was <40%.
4.5 Were the findings robust, e.g. as demonstrated through funnel plot or sensitivity analyses?	Yes	Funnel plot indicated no publication bias.
4.6 Were biases in primary studies minimal or addressed in the synthesis?	Yes	For the outcome of major bleeding, both fixed-effect model and random-effect model showed similar result, which indicates that the findings are robust.
Concerns regarding methods used to synthesize results	Unclear	There are some uncertainties regarding whether all analysis were predefined or not. However, overall, there are low concern regarding the synthesis and findings.

Phase 3: Judging risk of bias

Summary of concerns identified during the phase 2 assessment:

Domain	Concern	Rationale for concern
Concerns regarding specification of study eligibility criteria	Unclear	There is no information on whether the objectives and eligibility criteria were predefined and only studies available as full-length article was considered eligible. All other signalling questions were scored as "Yes".
Concerns regarding methods used to identify and/or select studies	Unclear	Since the full search strategy is not given, there is not enough information to assess whether it is likely that all relevant studies were identified.
Concerns regarding methods used to collect data and appraise studies	Low	All signaling questions were rated "Yes".
Concerns regarding the synthesis and findings	Unclear	There are some uncertainties regarding whether all analysis were predefined or not. However, overall, there are low concern regarding the synthesis and findings.

Signalling question	Rating	Reasoning
A. Did the interpretation of findings address all the concerns identified during the Phase 2 assessment?	No	All concerns identified were not addressed.
B. Was the relevance of identified studies to the review's research question appropriately considered	Probably Yes	
C. Did the reviewers avoid emphasizing results based on their statistical significance?	Yes	

Signalling question	Rating	Reasoning
Risk of bias	Unclear	Some concerns were identified and not addressed.

10.1.4 Data extraction forms

The detailed data extraction form for the systematic reviews are shown below.

Abdelhamid et al. (2020)

Characteristics of the systematic review	
Title	Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease (Review)
Author(s)	Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ, Deane KHO, Summerbell CD, Worthington HV, Song F, Hooper L.
Year of publication	2020
Journal	Cochrane Library: Cochrane Database of Systematic Reviews
Country of origin (corresponding author)	UK
Funding	Internal sources: University of East Anglia, UK. External sources: World Health Organization Nutrition Guidance Expert Advisory Group (they requested and funded the update and extension of this review); The National Institute for Health Research, via Cochrane Infrastructure funding to Cochrane Heart.
Reported conflict of interest	None known.
What is the main objective of the review?	To assess the effects of increased intake of fish- and plant-based omega-3 for all-cause mortality, cardiovascular events, adiposity and lipids.
Inclusion/Exclusion criteria	<p>Inclusion</p> <p>Type of study: RCTs that included diet advice or dietary supplementation to promote omega-3 fatty acid intake versus placebo, no supplementation, usual diet or lower-dose omega-3. One of the outcomes of interest had to be measured and be available (through publications or contact with authors). Only studies with follow-up for at least 12 months were included.</p> <p>Types of participants: Trials in adults (18 years or older, men and women) at any risk of cardiovascular disease (with or without existing cardiovascular disease) were eligible, including those in participants with increased risk of cancer, those undergoing or who have undergone coronary artery bypass grafting or angioplasty, and those with current or previous cardiovascular disease, nephritis in systemic lupus erythematosus, breast cysts, diabetes mellitus, rheumatoid arthritis, multiple sclerosis, psoriasis, hay fever, asthma or ulcerative colitis.</p>

	<p>Types of interventions: Dietary supplementation, a provided diet or advice on diet. The foodstuffs or supplements must have been oily fish; fish oils; linseed (flax), canola(rapeseed), perilla, purslane, mustard seed, candlenut, stillingia or walnut as a food, capsule, oil, made into a spreading fat or supplementing another food (such as bread or eggs). Refined EPA, DHA or ALAs, or concentrated fish or algal oils. Supplementation may have been in oil or capsule form or as foodstuffs provided to be consumed by mouth.</p> <p>Exclusion Trials using multiple risk factor interventions on lifestyle factors (such as weight reduction, smoking or physical activity goals), or differential dietary interventions not involving dietary fats, except where that other intervention was a direct replacement for polyunsaturated fats, or the effect of diet or supplementation could be separated out from the other interventions.</p>
Start and ending dates of the literature search	Searches were performed in 2019. As this was an update, date limits to the terms from the original search strategies were applied.
Population/Subjects	Adults with or without existing CVD from high-income countries living at home.
Intervention(s)	Dietary supplementation, a provided diet or advice on diet.
Comparator	Lower intake of LCn3 fats.
Primary outcome(s) of relevance	None relevant.
Secondary outcome(s) of relevance	Lipids
Tertiary outcome(s) of relevance	<p>Serious adverse events (any other reported illnesses) and side effects.</p> <p>Serious adverse events: any serious adverse event, bleeding, serious gastrointestinal events, pulmonary embolus or DVT, progression to advanced age-related macular degeneration, thrombophlebitis (no data identified), urolithiasis (no data identified).</p> <p>Side effects: withdrawal due to side effects, abdominal pain or discomfort, diarrhoea, nausea, any gastrointestinal side effect, skin problems, headache or worsening migraine, reflux, joint, lumbar and muscle pain, all side effects.</p>
Quality assessment tool(s)	The Cochrane criteria (Higgins, 2017).
Results of quality assessment	Summary risk of bias was low in 28 RCTs, moderate to high in the remainder.

Assessment of confidence of evidence	GRADE was performed for primary and secondary outcomes.
Data synthesis methodology	Dichotomous data were pooled using risk ratios (RR) to describe effect sizes and continuous data using mean differences (MD).
Number of included studies	86 RCTs, although the number of studies per outcome is lower, see results for overview.
Results (relevant) and the confidence in the evidence	<p>Lipids: duration of intervention was 12 to 72 months.</p> <ul style="list-style-type: none"> - High certainty evidence shows that long chain n-3 (LCn3) intake makes little or no difference to serum total cholesterol, 30 RCT with 38,469 participants. Mean Difference (IV, Random, 95% CI): 0.03 [0.01, 0.05]. Mean Difference (IV, Fixed, 95% CI): 0.01 [0.00, 0.02]. - High certainty evidence suggests that LCn3 intake has little or no effect on HDL cholesterol, 30 RCT, 46,604 participants. Mean Difference (IV, Fixed, 95% CI): 0.01 [0.00, 0.02]. - GRADE assessment suggests high certainty evidence that LCn3 intake makes little or no difference to LDL cholesterol. 25 RCTs, 43,454 participants. Mean Difference (IV, Random, 95% CI): 0.01 [-0.01, 0.03]. Mean Difference (IV, Fixed, 95% CI): 0.01 [-0.01, 0.03]. <p>Serious-adverse events:</p> <ul style="list-style-type: none"> - Bleeding (RR 1.12, 95% CI 0.91 to 1.37; I2 = 44%; 11 trials, 80,147 participants, 1324 events, duration of intervention: 12-72 months). Assessed as being very-low certainty evidence. Assessment of GRADE, reasons for downgrading: risk of bias: effect size changed direction (from harmful to protective) when analysis limited to trials at low summary risk of bias. Imprecision: 95% confidence intervals do not exclude large and important benefits or harms. - Serious gastrointestinal events (RR 1.34, 95% CI 0.64 to 2.80; I2=22%; 3 trials, 774 participants, 49 events). - Pulmonary embolus or DVT (RR 1.15, 95% CI 0.44 to 2.98; I2 =0%; 5 trials, 3546 participants, 20 events, duration of intervention 18-36 months). Assessed as being very-low certainty evidence. Assessment of GRADE, reasons for downgrading: Risk of bias: effect size suggested greater harm when analysis limited to trials at low summary risk of bias. Imprecision: 95% confidence intervals do not exclude large benefits or large harms. - Progression to advanced age-related macular degeneration (RR0.96, 95% CI 0.90 to 1.02; 1 trial, > 4000 participants, 2049 events). - Thrombophlebitis: no data identified - Urolithiasis: no data identified

	<p>Side effects</p> <ul style="list-style-type: none"> - Withdrawal due to side effects: the data suggest more participants taking LCn3 fats dropped out because of side effects (RR 1.16, 95% CI 0.99 to 1.36; I² = 1%; 23 trials, > 16,000 participants, 620 dropouts). - Increased abdominal pain or discomfort: data suggest an association with higher LCn3 (RR 1.05, 95% CI 0.91 to 1.20; I² = 16%; 9 trials, > 41,000 participants, 10,040 events). - Diarrhoea: the data suggested an increased risk with increased LCn3 (RR 1.02, 95% CI 0.87 to 1.19; I² = 49%; 13 trials, > 37,000 participants, 12,303 events). - Nausea: risk increased with LCn3 (RR 1.20, 95% CI 0.96 to 1.49; I² = 54%; 8 trials, > 35,000 participants, 7639 events). - Any gastrointestinal side effect: risk also appeared to increase with LCn3, albeit with very high heterogeneity (RR 1.10, 95% CI 0.97 to 1.26; I² = 74%; 33 trials, > 895,000 participants, 6651 events). - Skin problems, including itching or rashes: these were not affected by LCn3 in a meta-analysis with high heterogeneity (RR 1.11, 95% CI 0.52 to 2.37; I² = 68%; 9 trials, > 36,000 participants, 293 events). - Headache or worsening migraine: there were limited data on this outcome (RR 0.85, 95% CI 0.51 to 1.40; I² = 0%; 4 trials, 1526 participants, 60 events). - Reflux: there were limited data (RR 1.23, 95% CI 0.79 to 1.91; I² = 32%; 3 trials, > 8000 participants, 282 events). - Joint, lumbar and muscle pain: meta-analysis of data from three trials suggested that LCn3 had little or no effect on such pain (RR 0.95, 95% CI 0.74 to 1.23; > 27,000 participants, 989 people experienced pain). - All side effects: there was no suggestion that LCn3 increased or decreased all side effects combined in a meta-analysis with very high heterogeneity (RR 1.01, 95% CI 0.95 to 1.08; I² = 79%; 14 trials, > 39,000 participants, 9863 people with at least one side effect).
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Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo

<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age/ Gender</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect</i>
<i>ADCS 2010 (Alzheimer's Disease Cooperative Study) RCT, parallel</i>	<i>USA</i>	<i>18 months</i>	<i>Individuals with mild to moderate Alzheimer's disease. N: 238 intervention, 164 control.</i>	<i>Supplement (capsule). Intervention: 2 × 1 g algal-derived DHA capsules/day, each capsule contain 45%-55% of (950 mg soft-gel capsules, which contain</i>	<i>Adverse events (high vs low LCn3 fats); Pulmonary embolus (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats).</i>

			<p>Mean age in years (SD): 76 (9.3) intervention, 76 (7.8) control.</p> <p>Men: 52.9% intervention, 40.2% control.</p>	<p>approximately 510 mg DHA). Dose: +DHA 1.02 g/day.</p> <p>Control: 2 × 1 g placebo capsules/day (made up of corn or soy oil).</p>	
Afford 2013 RCT, parallel	Canada	12 months	<p>People with symptomatic paroxysmal or persistent atrial fibrillation.</p> <p>N: 165 intervention, 172 control (Analysed, intervention: 153 control: 163).</p> <p>Mean age in years (SD): 60 (12) intervention, 62 (13) control.</p> <p>Men: 69% intervention, 65% control.</p>	<p>Supplement (fish oil).</p> <p>4 × 1 g enteric-coated fish oil capsules/day (1.6 g/day EPA + 0.8 g/day DHA). Dose: 2.4 g /day EPA + DHA.</p> <p>Control: 4 × 1 g matching placebo capsules, 4 g/day safflower oil.</p>	Bleeding (high vs low LCn3 fats); CRP (data not usable)
Ahn 2016 RCT, parallel	South Korea	12 months	<p>Statin-treated coronary artery disease (CAD) patients undergoing percutaneous coronary intervention.</p> <p>N: 38 intervention, 36 control.</p> <p>Mean age in years (SD): 59.6 (9.1) intervention, 60.7 (0.8) [sic] control.</p> <p>Men: 63.2% intervention, 72.2% control.</p>	<p>Supplement (capsule).</p> <p>Intervention: 3 g of ω-3 PUFA containing 1395 mg of EPA and 1125 mg of DHA/day. Dose: +2.52 g /day EPA + DHA.</p> <p>Control: unclear whether control group were given placebo or only statins.</p>	Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats).

<p><i>AlphaOmega - EPA+DHA 2010</i></p> <p><i>RCT, 3 intervention arms. *Only data relevant for EPA/DHA is included here.</i></p>	<p><i>Netherlands</i></p>	<p><i>40 months</i></p>	<p><i>60–80-year-olds with previous myocardial infarction.</i></p> <p><i>N: 1192 EPA/DHA intervention, 1236 control.</i></p> <p><i>Men: 78.1% intervention, 78.7% control.</i></p> <p><i>Mean age in years (SD): 69.1 (5.6) intervention, 68.9 (5.6) control.</i></p>	<p><i>Supplementary margarine</i></p> <p><i>Intervention: 20 g/day enriched margarine incorporating 400 mg EPA-DHA (240 mg EPA and 160 mg DHA). Dose: average achieved 376 mg/day EPA + DHA.</i></p> <p><i>Control: 20 g/day margarine. No additional n-3 PUFAs. Identical margarine (oleic acid) placebo.</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats).</i></p>
<p><i>AREDS2 2014 (Age-Related Eye Disease Study 2)</i></p> <p><i>RCT, parallel, 2 × 2 factorial</i></p>	<p><i>USA</i></p>	<p><i>5 years</i></p>	<p><i>People aged 50-85 years at high risk of progression to advanced age-related macular degeneration.</i></p> <p><i>N: 2147 intervention (1068 DHA/EPA, 1079 DHA/EPA + lutein/zeaxanthin), 2056 control (1012 placebo, 1044 lutein/zeaxanthin).</i></p> <p><i>Age in years: intervention median 74.6 (IQR 11.1), control median 74 (IQR 11.1).</i></p> <p><i>Men: intervention 42.1%, control 44.4%.</i></p>	<p><i>Supplement (capsule).</i></p> <p><i>Intervention 350 mg/day DHA plus 650 mg/day EPA added to the standard AREDS supplement of Vitamin C (500 mg/day), Vitamin E (440 IU/day), beta-carotene (15 mg/d), zinc oxide (80 mg/day) and cupric oxide (2 mg/day). Dose: +1 g/day EPA + DHA.</i></p> <p><i>Control: standard AREDS supplement.</i></p>	<p><i>Progression to advanced age-related macular degeneration (high vs low LCn3 fats); skin problems (high vs low LCn3 fats).</i></p>
<p><i>ASCEND 2018</i></p> <p><i>RCT, parallel, 2 × 2 factorial (n-3 EPA + DHA vs MUFA) also</i></p>	<p><i>UK</i></p>	<p><i>7.4 years</i></p>	<p><i>People with diabetes, without apparent vascular disease (94% of participants in both arms had type 2 DM).</i></p>	<p><i>Supplement (capsule).</i></p> <p><i>Intervention: 840 mg /day EPA+DHA (460 mg /day EPA plus</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats).</i></p>

<i>randomised to aspirin vs placebo)</i>			<p><i>N: 7740 intervention, 7740 control.</i></p> <p><i>Age in years (SD): intervention 63.3 (9.2), control 63.3 (9.2).</i></p> <p><i>Men: intervention 62.6%, control 62.6%.</i></p>	<p><i>380 mg /day DHA) as 1 capsule daily.</i></p> <ul style="list-style-type: none"> <i>Arm 1: omega-3 (1 g/d: 0.41 g EPA, 0.34 g DHA) and placebo tablets for aspirin.</i> <i>Arm 3: omega-3 (1 g/d) and aspirin (100 mg/d) Control: 1 capsule /day of olive oil.</i> <i>Arm 2: aspirin (100 mg/d) and olive oil placebo capsule.</i> <i>Arm 4: olive oil placebo and placebo tablets for aspirin.</i> 	
<i>Berson 2004</i> <i>RCT, parallel</i>	<i>USA</i>	<i>48 months</i>	<p><i>People with retinitis pigmentosa.</i></p> <p><i>N: 221 randomised overall, analysed 105 intervention, 103 control.</i></p> <p><i>Mean age in years (SD): 37.8 (6.5) intervention, 36.0 (7.2) control. Age range: unclear (18-55 inclusion criterion).</i></p> <p><i>Men: 48% intervention, 54% control.</i></p>	<p><i>Supplement (DHA capsules).</i></p> <p><i>Intervention: 6 × 500 mg capsules/day of DHA (1.2 day DHA plus 1.8 g vegetable oil) plus < 0.0006 mg/day tocopherols plus 15,000 IU retinyl palmitate (vitamin A). Dose: 1.2 g/day DHA.</i></p> <p><i>Control: 6 × 500 mg capsules /day of soy and corn oils (half each) with 120 mg /day ALA, plus < 0.0006 mg /day tocopherols plus 15000 IU retinyl palmitate (vitamin A).</i></p>	<i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</i>
<i>Brox 2001</i> <i>RCT, parallel, 3 arms</i>	<i>Norway</i>	<i>14 months</i>	<i>People with moderate hypercholesterolaemia (mean baseline TG 204 mg/dL).</i>	<p><i>Supplement (oil).</i></p> <p><i>Intervention: seal oil – 15 mL /day (2.6 g, 1.1 g /day EPA + 1.5 /day DHA) (total n-3 3.9 g/d, total PUFA</i></p>	<i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats);</i>

			<p><i>N: 40 seal oil, 40 cod liver oil, 40 control (numbers analysed vary by outcome).</i></p> <p><i>Mean age in years: 53.2 seal oil, 55.0 cod liver oil, and 55.8 control. Age range: 43-66 years.</i></p> <p><i>Men: 53% seal oil, 50% cod liver oil, 48% control.</i></p>	<p><i>4.2 g/d): Seal oil dose: EPA + DHA 2.6 g/day. Cod liver oil – 15 mL /day (3.3 g, 1.5 g /day EPA + 1.8 g /day DHA) (total n-3 4.1 g/d, total PUFA 4.35 g/d): cod liver oil dose: EPA + DHA 3.3 g/day.</i></p> <p><i>Control: no supplement.</i></p>	
<p><i>Caldwell 2011</i></p> <p><i>RCT, parallel</i></p>	<p><i>USA</i></p>	<p><i>12 months</i></p>	<p><i>Participants with non-cirrhotic NASH (53% obese, mean 3.1 metabolic syndrome criteria, 35% diabetic, mean TG 191 mg/dL).</i></p> <p><i>N: 20 intervention, 21 control (analysed 17 intervention, 17 control).</i></p> <p><i>Mean age in years (SD): 46.4 (12.1) intervention, 47.2 (12) control. Age range: 25-72 years.</i></p> <p><i>Men: 35.3% intervention, 41.2% control.</i></p>	<p><i>Supplement (capsule).</i></p> <p><i>Intervention: 3 × 1 g fish oil capsules /day (Nordic Natural) for a total 2.1 g /day n-3, each capsule contained 70% of n-3 (1050 mg EPA, 750 mg DHA + 300 mg other n-3). Dose: 1.8 g /day EPA + DHA.</i></p> <p><i>Control: 3 × 1 g /day identical placebo (soybean) capsules containing 8% fish oils.</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</i></p>
<p><i>DART 1989 (Diet And Reinfarction Trial)</i></p> <p><i>RCT, parallel, 2 × 2 × 2 factorial</i></p>	<p><i>UK</i></p>	<p><i>2 years</i></p>	<p><i>Men recovering from myocardial infarction.</i></p> <p><i>N: 1015 intervention, 1018 control.</i></p> <p><i>Mean age, SD: 56.7 intervention, 56.4 control (SDs not stated).</i></p>	<p><i>Dietary advice (to eat more oily fish).</i></p> <p><i>Intervention: advised to eat at least 2 weekly portions of 200-400 g fatty fish (mackerel, herring, kipper, pilchard, sardine, salmon, and trout). If this was not possible, given MaxEPA capsules, 3 /day</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); Pulmonary embolus (high vs low LCn3 fats);</i></p>

				<p>(0.5 g EPA/d). 191/883 participants were taking MaxEPA at 2 years. Advice was reinforced 3-monthly. Dose: aimed for 0.5 g /day EPA.</p> <p>Control: no such dietary advice or capsules.</p>	
<p><i>Derosa 2016</i> RCT, parallel</p>	Italy	18 months	<p>White overweight/obese people with IFG or IGT (mean baseline TG 182 mg/dL).</p> <p>N: 138 intervention, 143 control (analysed 128 intervention, 130 control).</p> <p>Mean age in years (SD): 53.4 (11.2) intervention, 54.8 (12.1) control.</p> <p>Men: 50.72% intervention, 48.95% control.</p>	<p>Capsule (n-3 PUFA).</p> <p>Intervention: 3 × 1 g capsule /day n-3 PUFAs (ethyl esters, each 1-g capsule of n-3 PUFAs contains highly concentrated ethyl esters of omega-3 FAs, primarily EPA, and DHA in the proportion of 0.9–1.5). Dose: unclear (approx 2-3 g/d)</p> <p>Control: placebo (a capsule containing sucrose, mannitol and mineral salts, magnesium stearate (a saturated fat) and silicon dioxide, used as anti-caking agents).</p>	<p>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</p>
<p><i>Deslypere 1992</i> RCT, 4 arms, (n-3 EPA + DHA (3 different doses) vs MUFA)</p>	Netherlands	12 months	<p>Healthy monks</p> <p>N: 14 high-, 15 medium-, 15 low-dose intervention, 14 control.</p> <p>Mean age in years (SD): 56.2 (16.5) (not reported by arm) Age range: 21-87. Men: 100%.</p>	<p>Capsules.</p> <p>Intervention 9 capsules (9 g vol)/d, of which 3, 6 or 9 were fish oil and any remainder were placebo (providing respectively 1.12; 2.24 or 3.37 g n-3 FA/day). Dose: 1.12 g/d; 2.24 g /day or 3.37 g /day EPA + DHA).</p>	<p>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</p>

				Control: 9 placebo capsules made up of olive oil and Palmoil with the same SFA, cholesterol and vitamin E as the fish oil capsules.	
<i>DIPP 2015 (Dietary Intervention for Patients Polypectomized for tumours of the colorectum).</i> <i>RCT, parallel, 2 arms</i>	Japan	24 months	<i>People previously polypectomised for colorectal tumours.</i> <i>N: 104 intervention, 101 control.</i> <i>Mean age in years (SD): 58.3 (9.5) intervention, 59.7 (8.9) control. Age range: 35-75.</i> <i>Men: 73.1% intervention, 74.3% control.</i>	<i>Advice + supplement (fish oil capsules).</i> <i>Intervention: advice to reduce total fat intake, decrease consumption of n-6 PUFAs, increase intake of n-3 PUFAs from fish/marine foods, increase intake of n-3 PUFAs from perilla oil rich in ALA, take 8 capsules of fish oil /day (equivalent to 96 mg /day of EPA and 360 mg /day of DHA). Dose: 456 mg /day EPA + DHA and unknown dose of ALA.</i> <i>Control: advice to decrease intake of fats/oils as a whole.</i>	<i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats);</i>
<i>DO IT 2010 (Diet and Omega-3 Intervention Trial on atherosclerosis).</i> <i>RCT, parallel, 2 × 2 factorial</i>	Norway	36 months	<i>Elderly men with longstanding dyslipidaemia or hypertension (a subset of Oslo Diet heart trial) (mean baseline TG 1.8 mmol/L).</i> <i>N: intervention 282 (140 n-3 capsules + 142 n-3 capsules and dietary advice), control 281 (142 placebo capsules + 139 placebo capsules and dietary advice).</i>	<i>Supplement/capsule (also dietary advice as the factorial intervention).</i> <i>Intervention: 2 × 2 capsules /day including 2.4 g /day of omega-3 PUFA (Pikasol, 0.84 g /day EPA plus 0.48 g /day DHA plus 8.4 mg /day tocopherols). Dose: 1.32 g /day EPA + DHA.</i> <i>Control: 2 × 2 capsules /day including 4 g /day corn oil (2.24 g</i>	<i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats);</i>

			<p>Mean age in years (SD): intervention 70.4 (2.9), control 69.7 (3.0) years Age range: 64-76 years.</p> <p>Men: intervention 100%, control 100%.</p>	<p>/day linoleic, 1.28 g /day oleic acid, 16 mg /day tocopherols).</p>	
<p>Dream Asbell 2018 (Dry Eye Assessment and Management (DREAM) Study).</p> <p>RCT, parallel</p>	USA	12 months	<p>Adults with dry eye.</p> <p>N: 349 intervention randomised, 186 control randomised.</p> <p>Mean age in years (SD): 58.3 (13.5) intervention, 57.5 (12.6) control.</p> <p>N: 349 intervention randomised, 186 control randomised.</p>	<p>Supplement.</p> <p>Intervention: omega-3 supplements (2000 mg EPA + 1000 mg DHA /day as 5 gel caps). Dose: 3.0 g /day LCn3.</p> <p>Control: olive oil supplements (5 gel caps).</p>	<p>Serious adverse events (high vs low LCn3 fats); Bleeding (high vs low LCn3 fats); Pulmonary embolus (high vs low LCn3 fats); Abdominal pain or discomfort (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats); Nausea (high vs low LCn3 fats); skin problems (high vs low LCn3 fats); Headache or worsening migraine (high vs low LCn3 fats); Reflux (high vs low LCn3 fats); Joint, lumbar, muscle pain (high vs low LCn3 fats).</p>
<p>Energise 2018 (ENabling Reduction of low-Grade Inflammation in</p>	USA	12 months	<p>People aged ≥ 70 years with self-reported walking or stair-climbing difficulty.</p>	<p>Supplement.</p> <p>Intervention</p>	<p>Serious GI events (high vs low LCn3 fats).</p>

<p><i>SEniors (ENRGISE) pilot study</i></p> <p><i>RCT, 2x2</i></p>			<p><i>N: 148 intervention randomised (122 fish oil only, 26 fish oil plus losartan), 141 control randomised (102 placebo only, 39 placebo plus losartan).</i></p> <p><i>Mean age in years (SD): 78 (5.6) intervention, 77 (5.3) control.</i></p> <p><i>Men: 53% intervention, 53% control.</i></p>	<ul style="list-style-type: none"> • <i>Arm 1: omega-3 fish oil (1.4 g /day for 6 months, discontinued if AF or intolerance at 6 months, increased to 2.8 g /day if IL-6 remained high at 6 months).</i> • <i>Arm 4: omega-3 plus losartan Dose: 1.2 g /day LCn3 (0.8 g /day EPA plus 0.4 g /day DHA), increasing to 2.4 g /day (1.6 g /day EPA plus 0.8 g /day DHA) in some from 6-12 months.</i> • <i>Control:</i> • <i>Arm 2: losartan 25 mg/d.</i> • <i>Arm 3: placebo corn oil (for omega-3) plus placebo cellulose (for losartan).</i> • <i>Arm 5: placebo corn oil (for omega-3).</i> • <i>Arm 6: placebo cellulose (for losartan).</i> <p><i>Fish oil and corn oil placebo sourced from Epax, 0.7 g gel capsules, identical shape, colour, weight.</i></p>	
<p><i>EPE-A 2014</i></p> <p><i>RCT, parallel, 3 arms</i></p>	<p><i>USA</i></p>	<p><i>12 months</i></p>	<p><i>People with NASH and NAFLD (mean BMI 33.6, mean TG 139 mg/dL, 30.7% diabetic).</i></p> <p><i>N: 86 intervention-high, 82 intervention low, 75 control</i></p>	<p><i>Supplement (capsule).</i></p> <p><i>Comparison 1: high EPA vs low EPA (unclear what replaced EPA)</i></p> <p><i>Comparison 2: EPA vs unclear (placebo contents not reported).</i></p> <p><i>Intervention-high: EPA-E 2.7 g/d,</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats); Abdominal pain or discomfort (high vs</i></p>

			<p>(analysed 64, 55, 55 respectively, ITT analysis for primary outcomes).</p> <p>Mean age in years (SD): 47.8 (11.1) intervention-high, 47.8 (12.5) intervention-low, 50.5 (12.5) control.</p> <p>Men: 33.7% intervention-high, 41.5% intervention-low, 42.7% control</p>	<p>3 × EPA-E 300 mg capsules. Dose: 2.7 g /day EPA + DHA Intervention-low: EPA-E 1.8 g/d, 2 × EPA-E 300 mg capsules + 1 placebo capsule Dose: 1.8 g /day EPA + DHA.</p> <p>Control: 3 × placebo capsules. The pills were identical with respect to size, colour and smell.</p>	<p>low LCn3 fats); Diarrhoea (high vs low LCn3 fats); Nausea (high vs low LCn3 fats); skin problems (high vs low LCn3 fats); skin problems (high vs low LCn3 fats); Headache or worsening migraine (high vs low LCn3 fats);</p>
<p>EPIC-1 2008 (EPANOVA in Crohn's disease, study 1) RCT, parallel, 2-arm</p>	<p>Canada, Europe, Israel, USA</p>	<p>52 weeks</p>	<p>Adults with quiescent Crohn's disease, CDAI score < 150.</p> <p>N: 188 intervention, 186 control.</p> <p>Mean age in years (SD): 40.5 (15.2) intervention, 38.2 (13.1) control Age range: 18-70 years Smokers: 30.6% intervention.</p> <p>Men: 48.1% intervention, 41.1% control.</p>	<p>Supplement (capsule).</p> <p>Intervention: 2 × 2 1 g gelatin capsules omega-3 free FAs (Epanova- 2.2 g EPA, 0.8 g DHA). Dose: 3 g /day EPA + DHA.</p> <p>Control: 4 x1 g capsules MCT.</p>	<p>Bleeding (high vs low LCn3 fats); Abdominal pain or discomfort (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats); Nausea (high vs low LCn3 fats); skin problems (high vs low LCn3 fats); Headache or worsening migraine (high vs low LCn3 fats);</p>
<p>EPIC-2 2008 (EPANOVA in Crohn's Disease, Study 2) RCT, parallel, 2 arms</p>	<p>Canada, Europe, Israel, USA</p>	<p>58 weeks</p>	<p>Adults with a confirmed diagnosis of Crohn's Disease and a CDAI score < 150 who are responding to steroid induction therapy.</p> <p>N: intervention, 189, control 190 (187 intervention, 188 control analysed).</p>	<p>Supplement (capsule).</p> <p>Intervention: 2 × 2 1 g gelatin capsules omega-3 free FAs (Epanova) providing total dose ~2.2 g /day EPA, 0.8 g /day DHA. Dose: ~3.0 g /day EPA + DHA.</p>	<p>Bleeding (high vs low LCn3 fats); Abdominal pain or discomfort (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats); Nausea (high vs low LCn3 fats); skin problems (high vs low</p>

			<p>Mean age in years (SD): 38.5 (13.8) intervention, 40.0 (13.6) years control. Age range: > 16 years.</p> <p>Men: 48.1% intervention, 41.1% control.</p>	<p>Control: 2 × 2 1 g capsules MCT oil.</p>	<p>LCn3 fats); Headache or worsening migraine (high vs low LCn3 fats);</p>
<p>Fostar 2016 (Fish Oil in knee OSTeoARthritis)</p> <p>RCT, parallel</p>	<p>Australia</p>	<p>24 months</p>	<p>Adults aged 40+ years with knee osteoarthritis.</p> <p>N: 101 intervention, 101 control.</p> <p>Mean age in years (SD): 60.8 (10) intervention, 61.1 (10). Age range: > 40.</p> <p>Men: 41% intervention, 60% control.</p>	<p>Supplementary food (enriched orange juice).</p> <p>Intervention: 1-3 × a day drink of fruit juice mixed with day total = 15 mL of fish oil supplement (18% EPA, 12% DHA, 4.5 g/day total omega-3). Dose: 4.5 g /day EPA + DHA.</p> <p>Control: liquid oral oil 15 mL Sunola oil/day (which contains fish oil 2 mL plus 13 mL canola oil) (total omega-3 fat: ≥ 0.45 g EPA + DHA from 15 mL).</p>	<p>Bleeding (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats); Nausea (high vs low LCn3 fats); Reflux (high vs low LCn3 fats);</p>
<p>Franzen 1993</p> <p>RCT, parallel</p>	<p>Germany</p>	<p>12 months</p>	<p>Adults with documented CHD.</p> <p>N: 15 intervention, 15 control.</p> <p>Mean age in years (SD): 52 (9) intervention, 54 (7) control.</p> <p>Gender: unclear.</p>	<p>Fish oil capsules.</p> <p>Intervention: 9 × 1 g capsules /day of fish oils (20% EPA, 15% DHA, 3.15 g /day total omega-3). Dose: 3.15 g /day EPA + DHA.</p> <p>Control: 9 × 1 g capsules /day olive oil (which contains 6.3 g/day MUFA, 1.35 g/day SFA, 1.35 g /day total omega-6 fat).</p>	<p>Total cholesterol (high vs low LCn3 fats);</p>

<p><i>GISSI-HF 2008</i> (Gruppo Italiano per la Sperimentazione della Streptochinasi nell'Infarto miocardico – Heart Failure)</p> <p><i>RCT, parallel, 2 arms</i></p>	<p><i>Italy</i></p>	<p><i>3.9 years</i></p>	<p><i>People with chronic heart failure.</i></p> <p><i>N: 3494 intervention, 3481 control.</i></p> <p><i>Mean age: 67 intervention, 67 control.</i></p> <p><i>Men: 77.8% intervention, 78.8% control.</i></p>	<p><i>Intervention: 1 capsule/d of 1 g n-3 mainly EPA and DHA as ethyl esters in the average ratio of 1:1.2. Dose: ~0.866 g/d EPA + DHA.</i></p> <p><i>Control: 1 g/d matching olive oil placebo capsule</i></p>	<p><i>Heammorhagic stroke</i></p>
<p><i>HARP 1995 (Harvard Atherosclerosis Reversibility Project (HARP))</i></p> <p><i>RCT</i></p>	<p><i>USA</i></p>	<p><i>24 months</i></p>	<p><i>People with CHD.</i></p> <p><i>N: 41 intervention, 39 control (99.9% follow-up at trial end).</i></p> <p><i>Mean age in years (SD): 62 (7) intervention, 62 (7) years control. Age range: 30-75.</i></p> <p><i>Men: 93.5% intervention, 92.9 % control.</i></p>	<p><i>Supplement (capsule).</i></p> <p><i>Intervention: 12 fish oil capsules /day in divided doses, preferably after meals. Each fish oil capsule contained 500 mg of n-3 polyunsaturated FAs composed of EPA (240 mg), DHA (160 mg) and other (100 mg) (mainly DPA) providing total daily dose of 6 g of n-3 FAs. Dose: 6 g /day LCn3.</i></p> <p><i>Control: olive oil capsules identical in appearance to the fish oil capsules.</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</i></p>
<p><i>HEARTS 2017 (Slowing HEART diSease with lifestyle and omega-3 fatty acids)</i></p> <p><i>RCT</i></p>	<p><i>USA</i></p>	<p><i>30 months</i></p>	<p><i>People with stable coronary artery disease on statins.</i></p> <p><i>N: 143 intervention randomised, 126 in ITT analysis, 142 control randomised, 114 in ITT analysis.</i></p>	<p><i>Supplement (capsule).</i></p> <p><i>Intervention: LCn3 ethyl esters from fish oil, 4 x 1000 mg capsules/day. 3.36 g /day LCn3 (1.86 g /day EPA, 1.5 g /day DHA).</i></p> <p><i>Control: nil (no placebo).</i></p>	<p><i>Serious GI events (high vs low LCn3 fats);</i></p>

			<p>Mean age in years (SD): 62.5 (7.8) intervention, 63.5 (7.6) years control.</p> <p>Men: 84.9% intervention, 85.1% control.</p>		
<p>JELIS 2007 (Japan Eicosapentaenoic acid Lipid Intervention Study)</p> <p>RCT, parallel, 2-arm</p>	Japan	5 years	<p>People with hypercholesterolaemia (mean baseline TG 1.7mmol/L).</p> <p>N: intervention, 9326, control 9319 (analysed intervention 9326, control 9319).</p> <p>Mean age in years (SD): 61 (8) intervention 61 (9) control. Age range: 40-75 years.</p> <p>Men: 32% intervention, 31% control.</p>	<p>Supplement (EPA capsule).</p> <p>Intervention: 3 × 2 × 300 mg capsules /day EPA ethyl ester (total dose of 1.8 g /day EPA), after meals. Dose: 1.8 g /day EPA.</p> <p>Control: nothing (though all in both groups received "appropriate" dietary advice).</p> <p>All participants in both groups were on statins.</p>	<p>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats); Bleeding (high vs low LCn3 fats); skin problems (high vs low LCn3 fats); Joint, lumbar, muscle pain (high vs low LCn3 fats);</p>
<p>Lorenz-Meyer 1996</p> <p>RCT, parallel, 2 arms</p>	Germany	12 months	<p>People with Crohn's disease in remission (but with a recent relapse).</p> <p>N: 70 intervention, 63 control.</p> <p>Mean age in years (SD): 29.5 (9.6) intervention, 31.8 (10.9) control. Age range: 17-62 years intervention, 17-65 years control.</p> <p>Men: 35.7% intervention, 27.0% control.</p>	<p>Supplement (fish oil).</p> <p>Intervention: 2 × 3 1 g gelatin capsules /day of ethylester fish oil concentrate (3.3 g /day EPA + 1.8 g /day DHA). Dose: 5.1 g /day EPA + DHA.</p> <p>Control: 2 × 3 1 g gelatin capsules /day of corn oil.</p>	<p>Abdominal pain or discomfort (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats); skin problems (high vs low LCn3 fats);</p>

<p><i>MARINA 2011</i> (Modulation of Atherosclerosis Risk by INcreasing dose of n-3 fatty Acids)</p> <p>RCT, parallel, 4 arms</p>	<p>UK</p>	<p>12 months</p>	<p>Non-smoking men and women aged 45-70 years.</p> <p>N: intervention. 279 in 3 groups (G1 0.45 g /day n = 94, G2 0.9 g /day n = 93, G3 1.8 g /day n = 92); control: 88 (analysed G1 0.45 g /day n = 81, G2 0.9 g /day n = 80, G3 1.8 g /day n = 80, control 71).</p> <p>Mean age in years (CI): G1: 55 (53, 56), G2: 55 (54, 56), G3: 55 (54, 57) intervention 55 (54,57) control. Age range: 45-70.</p> <p>Men: 38.7% intervention, 38.6% control.</p>	<p>Supplement (fish oil capsules).</p> <p>Intervention: 3 × 1 g oil gelatin capsule /day consisting of blend of EPA concentrate, DHA concentrate, refined olive oil and 0.1% peppermint oil. Providing a daily dose of: 0.45 g, 0.9 g, or 1.8 g /day (all with EPA/ DHA ratio of 1.51). Dose: 1.8 g /day EPA + DHA (G3 used for outcomes).</p> <p>Control: 3 gelatin capsules/ day containing refined olive oil + 0.1% peppermint oil.</p>	<p>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</p>
<p><i>Mita 2007</i></p> <p>RCT, parallel</p>	<p>Japan</p>	<p>2 years</p>	<p>Japanese people with type 2 diabetes (mean baseline TG 1.5mmol/L).</p> <p>N: intervention. 40, control: 41 (analysed 30, 30).</p> <p>Mean age in years (SD): 59 (11.2) intervention 61.2 (8.4) control.</p> <p>Men: 53% intervention, 67% control.</p>	<p>Supplement (EPA oil capsules).</p> <p>Intervention: 1800 mg /day EPA EPADEL capsules (Mochida Pharmaceutical Co Ltd Japan): 98% pure ethyl-ester EPA (unclear how many caps). Dose: ~1.8 g /day EPA.</p> <p>Control: no intervention.</p>	<p>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats);</p>
<p><i>NAT2 2013 (Nutritional AMD Treatment-2)</i></p> <p>RCT, parallel</p>	<p>France</p>	<p>36 months</p>	<p>People with early AMD.</p> <p>N: 150 intervention, 150 control.</p>	<p>Supplement (fish oil capsule).</p> <p>Intervention: 3 daily fish oil capsules containing 1110 total n-3</p>	<p>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</p>

			<p>Mean age in years (SD): 73.9 (6.6) intervention, 73.2 (6.8) control. Age range: 55-85.</p> <p>Men: 31.3% intervention, 39.5% control.</p>	<p>FAs (EPA: 270 mg /day DHA: 840 mg/d) and vit E: 6 mg/day. Dose: 1.1 g /day EPA + DHA.</p> <p>Control: 3 × 602 mg /day olive oil capsules containing 0.2 g total PUFA and vitamin E: 0.09 g/day.</p>	<p>Pulmonary embolus (high vs low LCn3 fats);</p>
<p>Nodari 2011 HF RCT, parallel</p>	<p>Italy</p>	<p>12 months</p>	<p>People with heart failure (non-ischaemic dilated cardiomyopathy).</p> <p>N: 67 intervention, 66 control (analysed, intervention: 67 control: 66).</p> <p>Mean age in years (SD): 61 (11) intervention, 64 (9) control.</p> <p>Men: 95.5% intervention, 84.9% control.</p>	<p>Supplement (Omacor).</p> <p>Intervention: 2 × 1 g /day Omacor (1.7 g /day EPA + DHA at a ratio of 0.9:1.5).</p> <p>Control: 2 × 1 g /day olive oil (gelatin capsules identical in appearance to Omacor).</p>	<p>Total cholesterol (high vs low LCn3 fats);</p>
<p>Nye 1990 Randomisation: parallel, 3 groups</p>	<p>New Zealand</p>	<p>1 year</p>	<p>People undergoing PTCA.</p> <p>N: 36 intervention, 37 control (also 35 allocated to arm 3, aspirin and dipyridamole).</p> <p>Mean age in years (SD): 54 (8) intervention, 55 (8) control years.</p> <p>Men: 78% intervention, 76% control.</p>	<p>Supplement (capsules).</p> <p>Intervention: MaxEPA capsules 12 /day (2.2 g EPA). Dose: 2.2 g /day EPA.</p> <p>Control: olive oil capsules, 12/d, identical to MaxEPA.</p> <p>Both capsules included vitamin E.</p>	<p>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats);</p>

<p><i>OFAMI 2001 (Omacor Following Acute Myocardial Infarction)</i></p> <p><i>RCT, parallel, 2 arms</i></p>	<p><i>Norway</i></p>	<p><i>2 years</i></p>	<p><i>Participants recruited 4-8 days after confirmed MI.</i></p> <p><i>N: 150 intervention, 150 control.</i></p> <p><i>Mean age in years (SD): 64.4 intervention, 63.6 control (no SD).</i></p> <p><i>Age range: 28-86 years intervention, 29-87 years control.</i></p> <p><i>Men: 77% intervention, 82% control.</i></p>	<p><i>Supplement (capsules).</i></p> <p><i>Intervention: 4 gelatin capsules of omega-3-acid ethyl esters 90, each is 1 g containing 850-882 mg EPA and DHA as concentrated ethylesters. Dose ~3.4- 3.5 g /day EPA + DHA.</i></p> <p><i>Control: corn oil capsules, 4/d, each contains 1 g of corn oil.</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); Pulmonary embolus (high vs low LCn3 fats);</i></p>
<p><i>OPAL 2010 (Older People And n-3 Long-chain PUFA)</i></p> <p><i>2-arm, parallel, RCT</i></p>	<p><i>England and Wales</i></p>	<p><i>12 months</i></p>	<p><i>Healthy cognitively normal adults aged 70-79 years.</i></p> <p><i>N: 434 intervention, 433 control (analysed 376 intervention, 372 control).</i></p> <p><i>Mean age in years (SD): 74.7 (2.5) intervention, 74.6 (2.7) control. Age range: 70-79 years.</i></p> <p><i>Men: 53.4% intervention, 56.6% control.</i></p>	<p><i>Supplement (capsules).</i></p> <p><i>Intervention: 2 × 650 mg capsule /day Ocean Nutrition vanilla flavoured soft gelatin capsule (total daily dose of 200 mg EPA and 500 mg DHA). Dose: 0.7 g /day EPA + DHA.</i></p> <p><i>Control: 2 × 650 mg olive oil capsule identical to intervention.</i></p>	<p><i>Abdominal pain or discomfort (high vs low LCn3 fats);</i></p>
<p><i>ORIGIN 2012 (Outcome Reduction with Initial Glargine Intervention)</i></p> <p><i>RCT, 2 × 2 factorial</i></p>	<p><i>40 trial locations in Europe and the Americas</i></p>	<p><i>72 months</i></p>	<p><i>People at high risk of CV events with IFG, IGT or diabetes (mean baseline TG 140 mg/dL, 88.4% had diabetes).</i></p>	<p><i>Supplement capsule (Omacor).</i></p> <p><i>Intervention: 1 gelatin capsule /day Omacor containing at least 900 mg ethyl esters of n-3 fats (465 mg EPA + 375 mg DHA). Dose: 0.84 g /day EPA + DHA.</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats); Bleeding (high vs low LCn3 fats); Abdominal pain or discomfort</i></p>

			<p><i>N: 6319 intervention, 6292 control (analysed, intervention: 6281 control: 6255).</i></p> <p><i>Mean age in years (SD): 63.5 (7.8) intervention, 63.6 (7.9) control.</i></p> <p><i>Men: 65.4% intervention, 64.7% control.</i></p>	<p><i>Control: 1 × 1 g gelatin capsule /day olive oil.</i></p>	<p><i>(high vs low LCn3 fats);</i></p>
<p><i>ORL 2013 (Omega-3 FAs randomised long-term)</i></p> <p><i>RCT- parallel, 3 arms</i></p>	<p><i>Japan</i></p>	<p><i>12 months</i></p>	<p><i>Japanese adults with hypertriglyceridaemia (mean baseline TG 263 mg/dL).</i></p> <p><i>N: 171 intervention (4 g TAK), 165 control (2 g TAK).</i></p> <p><i>Mean age in years (SD): 55.9 (10.12) intervention, 56 (10.95) control. Age range: 20-74.</i></p> <p><i>Men: 70.8% intervention, 71.5% control.</i></p>	<p><i>Supplement (TAK-085 capsules).</i></p> <p><i>Intervention: 1 × 2 /day capsule each containing 2 g of TAK-085 (1 g of FA in TAK-085 capsules contains approximately 465 mg of EPA-E plus 375 mg of DHA-E). Total dose of 1.86 g /day EPA + 1.5 g /day DHA. Dose: ~3.4 g /day EPA + DHA (difference of +1.7 g /day from control arm).</i></p> <p><i>Control: 1 capsule /day containing 2 g of TAK-085 (1 g of FA in TAK-085 capsules contains approximately 465 mg of EPA-E plus 375 mg of DHA-E). Total dose of 0.93 g /day EPA + 0.75 g /day DHA. Dose: 1.7 g /day EPA + DHA.</i></p>	<p><i>LDL (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats);</i></p>
<p><i>Puri 2015</i></p> <p><i>RCT, parallel</i></p>	<p><i>Australia, Canada, UK, USA</i></p>	<p><i>12 months</i></p>	<p><i>People with Huntington's disease.</i></p>	<p><i>Supplement (ethyl-EPA).</i></p> <p><i>Intervention: 2 × 2 × 500 mg capsules/d, total dose of 2 g /day ethyl-EPA (code name LAX-101,</i></p>	<p><i>Diarrhoea (high vs low LCn3 fats);</i></p>

			<p><i>N: 67 intervention, 68 control (analysed, intervention: 39 control: 44).</i></p> <p><i>Mean age in years (SD): 50 (9.3) intervention, 49 (9.0) control.</i></p> <p><i>Men: 57% intervention, 44% control.</i></p>	<p><i>purity 95%). Dose: 1.9 g /day EPA.</i></p> <p><i>Control: 2 × 2 × 500 mg capsules /day liquid paraffin.</i></p>	
<p><i>Raitt 2005</i></p> <p><i>RCT, parallel</i></p>	<p><i>USA</i></p>	<p><i>24 months</i></p>	<p><i>People with implantable cardioverter defibrillators and recent sustained VT/VF.</i></p> <p><i>N: 100 intervention, 100 control.</i></p> <p><i>Mean age in years (SD): 63 (13) intervention, 62 (13) control.</i></p> <p><i>Men: 86% intervention, 86% control.</i></p>	<p><i>Supplement (fish oil capsules vs olive oil capsules).</i></p> <p><i>Intervention: 1.8 g /day fish oil capsules (including ethyl esters of EPA and DHA, 0.76 g/ d EPA, 0.54 g /day DHA). Dose: 1.3 g /day EPA + DHA.</i></p> <p><i>Control: 1.8 g /day olive oil capsules (73% oleic acid).</i></p>	<p><i>Serious GI events (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats);</i></p>
<p><i>REDUCE-IT 2019</i></p> <p><i>(Reduction of cardiovascular events with EPA - intervention trial)</i></p> <p><i>RCT, parallel</i></p>	<p><i>USA, Netherlands, Ukraine, Russia, South Africa, Poland, India, Romania, Australia, New Zealand (westernised 71%,</i></p>	<p><i>4.9 years (median)</i></p>	<p><i>People (45 years+) with hypertriglyceridaemia, and with CVD or with DM and another risk factor, and on statin (58% had DM at baseline, mean baseline TG > 200 mg/dL).</i></p> <p><i>N: intervention 4089 randomised, control 4090 randomised (analysed, intervention: 4083 control: 4077).</i></p> <p><i>Age median (IQ range) years: median 64 (57-69) intervention, 64</i></p>	<p><i>Supplement.</i></p> <p><i>Intervention: EPA ethyl ester derived from fish oil (AMR101 4 g/d, Amarin), 3.99 g /day EPA plus 8 mg /day vitamin E (2 capsules twice/day).</i></p> <p><i>Control: 3.73 g /day light liquid paraffin oil in 4 capsules (2 capsules twice a day).</i></p>	<p><i>HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats); Serious adverse events (high vs low LCn3 fats); Bleeding (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats); Nausea (high vs low LCn3 fats); Reflux (high vs low LCn3 fats);</i></p>

	<i>Eastern Europe 26%, Asia Pacific 3%</i>		<i>(57-69) control. Age range: not reported those with CVD included if at least 45 years, those with DM if at least 50 years old, 45.4% intervention and 46.6% control groups aged 65+ years.</i> <i>Men: 71.6% intervention, 70.8% control.</i>		
<i>Risk and prevention 2013</i> <i>RCT, parallel</i>	<i>Italy</i>	<i>60 months</i>	<i>Patients with multiple CV risk factors (59.9% had diabetes).</i> <i>N: 6244 intervention, 6269 control (analysed, intervention: 6239 control: 6266).</i> <i>Mean age in years (SD): 63.9 (9.3) intervention, 64.0 (9.6) control.</i> <i>Men: 62.3% intervention, 60.6% control.</i>	<i>Supplement (n-3 capsules).</i> <i>Intervention: 1 g /day n-3 capsules polyunsaturated FA ethyl esters (EPA and DHA content 850-882 mg with an average ratio of 1.0:1.2). Dose: ~0.87 g /day EPA + DHA.</i> <i>Control: 1 g /day olive oil capsules.</i>	<i>Bleeding (high vs low LCn3 fats); skin problems (high vs low LCn3 fats); Joint, lumbar, muscle pain (high vs low LCn3 fats);</i>
<i>Rossing 1996</i> <i>RCT, parallel</i>	<i>Denmark</i>	<i>12 months</i>	<i>Adults with insulin-dependent DM, diabetic nephropathy and normal BP (mean baseline TG 1.0 mmol/ L).</i> <i>N: 18 intervention, 18 control (analysed, 17 intervention, 15 control).</i> <i>Mean age (SD) years: 32 (7) intervention, 34 (10) control.</i> <i>Age range: 18-55 years.</i>	<i>Supplement.</i> <i>Intervention: cod-liver oil emulsion. EPA 2 g, DHA 2.6 g, total PUFA 4.6 g/day. Dose: 4.6 g /day EPA + DHA.</i> <i>Control: olive oil emulsion.</i>	<i>Total cholesterol (high vs low LCn3 fats); LDL (high vs low LCn3 fats); Nausea (high vs low LCn3 fats);</i>

<p><i>Sandhu 2016</i></p> <p><i>RCT, parallel, 5 arms (combined groups 4 and 5 omega-3-acid ethyl esters (Lovaza) n-3 ± raloxifene vs control groups 1 and 3 ± raloxifene),</i></p>	<p>USA</p>	<p>24 months</p>	<p><i>Healthy postmenopausal women (50% normal weight, 30% overweight, 20% obese) with high breast density detected on their routine screening mammograms.</i></p> <p><i>N: 54 + 53 intervention, 53 + 53 control.</i></p> <p><i>Mean age in years (SD): 56.56 (6.9) + 57.85 (5.1) intervention, 57.11 (5.9) + 57.68 (5.1) control.</i></p> <p><i>Men: 0% intervention, 0% control.</i></p>	<p><i>Supplement (n-3 capsules).</i></p> <p><i>Intervention: group 4, Lovaza 4 g/day. Lovaza is the FDA-approved n-3 FA formulation containing 465 mg of EPA + 375 mg of DHA per gram, total dose; 1860 mg /day EPA, 1500 mg /day DHA. Group 5 as group 4 plus 30 mg raloxifene/day. Dose: 3.36 g /day EPA + DHA.</i></p> <p><i>Control: group 1, no treatment; group 3, 30 mg raloxifene/day.</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats); skin problems (high vs low LCn3 fats);</i></p>
<p><i>SCIMO 1999 (Study on prevention of coronary atherosclerosis with marine omega-3 FAs)</i></p> <p><i>RCT, parallel</i></p>	<p>Germany</p>	<p>2 years</p>	<p><i>People with angiographically proven coronary artery disease.</i></p> <p><i>N: 112 intervention, 111 control (analysed 82 intervention, 80 control).</i></p> <p><i>Mean age in years (SD): 57.8 (9.7) intervention, 58.9 (8.1) control.</i></p> <p><i>Men: 82% intervention, 78.6% control.</i></p>	<p><i>Supplement (capsule).</i></p> <p><i>Intervention: concentrated fish oil capsules, 6 x 1 g capsules /day for first 3 months, 3 x 1 g /day for rest of trial (4 g /day EPA +DHA + DPA + ALA for first 3 months, then 2 g/d). Dose: ~2 g /day LCn3.</i></p> <p><i>Control: capsules containing fat that replicated the fat composition of the average European diet, 6 /day for first 3 months, 3 /day for rest of trial, opaque soft gelatin capsules identical to fish capsules in identical screw-top containers.</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats); Abdominal pain or discomfort (high vs low LCn3 fats); skin problems (high vs low LCn3 fats);</i></p>

<p><i>Shinto 2014</i></p> <p><i>RCT, parallel</i></p>	<p>USA</p>	<p>12 months</p>	<p>People aged ≥ 55 with probable Alzheimer dementia diagnosis.</p> <p>N: 13 intervention, 13 control.</p> <p>Mean age in years (SD): 75.9 (8.1) intervention, 75.2 (10.8) control.</p> <p>Men: 61% intervention 46% control.</p>	<p>Fish oil capsules.</p> <p>Intervention: 3 \times 1 g capsules /day of fish oils (975 mg EPA, 675 mg DHA/d). Dose: 1.65 g /day EPA + DHA.</p> <p>Control: 3 \times 1 g capsules /day soybean oil (which contains 5% fish oil).</p>	<p>Diarrhoea (high vs low LCn3 fats);</p>
<p><i>SHOT 1996 (SHunt Occlusion Tria)</i></p> <p><i>RCT, parallel</i></p>	<p>Norway</p>	<p>1 year</p>	<p>People admitted for CABG.</p> <p>N: 317 intervention, 293 control.</p> <p>Mean age in years (SD): 59.9 (8.7) intervention, 59.4 (8.8) control.</p> <p>Men: 86% intervention, 88% control.</p>	<p>Supplement (capsule).</p> <p>Intervention: 4 fish-oil concentrate soft gelatin capsules /day containing 51% EPA and 32% DHA ethyl esters and 3.7 mg vitamin E as an antioxidant. Dose: 3.3 g /day EPA + DHA.</p> <p>Control: no treatment.</p>	<p>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats); Bleeding (high vs low LCn3 fats);</p>
<p><i>SMART 2013 (SMART trial (from the Smart Foods Centre))</i></p> <p><i>RCT, 3-arm parallel</i></p>	<p>Australia</p>	<p>12 months</p>	<p>Overweight adults (mean baseline TG 1.3 mmol/L).</p> <p>N: fish + S intervention 41, fish 43, control 42 (analysed, fish + S intervention 21, fish 25, control 18).</p> <p>Mean age (SD) years: unclear by arm, overall 45.1 (8.4).</p> <p>Men: 27% fish + S intervention, 23% fish intervention, 28% control.</p>	<p>Supplement and food.</p> <p>Intervention, fish + S: hypocaloric diet aiming at 30% E from fat, 25% E from protein, 45% E from CHO, plus 180 g fish/week plus capsules including 420 mg /day EPA + 210 mg /day DHA. Dose: 0.63 g /day EPA + DHA.</p> <p>Intervention, fish: hypocaloric diet aiming at 30% E from fat, 25% E from protein, 45% E from CHO,</p>	<p>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</p>

				<p><i>plus 180 g fish/week plus capsules including 1 g olive oil/d.</i></p> <p><i>Control: hypocaloric diet aiming at 30% E from fat, 25% E from protein, 45% E from CHO, plus capsules including 1 g olive oil/d.</i></p>	
<p><i>Sofi 2010</i></p> <p><i>2-arm, parallel RCT</i></p>	<p><i>Italy</i></p>	<p><i>12 months</i></p>	<p><i>People with NAFLD patients (mean TG 143 mg/dL, mean BMI 29.3).</i></p> <p><i>N: 6 intervention, 5 control.</i></p> <p><i>Median age: 55 intervention, 54 control. Age range: 30-41 intervention, 42-70 control.</i></p> <p><i>Men: 66.7% intervention, 100% control.</i></p>	<p><i>Supplement (oil).</i></p> <p><i>Comparison: EPA + DHA vs MUFA</i></p> <p><i>Intervention: 6.5 mL /day olive oil enriched with n-3 containing 0.47 g EPA, 0.24 g DHA plus dietary recommendations. Dose: 0.83 g /day EPA + DHA.</i></p> <p><i>Control: 6.5 mL /day olive oil plus dietary recommendations.</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</i></p>
<p><i>SU.FOL.OM3 2010</i></p> <p><i>(supplementation en Folates et omega-3)</i></p> <p><i>RCT, 2x2 factorial</i></p>	<p><i>France</i></p>	<p><i>4 years</i></p>	<p><i>People with a history of MI, unstable angina or ischaemic stroke.</i></p> <p><i>N: control: 1248, intervention: 1253.</i></p> <p><i>Mean age in years (SD): 61.1 (8.8) intervention, 60.8 (8.7) control.</i></p> <p><i>Men: 80.85% intervention, 78.25% control.</i></p>	<p><i>Intervention: 2 gelatin capsules Pierre Fabre omega-3 (400 mg/d EPA and 200 mg/d DHA)Control: 2 gelatin capsules/d placebo (liquid paraffin with fish flavour)</i></p>	<p><i>Heammorrhagic stroke</i></p>

<p><i>Tande 2016</i></p> <p><i>2-arm, parallel RCT</i></p>	<p>Norway</p>	<p>12 months</p>	<p><i>Healthy male and female volunteers with BMI 25-35 kg/m² (mean baseline TG 1.4 mmol/L).</i></p> <p><i>N: 64 intervention, 63 control (50 intervention, 50 control analysed).</i></p> <p><i>Mean age in years (SD): 50.7 (7.7) intervention, 49 (9.4) control.</i></p> <p><i>Men: 42% intervention, 43 % control.</i></p>	<p><i>Supplement (capsule).</i></p> <p><i>Intervention: 2 × 500 mg Calanus oil capsules twice daily to provide a daily dose of 2 g. The Calanus oil contained approximately 85% wax ester with a sum of neutral lipids > 90%. Dose: 2 g /day EPA + DHA.</i></p> <p><i>Control: identical capsules of olive oil. Compositional analysis indicated that the FA content of the olive oil was primarily oleic acid (76.9%), palmitic acid (10.2%), and linoleic acid (7.7%).</i></p>	<p><i>HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats); skin problems (high vs low LCn3 fats);</i></p>
<p><i>THIS DIET 2008 (The Heart Institute of Spokane diet study)</i></p> <p><i>RCT, parallel</i></p>	<p>USA</p>	<p>24 months</p>	<p><i>Recent survivors of 1st MI (within < 6 weeks).</i></p> <p><i>N: 51 intervention, 50 control.</i></p> <p><i>Mean age in years (SD): 58 (10) intervention, 58 (9) control.</i></p> <p><i>Men: 80% intervention, 68% control.</i></p>	<p><i>Dietary advice (to follow a Mediterranean style diet high in n-3).</i></p> <p><i>Intervention: Mediterranean-style diet high in n-3. Dose: ~1.5 g /day omega-3 fat, or 0.31% E by intake assessment.</i></p> <p><i>Control: dietary advice (to follow the American Heart Association Step II diet).</i></p>	<p><i>HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</i></p>
<p><i>VITAL 2019 (Vitamin D and omega-3 trial)</i></p> <p><i>RCT, parallel 2x2</i></p>	<p>USA</p>	<p>5.3 years (median)</p>	<p><i>Multi-ethnic population of > 25,000 apparently healthy adults (men ≥ 50 years, women ≥ 55 years) without cancer or CVD at baseline.</i></p>	<p><i>Supplement.</i></p> <p><i>Intervention:</i></p> <ul style="list-style-type: none"> • <i>Arm 1: omega-3, 1 capsule/d, Omacor fish oil, EPA + DHA 840 mg/d: 465 mg EPA; 375</i> 	<p><i>Bleeding (high vs low LCn3 fats); Abdominal pain or discomfort (high vs low LCn3 fats); Diarrhoea (high vs low LCn3 fats);</i></p>

			<p><i>N: 12,933 intervention, 12,938 control (analysed intervention 12,933, control 12,938).</i></p> <p><i>Mean age in years (SD): 67.2 (7.1) intervention, 67.1 (7.1) control.</i></p> <p><i>Men: 49.4% intervention, 49.5% control.</i></p>	<p><i>mg DHA provided in calendar packs and placebo D3.</i></p> <ul style="list-style-type: none"> <i>Arm 3: omega-3 as in Arm 1 and vitamin D3 (1/d, 2000 IU).</i> <i>Control:</i> <i>Arm 2: placebo omega-3 and vitamin D3 (1/d, 2,000IU).</i> <i>Arm 4: placebo omega-3 and placebo D3.</i> <p><i>Dose: 840 mg /day LCn3, or 0.38% E.</i></p>	<p><i>Nausea (high vs low LCn3 fats);</i></p>
<p><i>Weinstock-Guttman 2005</i></p> <p><i>RCT, parallel</i></p>	<p><i>USA</i></p>	<p><i>12 months</i></p>	<p><i>Population: adults with MS.</i></p> <p><i>N: 15 intervention, 16 control (analysed, intervention: 13, control: 14).</i></p> <p><i>Mean age in years (SD): 39.9 (10.0) intervention, 45.1 (7.7) control.</i></p> <p><i>Men: 15.4% intervention, 14.3% control.</i></p>	<p><i>Dietary advice plus supplement.</i></p> <p><i>Intervention: 1.98 g /day EPA, 1.32 g /day DHA supplements + low fat diet (< 15% total calories).</i></p> <p><i>Dose: 3.3 g /day EPA + DHA.</i></p> <p><i>Control: one 1 g olive oil placebo capsules 6 times/d, moderate-fat diet (< 30% total calories) (AHA Step 1 diet).</i></p>	<p><i>HDL (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</i></p>
<p><i>WELCOME 2015 (Wessex Evaluation of fatty Liver and Cardiovascular Markers in NAFLD with Omacor Therapy)</i></p> <p><i>RCT, parallel</i></p>	<p><i>UK</i></p>	<p><i>15-18 months</i></p>	<p><i>Patients with NAFLD (mean TG 1.4mmol/L, mean BMI 32.0, 9% diabetic).</i></p> <p><i>N: 51 intervention, 52 control (analysed, 47 intervention, 48 control).</i></p>	<p><i>Supplement (Omacor capsules).</i></p> <p><i>Intervention: 4 g OMACOR /day (providing 1.84 g EPA, 1.52 g DHA as ethyl esters). Dose: 3.36 g /day EPA + DHA.</i></p> <p><i>Control: 4 g olive oil capsules /day (providing; ALA 1%, oleic acid 67%, palmitic acid 15%, stearic acid 2%, n-6 fat: 15%).</i></p>	<p><i>Total cholesterol (high vs low LCn3 fats); LDL (high vs low LCn3 fats);</i></p>

			<p><i>Mean age in years (SD): 48.6 (11.1) intervention, 54 (9.6) control.</i></p> <p><i>Men: 49% intervention, 67% control.</i></p>		
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Chua et al. (2012)

Characteristics of the systematic review	
Title	Relationship of Dietary Intake of Omega-3 and Omega-6 Fatty Acids with Risk of Prostate Cancer Development: A Meta-Analysis of Prospective Studies and Review of Literature
Author(s)	Michael E. Chua, Maria Christina D. Sio, Mishell C. Sorongon, and Jun S. Dy
Year of publication	2012
Journal	Prostate Cancer
Country of origin (corresponding author)	Philippines
Funding	No information
Reported conflict of interest	None
What is the main objective of the review?	To give a systematic review through quality assessment of all available literatures regarding the association of omega fatty acids and prostate cancer.
Inclusion/Exclusion criteria	Inclusion criteria: Studies that described the effects of dietary consumption of omega-3 and/or omega-6 fatty acids (with or without their derivatives) on prostate cancer incidence; studies with prospective cohort study design with human study population; studies that described the effects of exposure to omega-3 and/or omega-6 with different levels of exposure.

	Exclusion criteria: Animal studies and in vitro experimental studies; case-control studies; review articles and letters to the editors.				
Start and ending dates of the literature search	Up to June 2011				
Population/Subjects	Males, adults				
Intervention(s)	Different levels of omega-3 and/or omega-6 intake				
Outcome(s) of relevance	Prostate cancer development				
Quality assessment tool(s)	Newcastle-Ottawa Quality Assessment Scale (NOQAS) from Cochrane Collaboration for cohort studies				
Results of quality assessment	All included studies were scored to have high quality (≥ 8).				
Data synthesis methodology	The reported RRs or ORs were used to estimate the risk ratio of prostate cancer specific mortality or prostate cancer incidence and its subcategories among highest and lowest dietary intake of omega-3 fatty acids, omega-6 fatty acids, and their components. Before estimating the summary of risk ratios, test for heterogeneity was done using Cochran's chi-square test (Q) to assess the consistency of associations. For homogeneous studies, a fixed effect model of analysis was applied. For significant heterogeneity ($P \leq 0.10$), random effect model was used for analysis instead of the fixed effects model.				
Number of included studies	8 cohort studies, only 4 with EPA and/or DHA intake.				
Results (relevant) and the confidence in the evidence	A slightly positive association was noted on dietary long-chain n-3PUFA, composed of EPA and DHA with prostate cancer risk (pooled RR: 1.135; 95% CI: 1.008, 1.278; $P=0.036$); however, when two other cohort studies with data of EPA and DHA, both analysed separately, were included into the pool, the association became not significant (RR: 1.034; 95% CI: 0.973, 1.096; $P=0.2780$).				
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo					
<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect</i>
<i>Schurman et al., 1999</i>		<i>6.3 years</i>	<i>58,279 participants in total. Age: 55-69 years old.</i>	<i>Omega 3 (ALA, EPA, DHA) and Omega 6 (LA, AA)</i>	<i>Prostate cancer</i>

<i>Leitzmann et al., 2004</i>		<i>14 years</i>	<i>44,856 participants in total. Age: 40-75 years old.</i>	<i>Omega 3 and Omega 6 and ALA</i>	
<i>Park et al., 2007</i>		<i>8 years</i>	<i>82,483 participants. Age: >45 years</i>	<i>Omega 3 (DHA and EPA), ALA, Omega 6</i>	
<i>Wallstrom et al., 2007</i>		<i>11 years</i>	<i>10,564 participants in total. Age: 45-73 years old.</i>	<i>Omega 3 and Omega 6</i>	
<i>Chavarro et al., 2008</i>		<i>19 years</i>	<i>20,167 participants in total. Age: 40-84 years old.</i>	<i>Omega 3 (long-chain n-3)</i>	
Comments: No specific doses are reported.					

Downie et al. (2019)

Characteristics of the systematic review	
Title	Omega-3 and omega-6 polyunsaturated fatty acids for dry eye disease (Review)
Author(s)	Downie LE, Ng SM, Lindsley KB, Akpek EK
Year of publication	2019
Journal	Cochrane Database of Systematic Reviews
Country of origin (corresponding author)	Australia
Funding	<ul style="list-style-type: none"> • National Eye Institute, National Institutes of Health, USA. • Methodological support provided by the Cochrane Eyes and Vision US Project, which is funded by Grant UG1EY020522. • National Institute for Health Research, UK. • Richard Wormald, Co-ordinating Editor for Cochrane Eyes and Vision (CEV) acknowledged financial support for his CEV research sessions from the Department of Health through the award made by the National Institute for Health Research to Moorfields Eye

	<p>Hospital NHS Foundation Trust and UCL Institute of Ophthalmology for a Specialist Biomedical Research Centre for Ophthalmology.</p> <ul style="list-style-type: none"> This review was supported by the National Institute for Health Research, via Cochrane Infrastructure funding to the CEV UK editorial base.
Reported conflict of interest	SMN and KL received salary support from Cochrane Eyes and Vision @ US Project, funded by the National Eye Institute, at the National Institutes of Health. LED is the senior author on two studies considered in this review; in view of this, a third (independent) person verified data extraction and risk of bias assessment for these studies.
What is the main objective of the review?	Assess the effects of omega-3 and omega-6 polyunsaturated fatty acid (PUFA) supplements on dry eye signs and symptoms, and to document any potential treatment-related adverse events.
Inclusion/Exclusion criteria	<p>RCTs involving dry eye participants, in which omega-3 and/or omega-6 supplements were compared with a placebo/control supplement, artificial tears, or no treatment, were included. We included head-to-head trials comparing different forms or doses of PUFAs.</p> <p>No restrictions on publication year or language.</p>
Start and ending dates of the literature search	The searches were performed February 2018. No restrictions were applied to the year of publication.
Population/Subjects	Adults with diagnosis of dry eye disease.
Intervention(s)	Omega-3 and/or omega-6 supplements.
Comparator	Lower levels of omega-3 and/or omega-6 supplements, placebo/control supplement or no treatment.
Primary outcome(s)	Subjective improvement in dry eye symptoms (e.g. dryness, scratchiness, foreign body sensation, burning).
Secondary outcome(s)	Secondary outcomes included a range of clinical measures (ocular surface staining, aqueous tear production, tear film stability, change in frequency of use of artificial tears, change in conjunctival goblet cell density, change in the proportion of participants with improved blurred vision symptoms, change in ocular surface inflammatory biomarkers, change in tear osmolarity) and side effects.
Adverse outcomes considered	Adverse events were documented as reported in the included studies. Specifically, incidence of cancer and gastrointestinal disorders, such as diarrhea, were reported, as well as ocular adverse events such as an increase in blurred vision.
Quality assessment tool(s)	Risk of bias of included studies were evaluated according to the guidelines provided in Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2017).

Results of quality assessment	Half of studies had high risk of bias in one or more domains.					
Data synthesis methodology	Risk Ratio with 95% CI. Meta-analysis was not performed due to substantial heterogeneity ($I^2 = 76\%$).					
Number of included studies	34 RCTs, whereas 3 of the studies included data on adverse effects.					
Confidence in the evidence	<p>The certainty of the evidence was performed by Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.</p> <p>For gastrointestinal disorders at 1-month (with actual follow-up ranging from 3 to 12 months), the confidence in the evidence was low. This was based on three studies, reporting gastrointestinal disorders in between 5% and 19% of participants in the omega-3 group and between 0% and 24% of participants in the placebo group.</p>					
Results (relevant)	<p>The most common side effect was temporary gastrointestinal problems.</p> <p>Of the nine studies that investigated long-chain omega-3 fatty acid supplementation relative to placebo and reported adverse events, six trials reported that participants in the omega-3 group experienced gastrointestinal disorders (including diarrhoea) and one trial reported that participants in the comparator group experienced similar effects. Three of these studies provided sufficient data for a meta-analysis related to gastrointestinal adverse effects. The statistical heterogeneity ($I^2 = 76\%$) was substantial. Gastrointestinal disorders were reported in between 5% and 19% of participants in the omega-3-treated group and between 0% and 24% of participants in the placebo group.</p> <p>The certainty of the body of evidence (according to the GRADE system) was downgraded by two levels to low, as results were inconsistent among studies and few events accounted for the wide confidence intervals reported (imprecision).</p>					
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo						
<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age/ Gender</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect</i>	<i>Serious adverse effect</i>
<i>Asbell 2018,</i>	<i>USA</i>	<i>12 months/</i>	<i>535 participants in total, 349 participants in the</i>	<i>Omega-3 supplementation: a</i>	<i>The percentage of patients with at least one non-serious adverse event</i>	<i>The percentage of patients with</i>

<p><i>randomized, controlled trial</i></p>			<p><i>omega-3 treatment group; 186 participants in the placebo group.</i></p> <p><i>Age (mean ± SD, range): 58.3 ± 13.5 years in the omega-3 treatment group; 57.5 ± 12.6 years in the control group.</i></p> <p><i>Gender: 65 men and 284 women in the omega-3 treatment group; 36 men and 150 women in the control group.</i></p>	<p><i>daily dose of 2000 mg EPA and 1000 mg DHA.</i></p> <p><i>Placebo: daily dose of 5000 mg olive oil</i></p>	<p><i>was similar in the active supplement group and the placebo group (61.9% and 60.8%, respectively; p=0.87), as was the percentage of patients with an episode of bleeding (2.0% and 1.6%, respectively; p=1.00). A higher percentage of patients reported diarrhoea in the active supplement group than in the placebo group (4.9% and 1.6%, respectively; p=0.09).</i></p>	<p><i>at least one serious adverse event was 6.0% in the active supplement group and 8.1% in the placebo group (p =0.31).</i></p>
<p><i>Bhargava 2016a; randomized, parallel-group, controlled trial</i></p>	<p><i>India</i></p>	<p><i>6 months</i></p>	<p><i>130 participants in total, 65 participants in each treatment groups.</i></p> <p><i>Age (mean ± SD, range): 47.7 ± 3.8 (range 24 to 68) years in the omega-3 treatment group; 48.9 ± 4.5 (range 21 to 70) years in the control group.</i></p> <p><i>Gender: 25 men and 40 women in the treatment group; 27 men and 38</i></p>	<p><i>Omega-3 supplementation: Daily dose of 720 mg EPA and 480 mg DHA.</i></p> <p><i>Placebo: Oral capsules containing olive oil (dose not reported).</i></p>	<p><i>4 participants in the placebo group (n = 65) experienced transient skin rashes that were not severe enough to warrant dis-continuation from the study. Eight participants in the omega-3intervention group (n = 65) experienced gastric intolerance.</i></p>	<p><i>Not reported</i></p>

			women in the control group.			
Deinema 2017; randomized, parallel-group, controlled trial	Australia	90 days/ baseline, 30, 60, and 90 days	60 participants in total, 20 in each of the 3 intervention groups. Age (mean \pm SE, range): 39.4 \pm 3.4 years in the fish oil group; 42.3 \pm 3.8 years in the krill oil group; 46.2 \pm 4.5 years in the placebo group. Gender: 47% (n = 9) female in the fish oil group; 72% female (n = 13) in the krill oil group; 82% female (n= 14) in the placebo group.	Omega-3 supplementation: Daily dose of 1000 mg EPA and 500 mg DHA from fish oil capsules (triglycerideomega-3 PUFAs). Daily dose of 945 mg EPA and 510 mg DHA from krill oil capsules (phospholipidomega- 3 PUFAs). Placebo: Daily dose of 1500 mg olive oil.	The three (olive oil, fish oil and krill oil) interventions were generally well tolerated. Of the 53 adverse events noted, 51 were considered mild (i.e., awareness of symptoms or signs but well tolerated) and 2 were graded as moderate (i.e., discomfort interfering with normal activity). The most frequently reported adverse events were colds (24.5%), sore throat (11.3%), headache/migraine (11.3%), and gastrointestinal events (e.g., nausea, abdominal discomfort, bloating, stomach cramps, heartburn, and gastroenteritis; 13.2%). Based on the principal investigator's (L.E.D.'s) assessment, 72% were deemed to have no potential association with the study supplements and 28% of the adverse events were considered to be potentially related to the study supplements.	None
Comments:						

Fu et al. (2015)

Characteristics of the systematic review	
Title	Effect of Individual Omega-3 Fatty Acids on the Risk of Prostate Cancer: A Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies
Author(s)	Yuan-Qing Fu, Ju-Sheng Zheng, Bo Yang, and Duo Li
Year of publication	2015
Journal	J Epidemiol
Country of origin (corresponding author)	China
Funding	National Natural Science Foundation of China (NSFC, grant number81273054); the PhD. Programs Foundation of Ministry of Education of China (grant number 20120101110107); and the National Basic Research Program of China (grant number973 Program 2011CB504002).
Reported conflict of interest	None
What is the main objective of the review?	To estimate the trend and quantify the association for both dietary intakes and blood concentration of individual n-3 PUFAs with prostate cancer (PCa) risk based on prospective studies only.
Inclusion/Exclusion criteria	<p>Inclusion criteria:</p> <p>Prospective study design (including prospective cohort, nested case-control, and case-cohort studies); exposure of interest (dietary n-3 PUFAs or blood n-3 PUFAs concentrations); endpoint (incident PCa in males); and reporting of risk estimate (relative risk, odd ratio, or hazard ratio) of PCa with corresponding 95% confidence intervals (CIs) for individualn-3 PUFA exposure.</p> <p>Exclusion criteria:</p> <p>Retrospective or cross-sectional studies, animal or cell culture studies, reviews, editorials, and commentaries.</p>
Start and ending dates of the literature search	Up to February 2014
Population/Subjects	Males. Blood n-3 PUFAs, age 27-84. Dietary n-3 PUFAs, age 27-80.

Intervention(s)/exposure	Dietary n-3 PUFAs or blood n-3 PUFAs concentrations				
Comparator	Lower levels in blood or lower dietary intake of n-3 PUFAs				
Outcome(s) of relevance	Prostate cancer				
Quality assessment tool(s)	Newcastle-Ottawa criteria				
Results of quality assessment	Moderate quality: 3, High quality: 13				
Assessment of confidence in evidence	Not done.				
Data synthesis methodology	A 2-stage random-effects dose-response meta-analysis was performed to examine a potential curvilinear association between dietary n-3 PUFA exposure and risk of PCa, using restricted cubic splines with three knots at fixed percentiles (25%, 50%, and 75%) of dietary n-3 PUFA distribution.				
Total number of included studies	16, 7 nested case-control studies (7 blood/0 diet), 6 cohorts (0 blood/6 diet), 3 case-cohorts (2 blood/1 diet).				
Follow-up	From 1.9 to 16 years.				
Number of participants	Blood n-3 PUFA: >450,000 (<i>n</i> not reported for one study). Dietary n-3 PUFA: >850,000				
Results (relevant) and the confidence in the evidence	<p>Blood concentration of docosahexaenoic acid, but not eicosapentaenoic acid, showed marginal positive association with PCa risk (relative risk for 1% increase in blood docosahexaenoic acid concentration: 1.02; 95% confidence interval, 1.00–1.05; I²= 26%; P= 0.11 for linear trend), while dietary docosahexaenoic acid intake showed a non-linear positive association with PCa risk (P< 0.01).</p> <p>Subgroup analyses indicated that blood eicosapentaenoic acid concentration and blood docosahexaenoic acid concentration were positively associated with aggressive PCa risk and nonaggressive PCa risk, respectively. Among studies with nested case-control study designs, a 0.2% increase in blood docosapentaenoic acid concentration was associated with a 3% reduced risk of PCa (relative risk 0.97; 95%confidence interval, 0.94–1.00; I²= 44%; P= 0.05 for linear trend).</p>				
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo					
<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect</i>

<i>Shuurman et al., 1999.</i> <i>Case-cohort</i>	<i>Netherland</i>	<i>6.3 years</i>	<i>58,279 participants in total.</i> <i>Age: 55-69 years old.</i>	<i>EPA, DHA</i>	<i>Prostate cancer</i>
<i>Wallstrom et al., 2007</i> <i>Prospective cohort</i>	<i>Sweden</i>	<i>11 years</i>	<i>10,564 participants in total.</i> <i>Age: 45-73 years old.</i>	<i>EPA, DHA, EPA+DHA</i>	<i>Prostate cancer</i>
<i>Park et al., 2007</i> <i>Prospective cohort</i>	<i>USA</i>	<i>8 years.</i>	<i>82,483 participants in total.</i> <i>Age: >45 years old.</i>	<i>EPA, DHA</i>	<i>Prostate cancer</i>
<i>Pelser et al., 2013</i> <i>Prospective cohort</i>	<i>USA</i>	<i>9 years</i>	<i>288,268 participants in total.</i> <i>Age: 50-71 years old.</i>	<i>EPA, DHA, EPA+DHA</i>	<i>Prostate cancer</i>
<i>Männistö 2003</i> <i>Nested case-control</i>	<i>Finland</i>	<i>5-8 years</i>	<i>290,406 participants in total.</i> <i>Age: 50-59 years old.</i>	<i>DHA, EPA</i>	<i>Prostate cancer</i>
<i>Leitzmann et al., 2004</i> <i>Prospective</i>	<i>USA</i>	<i>14 years</i>	<i>47,866 participants in total.</i> <i>Age: 40-75 years old.</i>	<i>EPA, DHA, EPA+DHA</i>	<i>Prostate cancer</i>
<i>Bassett et al., 2013</i> <i>Case-cohort</i>	<i>Australia</i>	<i>8.9 years</i>	<i>17,045 participants in total.</i> <i>Age: 27-80 years old.</i>	<i>EPA, DHA</i>	<i>Prostate cancer</i>
Comments: Doses were not given. DHA and EPA levels in the blood were reported.					

Irving et al. (2016)

Characteristics of the systematic review	
Title	Polyunsaturated fatty acid supplementation for schizophrenia (Review)
Author(s)	Irving CB, Mumby-Croft R, Joy LA

Year of publication	2006
Journal	Cochrane Library: Cochrane Database of Systematic Reviews
Country of origin (corresponding author)	UK
Funding	Internal sources: Cochrane Schizophrenia Group General Fund, UK; School of Business, Oxford Brookes University, UK. External sources: No sources of support supplied.
Reported conflict of interest	None known.
What is the main objective of the review?	To assess the effects of essential fatty acid (EFA) supplementation of antipsychotic treatment for schizophrenia-like illnesses. To investigate the evidence for a difference between the effects of omega-3 and omega-6 EFA supplementation of neuroleptic treatment for schizophrenia-like illnesses. To investigate the effects of pure preparations of EFA such as the omega-3 eicosapentaenoic acid compared with a mixture of EFA's such as the 'fish oil' or 'evening primrose oil' preparations sold over the counter in pharmacies or drug stores.
Inclusion/Exclusion criteria	Inclusion Types of studies: All relevant randomised controlled trials. For crossover RCTs, only data up to the point of first cross-over. Types of participants: People with schizophrenia or similar chronic mental illnesses, diagnosed by any criteria, irrespective of gender, age or race. Types of interventions: Any type of polyunsaturated fatty acid supplementation (omega 3 PUFAs including EPA, its ester, ethyl-eicosapentaenoic acid (E-EPA) and DHA; omega 6 PUFAs including gamma-linolenic acid) of a standard neuroleptic care. Exclusion: Qasi-randomised trials.

Start and ending dates of the literature search	This is an update of previous systematic reviews, and the search was performed in 2006.
Population/Subjects	People with schizophrenia or similar chronic mental illnesses, diagnosed by any criteria, irrespective of gender, age or race. Participants: both sex, >18 years.
Intervention(s)	Any type of polyunsaturated fatty acid supplementation (omega 3 PUFAs including EPA, its ester, ethyl-eicosapentaenoic acid (E-EPA) and DHA; omega 6 PUFAs including gamma-linolenic acid) of a standard neuroleptic care.
Comparator	Placebo
Duration of intervention	12-16 weeks
Primary outcome(s) of relevance	Adverse effects: No clinically important general adverse effects.
Secondary outcome(s) of relevance	Adverse effects: Not any general adverse effects, average endpoint general adverse effect score, average change in general adverse effect scores, no clinically important change in specific adverse effects, not any change in specific adverse effects, average endpoint specific adverse effects, and average change in specific adverse effects.
Quality assessment tool(s)	The Cochrane Collaboration Handbook (Higgins, 2008) tool.
Results of quality assessment	In half of the trials there was a high risk of other sources of bias. In two studies there was a high risk of selective reporting, and in one study there was a high risk of incomplete data assessment. In half of the studies the adequate sequence generation was unclear. In three studies the allocation concealment was unclear. In two studies the blinding was unclear. In three studies it was unclear if incomplete data was addressed, and in half of the studies it was unclear if they were free of other bias. With so few good quality data, all findings can still only be considered as hypothesis generating.
Data synthesis methodology	Meta-analysis, risk ratio with 95% CI.
Number of included studies	Eight RCTs. Between one and six RCTs had data on the different relevant outcomes for our assessment (see "Results" for details).

Assessment of confidence in the evidence	<p>The confidence in evidence is not assessed using established methods, however, it is discussed in the text.</p> <p>Overall, the methodological quality of the eight included studies were considered as good by the authors. However, they also pinpointed that the studies were small and short. Only one study randomised over 100 people and only one study was over three months in duration. Despite good methodological quality, data reporting was poor, and the quality of evidence suffers. The authors concluded that with so few good quality data, all findings can still only be considered as hypothesis generating.</p>
Results (relevant)	<p>Any dose omega-3 (E-EPA or EPA vs placebo)</p> <p>Adverse effects: only one study reported data for movement disorders. Data suggests no effect of omega-3 supplementation.</p> <p>Leaving the study early: The studies have low or no attrition (<10% total) (n=679, 6 RCTs, RR 0.85, CI 0.51 to 1.40).</p> <p>Specific dose omega-3 (E-EPA) vs placebo</p> <p>Adverse effects:</p> <ul style="list-style-type: none"> - Experiencing at least one adverse effect (only short-term studies): no differences between groups are found for any dose. Data for 1g/day: n=63, 1 RCT, RR 0.67, CI 0.37 to 1.20. Data for 4 g/day: n=58, 1 RCT, RR 1.15, CI 0.72 to 1.82. - Diarrhoea: Data from two studies, one short term and one medium term, do not show that people receiving less than 1 g/day of E-EPA are more likely to experience diarrhoea than those on placebo (n) 150, 2 RCTs, RR 2.04, CI 0.91 to 4.54). The data are heterogeneous and the results from only the medium-term study suggests that there may be a link with the use of low dose E-EPA supplementation and diarrhoea (n=87, 1 RCT, RR 17.39, CI 1.03 to 292). - Nausea: only data from one short-term study were available. Use of E-EPA was not clearly associated with nausea. - Liver and biliary tract problems, metabolic and nutritional difficulties (e.g. weight gain) and musculoskeletal adverse effects: No differences are apparent for any dose of E-EPA supplementation compared with placebo. The data is based on one short-term RCT. - Psychosexual difficulties, infections: No statistically significant differences were found, however it appears as though there is an effect. Based on one short-term study only. - Rashes, urinary problems and "any other" adverse effects: These effects were rare and not different for any dose. Based on one short-term study. - Leaving the study early: Data for <1 g/day: No differences in numbers leaving the study early were found (n=150, 2 RCTs, RR 1.61, CI 0.71 to 3.67). Data are based on one short-term and one medium-term study

	<ul style="list-style-type: none"> - Leaving the study early: Data for 2 g/day: no differences between the groups (n=307, 3 RCTs, RR 1.17, CI 0.54 to 23.94). Data are based on two short-term studies and one medium-term. - Leaving the study early: Data for 3 g/day: no differences between groups. (n=40, 1 RCT, RR 3.00t, CI 0.13 to 69.52). <p>Heterogeneity and publication bias were not assessed due to small number of studies.</p>				
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo					
<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age/ Gender</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect (comparison of a specific dose of E-EPA versus placebo)</i>
<i>Fenton 2001</i>	<i>Not reported</i>	<i>16 weeks</i>	<p><i>90 participants in total, only data from 87 participants.</i></p> <ul style="list-style-type: none"> - <i>Ethyl-eicosapentaenoic acid, 500 mg/day + vitamin E, n=43.</i> - <i>Mineral oil placebo + vitamin E, n=44.*</i> <p><i>Age: 18-65 years, mean ~40 years.</i></p> <p><i>Sex: 70 men, 20 women.</i></p>	<i>Ethyl-eicosapentaenoic acid and placebo (mineral oil).</i>	<i>Gastrointestinal (diarrhoea); Infections and respiratory system.</i>
<i>Peet 2002</i>		<i>12 weeks</i>	<p><i>122 participants in total, only 115 participants analysed.</i></p>	<i>Ethyl-eicosapentenoic acid and placebo.</i>	<i>Experiencing at least one adverse effect (short term); gastrointestinal (diarrhoea); gastrointestinal (nausea, short term); liver and biliary tract (short term); metabolic and nutritional (short term); musculoskeletal (short term); psychiatric (short</i>

		<ul style="list-style-type: none"> - Ethyl-eicosapentenoic acid, 1 g/day, n=32 - Ethyl-eicosapentaenoic acid, 2 g/day, n=32. - Ethyl-eicosapentaenoic acid, 4 g/day, n=27. - Placebo, n=31. <p>Age: ~18 – 65 years.</p> <p>Sex: 66 men, 39 women.</p>		<p>term); reproductive (short term); Infections and respiratory system; skin (short term); urinary (short term); vision (short-term); other adverse outcomes (short term)</p>
Comments:				

Mocellin et al. (2016)

Characteristics of the systematic review	
Title	A systematic review and meta-analysis of the n-3 polyunsaturated fatty acids effects on inflammatory markers in colorectal cancer
Author(s)	Michel C. Mocellin, Carolina Q. Camargo, Everson Araujo Nunes, Giovanna M.R. Fiates, Erasmo B.S.M. Trindade
Year of publication	2016
Journal	Clinical nutrition
Country of origin (corresponding author)	Brazil
Funding	The Graduate Program in Nutrition e Federal University of Santa Catarina, Brazil and Coordination of Improvement of Higher Education Personnel (CAPES)/Social Demand Program for grating scholarships to the first two authors.

Reported conflict of interest	None
What is the main objective of the review?	To evaluate the effects of n-3 PUFA on inflammatory mediators in colorectal cancer (CRC) patients.
Inclusion/Exclusion criteria	<p>Inclusion: controlled or randomized clinical trials performed in humans; use of n-3 polyunsaturated fatty acids as intervention, isolated or added in dietary formulas or as lipid emulsion; sample composed of subjects with over 18 years of age and only affected by malignant colorectal neoplasm; and those trials that had assessed the cytokines or acute phase proteins levels in vivo.</p> <p>Exclusion: Trials that did not meet the inclusion criteria, duplicated or triplicated publications from the same trial, as well as, trials that were originally published in languages other than English, Spanish or Portuguese.</p>
Start and ending dates of the literature search	Start not reported, end of literature search September 2014.
Population/Subjects	Human adults at 18 years and above with malignant colorectal neoplasm/colorectal cancer
Intervention(s)	See inclusion criteria.
Outcome(s) of relevance	Inflammatory mediators (TNFa, IL-6, CRP, albumin)
Quality assessment tool(s)	The Cochrane Collaboration's tool for assessing quality and risk of bias and CONSORT-based checklist.
Results of quality assessment	Three trials were completely free of bias. In all trials attrition or reporting bias were not identified. In one, the information was not sufficient to judge it as free of detection bias, and in another potential selection bias was detected. Two did not have allocation concealment and six used adequate techniques for sequence generation.
Data synthesis methodology	<p>Individual analysis was performed for each inflammatory mediator/marker. Pooled and stratified analyses were made. The trials were primary stratified in two categories: surgical or chemotherapy trials.</p> <p>The random effects meta-analysis package from STATA was used with the inverse-variance method to test the significance of the analysis.</p>
Number of included studies	9 trials

Results (relevant) and the confidence in the evidence	<p>Benefits on some inflammatory mediators with the use of n-3 PUFA on CRC patients were suggested, but these benefits are specific to certain supplementation protocols involving duration, dose and route of administration, and also, the concomitant anti-cancer treatment adopted.</p> <p>N-3 PUFA reduce the levels of IL-6 (SMD -2.34; 95% CI -4.37, -0.31; p ¼ 0.024) and increase albumin (SMD 0.31; 95% CI 0.06, 0.56; p ¼ 0.014) in overall analyses.</p> <p>In stratified analyses, reduction in IL-6 levels occurs in surgical patients that received 0.2 g/kg of fish oil parenterally at postoperative period (SMD -0.65; 95% CI -1.06, -0.24; p ¼ 0.002), while, increase in albumin concentration occurs in surgical patients that received 2.5 g/d of EPA + DHA orally at preoperative period (SMD 0.34; 95% CI 0.02, 0.66; p ¼ 0.038).</p> <p>In patients undergoing chemotherapy, the supplementation of 0.6 g/d of EPA + DHA during 9 week reduces CRP levels (SMD -0.95; 95% CI -1.73, -0.17; p ¼ 0.017), and CRP/albumin ratio (SMD -0.95; 95% CI -1.73, -0.18; p ¼ 0.016).</p>
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Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo

<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age/ Gender</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect (comparison of a specific dose of E-EPA versus placebo) (H: higher level; L: lower level; Un: unchanged)</i>
<i>Zhu et al., 2012 Clinical trial, parallel</i>	<i>China</i>	<i>7 postoperative days</i>	<i>Surgical patients</i>	<i>EPA+DHA, 0.2 g/kg per day (parenteral)</i>	<i>TNF-α (L); IL-6 (L)</i>
<i>Liang et al., 2008 Clinical trial, parallel</i>	<i>China</i>	<i>7 postoperative days</i>	<i>Surgical patients</i>	<i>EPA+DHA, 0.2 g/kg per day (parenteral)</i>	<i>TNF-α (Un); IL-6 (Un)</i>
<i>Mocellin et al., 2013 Clinical trial, parallel</i>	<i>Brazil</i>	<i>9 weeks</i>	<i>Chemotherapy patients</i>	<i>EPA+DHA, 0.6 g per day (oral)</i>	<i>TNF-α (Un); IL-1β (Un); CRP(L); albumin (Un)</i>

<i>Pastore_Silva et al., 2012</i> <i>Clinical trial, parallel</i>	<i>Brazil</i>	<i>9 weeks</i>	<i>Chemotherapy patients</i>	<i>EPA+DHA, 0.6 g per day (oral)</i>	<i>TNF-α (Un); IL-6 (Un); IL-1β (Un); CRP (Un), albumin (Un)</i>
<i>Purasiri et al., 1994</i> <i>Clinical trial, parallel</i>	<i>UK</i>	<i>6 months</i>	<i>Follow-up</i>	<i>EPA+DHA, 2.4-4.8 g per day (oral)</i>	<i>TNF-α (L); IL-6 (L); IL-1β (L)</i>
<i>Braga et al., 2002</i> <i>Clinical trial, parallel</i>	<i>Italy</i>	<i>9 perioperative days or 5 preoperative days</i>	<i>Surgical patients</i>	<i>EPA+DHA, 3.3 g per day (Oral on preoperative days and Enteral on postoperative days)</i>	<i>IL-6 (L)</i>
<i>Sorensen et al., 2014</i> <i>Clinical trial, parallel</i>	<i>Denmark</i>	<i>14 perioperative days</i>	<i>Surgical patients</i>	<i>EPA+DHA, 3.0 g per day (Oral on preoperative days and Enteral on postoperative days)</i>	<i>CRP (Un); albumin (Un)</i>
<i>Horie et al 2006 intervention</i>	<i>Japan</i>	<i>5 preoperative days</i>	<i>Surgical patients</i>	<i>EPA+DHA, 2.5 g per day Enteral</i>	<i>albumin (H)</i>
<i>Trabal et al. 2010 intervention</i>	<i>Spain</i>	<i>12 weeks</i>	<i>Chemotherapy patients</i>	<i>EPA+DHA, 2 g per day Enteral</i>	<i>albumin (Un)</i>
Comments: <i>L</i> = Lower levels than control, <i>H</i> = Higher levels than control, <i>Un</i> =unchanged compared to control group					

Newberry et al. (2016)

Characteristics of the systematic review	
Title	Omega-3 Fatty Acids and Maternal and Child Health: An Updated Systematic Review
Author(s)	Sydne J. Newberry, Mei Chung, Marika Booth, Margaret A. Maglione, Alice M. Tang, Claire E. O'Hanlon, Ding Ding Wang, Adeyemi Okunogbe, Christina Huang, Aneesha Motala, Martha Timmer, Whitney Dudley, Roberta Shanman, Tumaini R. Coker, Paul G. Shekelle

Year of publication	2016
Journal	Evidence Report/Technology Assessment Number 224
Country of origin (corresponding author)	USA
Funding	Prepared for: Agency for Healthcare Research and Quality U.S. Department of Health and Human Services 5600 Fishers Lane Rockville, MD 20857; Contract No. 290-2012-00006-I
Reported conflict of interest	None
What is the main objective of the review?	To update a prior systematic review on the effects of omega-3 fatty acids (n-3 FA) on maternal and child health and to assess the evidence for their effects on, and associations with, additional outcomes.
Inclusion/Exclusion criteria	<p>Inclusion criteria</p> <p>Population:</p> <p>“Healthy” pregnant women with or without a history of previous preterm birth or a birth of an infant small for gestational age and/or gestational hypertension, preeclampsia, or eclampsia.</p> <p>“Healthy” term or preterm infants.</p> <p>Intervention:</p> <p>N-3 PUFAs</p> <p>Comparators:</p> <p>Placebo, different n-3 sources, different n-3 concentrations, alternative n-3 fortified infant formula, soy-based infant formula, diet with different Vitamin K exposure.</p>

Maternal outcomes: blood pressure control, Peripartum depression, Gestational length, Birth weight.

Pediatric outcomes: Neurological/visual/cognitive development, Risk for ADHD, Risk for Autism spectrum disorders, Risk for learning disabilities, Risk for atopic dermatitis, Risk for allergies, Incidence of respiratory disorders, Incidence of specific adverse events reported in trials by study arm.

Timing/Duration of intervention or follow-up:

Maternal intervention: Interventions implemented anytime during pregnancy but preferably during the first or second trimester.

Follow-up duration is anytime during pregnancy (for maternal outcomes of pre/eclampsia or maternal hypertension); term (for outcomes related to birth weight, duration of pregnancy); or within the first 6 months postpartum (for the outcome of postpartum depression).

Infant exposures: Interventions implemented within one month of birth or exposures measured within 1 month of birth. Follow-up duration is 0 to 18 years.

Settings: Community-dwelling individuals seen by primary care physicians or obstetricians in private or academic medical practices. Community dwelling children seen in outpatient health care or educational settings.

Study type: RCTs, prospective cohort studies, and nested case control studies.

Exclusion criteria:

Cross-sectional, retrospective cohort, and case study designs.

Studies without measure of intake/exposure prior to outcome.

Not peer-reviewed studies.

	<p>Not in English language.</p> <p>Unpublished studies.</p> <p>Observational studies with enrollment sizes of less than 250 unless no other studies were identified for a particular outcome.</p> <p>Studies that reported exposures only as servings of fish without calculating n-3 FA intakes, study size, exposure duration, or other similar criteria, if the number of studies identified was very large.</p>
Start and ending dates of the literature search	January 1, 2000 to August 24, 2015.
Population/Subjects	<p>"Healthy" pregnant women with or without a history of previous preterm birth or a birth of an infant small for gestational age and/or gestational hypertension, preeclampsia, or eclampsia.</p> <p>"Healthy" term or preterm infants.</p>
Intervention(s)	<p>Pregnant women: Supplementation and/or diet type EPA, DHA, ALA.</p> <p>Children: during pregnancy vs post-delivery, route of delivery: via umbilical cord & placenta, mother's milk, child's supplementation (e.g., formula) &/or diet, and with vs without mother's intake via diet &/or supplementation during pregnancy &/or breastfeeding. EPA, DHA, ALA source was e.g., mother's milk, marine, plant.</p>
Outcome(s) of relevance	Adverse effects (tertiary outcome)
Quality assessment tool(s)	The Cochrane Risk of Bias tool.
Results of quality assessment	
Data synthesis methodology	All included studies were summarized narratively and in summary tables. Studies of different design were analysed separately and combined if appropriate. Populations, exposures, and outcomes were compared and contrasted across study designs, examining any differences in outcomes between interventional and observational studies. Meta-analyses were considered when there were at least three trials with similar population, intervention, and outcome measure. The Hartung-Knapp-Sidik-Jonkman method was used for

	random effects meta-analysis. When sufficient data were available and clinical heterogeneity was minimal, dose-response meta-analysis (for observational studies) or meta-regression on doses (for RCTs) were conducted. When new bodies of observational studies were added, possibility for random-effects multivariate dose-response meta-analysis was assessed,
Number of included studies	143; 94 RCTs and 48 observational studies.
Results (relevant) and the confidence in the evidence	<p><u>Maternal Exposures and Outcomes</u></p> <p>Gestational length and risk for preterm birth:</p> <ul style="list-style-type: none"> - Prenatal DHA or DHA-enriched fish oil supplementation had a small positive effect on length of gestation (moderate SoE), but no effect on risk for preterm birth (low SoE). - Prenatal EPA plus DHA-containing fish oil supplementation has no effect on length of gestation (low SoE). - Supplementation with DHA, or EPA plus DHA-, or DHA-enriched fish oil does not decrease risk for preterm birth (low SoE). <p>Birth weight and risk for low birth weight:</p> <ul style="list-style-type: none"> - Changes in maternal n-3 FA biomarkers were significantly associated with birth weight. - Prenatal algal DHA or DHA-enriched fish oil supplementation had a positive effect on birth weight among healthy term infants (moderate SoE), but prenatal DHA supplementation had no effect on risk for low birth weight (low SoE). - Prenatal EPA plus DHA or alpha-linolenic acid (ALA) supplementation had no effect on birth weight (low SoE). <p>Fetal, Infant, and Child Exposures and Outcomes</p> <p>Adverse events:</p> <ul style="list-style-type: none"> - Prenatal and infant supplementation with n-3 FA or fortification of food with n-3 FA did not result in any serious or nonserious adverse events (moderate SoE); with the exception of an increased risk for mild gastrointestinal symptoms. <p>Strength of evidence:</p>

	<p>Moderate: maternal adverse events; no significant difference in risk for serious adverse events (RCTs); increased risk for mild gastrointestinal symptoms but no other consistent non-serious adverse events (RCTs).</p> <p>Moderate: Infant adverse events; increased risk for mild gastrointestinal symptoms across studies but no other consistent non-serious adverse events (RCTs); No significant difference in risk for serious adverse events (RCTs).</p> <p>Low: Infant adverse events; No significant difference in risk for serious events associated with preterm birth (RCTs).</p>				
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo					
<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Participants</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect</i>
<p>7 RCTs</p> <p><i>Strength of evidence: low</i></p>			<p>Healthy pregnant women</p>	<p>Algal DHA or DHA-enriched fish oil supplementation</p>	<p>Risk for preterm birth. No significant effects on the incidence of preterm birth compared with placebo. Meta-analysis of 7 RCTs: OR 0.87 (95% CI 0.66, 1.15)</p>
<p>9 RCTs and 2 observational studies</p> <p><i>Strength of evidence: low</i></p>			<p>At-risk pregnant women</p>	<p>EPA+DHA fish oil supplementation</p>	<p>Risk for preterm birth.</p> <p>RCTs: No significant effects on the incidence of preterm birth compared with placebo. Meta-analysis of 9 RCTs: 0.86 (95% CI 0.65, 1.15).</p> <p>Observational studies showed mixed results.</p>
<p>16 RCTs</p> <p>10 observational studies</p>			<p>Healthy pregnant women</p>	<p>n-3 FA supplementation</p>	<p>Birth weight.</p> <p>RCTs: Significant increase in birth weight compared with placebo. Meta-analysis of 16 RCTs in update: WMD 74.8 (95% CI 12.4, 137.17) grams.</p>

<i>Strength of evidence: Moderate</i>					<i>Observational studies of dietary intake, supplement use, and biomarkers generally showed positive associations with birth weight.</i> <i>Original report: Mixed findings</i>
<i>12 RCTs</i> <i>3 observational studies</i> <i>Strength of evidence: Moderate</i>			<i>Healthy pregnant women</i>	<i>Algal DHA or DHA-enriched fish oil supplementation</i>	<i>Birth weight.</i> <i>RCTs: Significant Increase in birth weight compared with placebo. Meta-analysis of 12 RCTs: WMD 90.12 (95% CI 2.62, 177.62) grams.</i> <i>Observational studies showed associations between DHA intake and biomarkers and birth weight.</i> <i>Original report: mixed findings</i>
<i>5 RCTs</i> <i>4 observational studies</i> <i>Strength of evidence: low</i>			<i>Healthy pregnant women</i>	<i>EPA+DHA fish oil supplementation</i>	<i>Birth weight.</i> <i>RCTs: No significant effects on birth weight compared with placebo. Meta-analysis of 5 RCTs: WMD 37.89 (95% CI -19.53, 95.31) grams.</i> <i>Observational studies showed mixed associations with birth weight.</i> <i>Original report: no effects</i>
<i>4 RCTs</i>			<i>Healthy pregnant women</i>	<i>Algal DHA or DHA-enriched fish oil supplementation</i>	<i>Low birth weight.</i>

<i>Strength of evidence: low</i>					<i>RCTs: No significant effects on risk of low birth weight compared with placebo. Meta-analysis of 4 RCTs: OR 0.72 (95% CI 0.43, 1.11).</i>
<i>9 RCTs</i> <i>Strength of evidence: Moderate.</i>			<i>Pregnant or breastfeeding women</i>	<i>Supplementation with n-3 FA in the form of fish oil</i>	<i>Maternal adverse events, non-serious.</i> <i>RCTs: Increased risk for mild gastrointestinal symptoms but no other consistent non-serious adverse events. These RCTs were determined to be too heterogeneous to permit pooling.</i>
<i>4 RCTs</i> <i>Strength of evidence: Moderate</i>			<i>Pregnant or breastfeeding women</i>	<i>Supplementation with n-3 FA in the form of fish oil</i>	<i>Maternal events, serious.</i> <i>RCTs: No significant difference in risk for serious adverse events. These RCTs were determined to be too heterogeneous to permit pooling. These RCTs were determined to be too heterogeneous to permit pooling.</i>
<i>13 RCTs</i> <i>Strength of evidence: Moderate</i>			<i>Healthy term infants or preterm infants</i>	<i>Supplementation with n-3 FA in the form of fish oil alone or added to infant formula</i>	<i>Infant adverse events non-serious.</i> <i>Increased risk for mild gastrointestinal symptoms across studies but no other consistent non-serious adverse events. These RCTs were determined to be too heterogeneous to permit pooling.</i>
<i>6 RCTs</i> <i>Strength of evidence: Moderate</i>			<i>Healthy term infant</i>	<i>Supplementation with n-3 FA in the form of fish oil</i>	<i>Infant adverse events serious.</i> <i>No significant difference in risk for serious adverse events. These RCTs were determined to be too heterogeneous to permit pooling.</i>

<i>RCTs</i> <i>Strength of evidence: Low</i>			<i>Preterm infants</i>	<i>Supplementation with n-3 FA in the form of fish oil</i>	<i>Infant adverse events serious.</i> <i>No significant difference in risk for serious events associated with preterm birth. These RCTs were determined to be too heterogeneous to permit pooling.</i>
<i>3 RCTs, 5 observational studies, 4 biomarkers studies</i> <i>Strength of evidence: Low</i>			<i>Breastfeeding women or infants</i>	<i>Supplementation with DHA</i>	<i>Asthma and other respiratory illnesses: Wheeze</i> RCTs: No significant effect on risk for wheeze at 12 months; meta-analysis of 3 RCTs: OR 1.06 (95% CI 0.73, 1.54) Observational studies: showed Inconsistent associations with risk for wheeze across studies.
<i>3 RCTs, 7 observational studies</i> <i>Strength of evidence: Low</i>			<i>Breastfeeding women or infants</i>	<i>Supplementation of mothers or infants through formula fortification with any n-3 FA or exposure as assessed with biomarkers</i>	<i>RCTs: No significant (and inconsistent) effects on risk for atopic dermatitis/eczema across RCTs, consistent with observational studies.</i>
<i>3 RCTs, 2 observational studies</i> <i>Strength of evidence: Low</i>			<i>Breastfeeding women or infants</i>	<i>Supplementation of mothers or infants through formula fortification with any n-3 FA or exposure as assessed with biomarkers</i>	<i>RCTs: No significant effect on the risk for food or dust mite allergy and no association of breastmilk or infant biomarkers and risk for allergies across observational studies.</i>
<i>3 RCTs</i>			<i>Breastfeeding women or infants</i>	<i>Supplementation of mothers or infants through formula</i>	<i>RCTs: No significant effect on the risk for asthma and other respiratory illnesses</i>

<i>Strength of evidence: Moderate</i>				<i>fortification with any n-3 FA</i>	
<i>10 observational studies</i>			<i>Breastfeeding women or infants</i>	<i>Any n-3 FA exposures</i>	<i>Observational Studies: Inconsistent associations with risk for respiratory illness s across studies.</i>

Ren et al. (2021)

Characteristics of the systematic review	
Title	Systematic Literature Review and Meta-Analysis of the Relationship Between Polyunsaturated and Trans Fatty Acids During Pregnancy and Offspring Weight Development
Author(s)	Ren, K., Vilhjálmsdóttir, B.L., Rohde, J.F., Walker, K.C., Runstedt, S.E., Lauritzen, L., Heitmann, B.L., Specht, I.O.
Year of publication	2021
Journal	Frontiers in Nutrition
Country of origin (corresponding author)	Denmark
Funding	The Parker Institute, Bispebjerg and Frederiksberg Hospital was supported by a core grant from the Oak Foundation.
Reported conflict of interest	The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
What is the main objective of the review?	To evaluate whether levels of EPA, DHA, and trans fatty acids during pregnancy influenced offspring weight development.
Inclusion/Exclusion criteria	<p>Inclusion criteria for RCTs and observational studies:</p> <p>All years of publication were eligible. Languages were restricted to English and Chinese.</p> <p>Other inclusion criteria for RCTs:</p> <ul style="list-style-type: none"> - Population: Healthy pregnant women and children.

	<ul style="list-style-type: none"> - Intervention: DHA and/or EPA as supplement(s) during pregnancy. - Comparison: No treatment groups. - Outcomes: Primary outcome is offspring weight; secondary outcome is BMI. <p>Other inclusion criteria for observational studies:</p> <ul style="list-style-type: none"> - Associations between levels of fatty acid and weight or BMI at different age groups (weight at birth, aged 0–4 years, and aged 5–10 years) should be measured. - Associations between polyunsaturated and trans fatty acids consumption during pregnancy and offspring adiposity were presented based on fully adjusted models. - The sample tissue, from which levels of DHA and/or EPA were analyzed, should be maternal plasma or placental tissues. - The DHA content in the tissue should be expressed in percentage of total fatty acids. <p>Exclusion:</p> <p>Cross-sectional studies with no control group, chart reviews, case series, commentaries, self-reported dietary information studies, and animal studies.</p>
Start and ending dates of the literature search	Up to 2019
Population/Subjects	See inclusion criteria
Intervention(s)	Most of the studies used both DHA and EPA as supplements. Seven studies used DHA as the single supplement. The majority of the trials used fish oil.
Outcome(s) of relevance	Birth weight
Quality assessment tool(s)	The Cochrane risk of bias tool.
Results of quality assessment	Of the 19 RCTs, 6 RCTs had low risk of bias, 6 RCTs had unclear risk of bias, and 7 RCTs had high risk of bias.
Data synthesis methodology	Meta-analysis

Number of included studies	A total of 27 studies representing 19 trials. A total of 19 RCT studies were combined in a meta-analysis on birth weight.				
Results (relevant) and the confidence in the evidence	<p>A total of 6,408 infants were investigated in relation to birth weight. There was no indication of publication bias for the outcome birth weight.</p> <p>The meta-analysis showed significant overall higher birth weight in the n-3 LCPUFA-supplemented compared to the control groups, irrespective of intake level [mean differences (MD) 57.5 g, 95% CI 26.2–88.9, n=6,408, I²=19%].</p> <p>Stratified analysis of the effects of n-3 LCPUFA intervention doses showed a higher birth weight among the supplemented individual with doses higher than 650 mg/day compared to the control groups, but not among individuals supplied with lower doses (MD 87.5 g, 95% CI 52.3–122.6, n=3,831).</p> <p>The overall quality of the meta-analysis results for birth weight was rated as moderate quality.</p>				
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo					
<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect (birth weight)</i>
<i>Ramakrishnan et al. (2010)</i>	<i>Mexico</i>	<i>18–22 weeks of gestation until birth</i>	<i>Newborn: 487 in the intervention group, 486 in the control group.</i>	<i>Intervention: Algal DHA capsule, 400 mg/day. Control: olive oil capsules.</i>	<i>Birth weight</i>
<i>Carlson et al. (2014)</i>	<i>USA</i>	<i>8–20 weeks of gestation until birth</i>	<i>Newborn: 154 in the intervention group, 147 in the control group.</i>	<i>Intervention: 600 mg/day DHA Control: A mix of soybean and corn oil capsule</i>	<i>Birth weight</i>
<i>Mulder et al. (2014)</i>	<i>Canada</i>	<i>Before 16 weeks of gestation until birth</i>	<i>Newborn: 104 in the intervention group, 111 in the control group.</i>	<i>Intervention: 400 mg/day DHA</i>	<i>Birth weight</i>

				<i>Control: A mix of soybean and corn oil capsule</i>	
<i>Harris et al. (2015)</i>	<i>USA</i>	<i>16–20 weeks of gestation until birth</i>	<i>Newborn: 107 in the intervention group, 121 in the control group.</i>	<i>Intervention: 300 mg/day or 600mg/day DHA</i> <i>Control: olive oil</i>	<i>Birth weight</i>
<i>Smuts et al. (2003)</i>	<i>USA</i>	<i>24–28 weeks of gestation until birth</i>	<i>Newborn: 18 in the intervention group, 16 in the control group.</i>	<i>Intervention: 183.9±71.4 mg/day(mean±SD) (DHA enriched eggs)</i> <i>Control: regular eggs</i>	<i>Birth weight</i>
<i>Makrides et al. (2010)</i>	<i>Australia</i>	<i>After 21 weeks of gestation until birth</i>	<i>Newborn: 1,197 in the intervention group, 1,202 in the control group.</i>	<i>Intervention: 800 mg/day DHA+100 mg/day EPA</i> <i>Control: vegetable oil</i>	<i>Birth weight</i>
<i>Escolano-Margarit et al. (2011)</i>	<i>Germany, Spain, Hungary</i>	<i>20 weeks until birth</i>	<i>Newborn: 43 in the intervention group, 47 in the control group.</i>	<i>Intervention: 500 mg/day DHA+150 mg/day EPA</i> <i>Control: milk</i>	<i>Birth weight</i>
<i>Hauer et al. (2012)</i>	<i>Germany</i>	<i>15 weeks of gestation to 16 weeks post-partum</i>	<i>Newborn: 92 in the intervention group, 96 in the control group.</i>	<i>Intervention: 1,020 mg/day DHA+180 mg/day EPA</i> <i>Control: dietary counseling</i>	<i>Birth weight</i>

<i>Vinding et al. (2018)</i>	<i>Denmark</i>	<i>24 weeks of gestation to 1 week post-partum</i>	<i>Newborn: 304 in the intervention group, 301 in the control group.</i>	<i>Intervention: 888 mg/day DHA+1,320 mg/day EPA Control: olive oil</i>	<i>Birth weight</i>
<i>Bergmann et al. (2012)</i>	<i>Germany</i>	<i>Mid-pregnancy to 12 weeks post-partum</i>	<i>Newborn: 41 in the intervention group, 74 in the control group.</i>	<i>Intervention: 200 mg/day DHA+60 mg/day EPA Control: Combined supplement (basic supplement + FOS)</i>	<i>Birth weight</i>
<i>Van Goor et al. (2010)</i>	<i>Netherands</i>	<i>15.6–17.4 weeks of gestation to 12 weeks post-partum</i>	<i>Newborn: 42 in the intervention group, 36 in the control group.</i>	<i>Intervention: 220 mg/day DHA+34 mg/day EPA Control: soybean oil</i>	<i>Birth weight</i>
<i>Judge et al. (2011)</i>	<i>USA</i>	<i>24 weeks until birth</i>	<i>Newborn: 27 in the intervention group, 21 in the control group.</i>	<i>Intervention: 267 mg/day DHA+34 mg/day EPA (cereal bar) Control: corn oil cereal bar</i>	<i>Birth weight</i>
<i>Keenan et al. (2016)</i>	<i>USA</i>	<i>6 weeks supply during pregnancy</i>	<i>Newborn: 34 in the intervention group, 15 in the control group.</i>	<i>Intervention: 50 mg/day DHA+90 mg/day EPA Control: Soybean oil</i>	<i>Birth weight</i>
<i>Ostadrhimi et al. (2018)</i>	<i>Iran</i>	<i>20 weeks of gestation to 4 weeks post-partum</i>	<i>Newborn: 75 in the intervention group, 75 in the control group.</i>	<i>Intervention: 120 mg/day</i>	<i>Birth weight</i>

				<i>DHA+180mg/day EPA (fish oil capsule).</i>	
				<i>Control: liquid paraffin</i>	
<i>Huarte et al. (2015)</i>	<i>Spain</i>	<i>28 weeks until 16 weeks post-partum</i>	<i>Newborn: 56 in the intervention group, 54 in the control group.</i>	<i>Intervention: 320 mg/day DHA+72mg/day EPA (fish oil-enriched dairy).</i>	<i>Birth weight</i>
				<i>Control: dairy</i>	
<i>Malcolm et al. (2003)</i>	<i>UK</i>	<i>15 weeks of gestation until birth</i>	<i>Newborn: 28 in the intervention group, 27 in the control group.</i>	<i>Intervention: 200 mg/day DHA+36mg/day EPA (fish oil capsule).</i>	<i>Birth weight</i>
				<i>Control: sunflower oil</i>	

Sarmiento et al. (2016)

Characteristics of the systematic review	
Title	Polyunsaturated fatty acid supplementation for drug-resistant epilepsy (Review)
Author(s)	Sarmiento Vasconcelos V, Macedo CR, de Souza Pedrosa A, Pereira Gomes Morais E, Porfírio GJM, Torloni MR
Year of publication	2016
Journal	Cochrane Database of Systematic Reviews
Country of origin (corresponding author)	Brazil
Funding	Internal sources:

	<p>Brazilian Cochrane Centre, Brazil.</p> <p>External sources:</p> <ul style="list-style-type: none"> - National Institute for Health Research (NIHR), UK. This review was supported by the National Institute for Health Research, via Cochrane Infrastructure funding to the Epilepsy Group.
Reported conflict of interest	None known.
What is the main objective of the review?	To assess the effectiveness and tolerability of omega-3 polyunsaturated fatty acids (EPA and DHA) in the control of seizures in people with refractory epilepsy.
Inclusion/Exclusion criteria	<p>Inclusion</p> <p>Types of studies: All randomised and quasi-randomised studies using PUFAs for the treatment of drug-resistant epilepsy.</p> <p>Types of participants: All individuals (adults and children) with a diagnosis of drug-resistant epilepsy, irrespective of their seizure type or epilepsy syndrome. A diagnosis of 'drug-resistant' was defined as unsatisfactory seizure control despite the use of at least two antiepileptic drugs in adequate dosages.</p> <p>Types of interventions: Supplementation with PUFAs belonging to the omega-3 series (EPA and DHA) at any dosage and over any period of time, combined with antiepileptic drugs.</p> <p>The intervention was compared to no supplementation, placebo treatment or other treatment.</p> <p>Exclusion: People under dietary treatments (e.g. ketogenic diet) or who have previously used PUFA supplements were not included.</p>
Start and ending dates of the literature search	Up to November 2015.
Population/Subjects	See inclusion criteria.

Intervention(s)	See inclusion criteria.
Outcome(s) of relevance	Gastrointestinal effects, nausea and diarrhoea. Levels of HDL and LDL.
Quality assessment tool(s)	The risk of bias was assessed according to the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011).
Results of quality assessment	<p>One study had unclear RoB for random sequence generation, allocation concealment and blinding of outcome assessors, and high RoB for incomplete outcome data.</p> <p>One study had high risk of bias for random sequence generation, allocation concealment and blinding of outcome assessment.</p> <p>One study had low RoB for all domains.</p>
Data synthesis methodology	For meta-analysis of dichotomous data, the Mantel-Haenszel method was used. For continuous data, the inverse variance method was used. The fixed-effect model for meta-analyses was used if studies were homogeneous.
Total number of included studies	3 RCTs
Results (relevant) and the confidence in the evidence	<p>There were no dropouts due to non-compliance in two of the studies. There was one loss due to gastrointestinal effects (nausea and indigestion) and two losses due to increased seizure severity or status epilepticus, all in the placebo groups of the two studies.</p> <p>Two studies analysed gastrointestinal effects and there were no significant differences between PUFA versus placebo (RR 0.78, 95% CI 0.32 to 1.89) (IS = 8%). The quality of the evidence (GRADE) was low.</p> <p>The most frequent side effects were nausea and diarrhoea (details of the severity of these symptoms were not given).</p> <p>Changes in plasma lipid profile (triglycerides, low- and high-density lipoprotein cholesterol) were not reported in the included studies.</p> <p>There were a few reports of other adverse events, all in one study. In the PUFA group there was one case of sleepiness, one participant complained of fatigue and breathlessness and one had recurrence of depression and paranoia. In the placebo group there were two cases of sleepiness and one case of aggression and fatigue.</p> <p>One of the studies did not provide any information on adverse effects.</p>
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo	

<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age/ Gender</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect</i>
<i>Bromfield 2008</i>	<i>USA</i>	<i>12 weeks</i>	<i>Adults (13 PUFA, 14 Placebo), 9 men and 12 women (5 without information), mean age 37 (range 22 to 62 years).</i>	<i>PUFA group: one capsule (1.1 g) taken twice a day, total dose 2.2 g PUFAs daily (EPA plus DHA in a 3:2ration). Placebo: identical capsules containing a mineral oil</i>	<i>Gastrointestinal effects, no significant differences</i>
<i>Yuen 2005</i>	<i>UK</i>	<i>12 weeks</i>	<i>58 adults (30 PUFA, 28 placebo), 35 men and 22 women, mean age 38.5 (range 19 to 65 years).</i>	<i>PUFA group: 1000 mg fish oil capsules, total daily dose 1.7 g omega-3 PUFAs (1 g EPA and 0.7 g DHA). Each capsule contained 171 mg EPA and 112 mg DHA, and < 100 IU vitamin A and < 40 IU vitamin D). Placebo: matching capsules containing mixed oils (palm olein 70%, rapeseed oil 15%, sunflower oil 15%).</i>	<i>Gastrointestinal effects, other side effects., no significant effects</i>
<i>Comments: No significant differences between placebo and PUFA treatment group (RR 0.78, 95% CI 0.32 to 1.89) (IS = 8%). Low quality of evidence due to unclear risk of bias and high imprecision.</i>					

Su et al. (2021)

Characteristics of the systematic review

Title	Omega-3 Polyunsaturated Fatty Acid Supplementation in Patients with Lower Extremity Arterial Disease
Author(s)	Min-I Su, Ying-Chih Cheng, Yu-Chen Huang & Cheng-Wei Liu
Year of publication	2021
Journal	Journal of the American College of Nutrition
Country of origin (corresponding author)	Taiwan
Funding	Not reported
Reported conflict of interest	None
What is the main objective of the review?	To investigate the effect of omega-3 PUFA supplements on outcomes in lower extremity arterial disease (LEAD) patients.
Inclusion/Exclusion criteria	<p>Inclusion criteria:</p> <p>Patients with LEAD, RCTs; clinical trials in which omega-3 PUFA supplementation was administered, and clinical outcomes were measured at baseline and at the end of the intervention in both treatment and control groups; published in English.</p> <p>Exclusion criteria:</p> <p>Articles reporting individual cases or series of cases and conference abstracts for which no full text was available.</p>
Start and ending dates of the literature search	No information (All studies included before February 2020)
Population/Subjects	Patients with Lower Extremity Arterial Disease (LEAD)
Intervention(s)	See inclusion criteria
Outcome(s) of relevance	<p>Changes in lipid profiles, including LDL and HDL.</p> <p>CRP</p>

Quality assessment tool(s)	The Cochrane Collaboration's tool.				
Results of quality assessment	Most of the studies had a low risk of bias.				
Data synthesis methodology	For continuous outcomes, standardized mean differences (SMDs; Hedges'g) with 95% confidence intervals were calculated to estimate the exact effect size across different studies reporting different variables.				
Number of included studies	16 RCTs				
Results (relevant) and the confidence in the evidence	No significant association was found for n-3 PUFAs and CRP, HDL or LDL. The grade quality was moderate for C-reactive protein; low for LDL and HDL.				
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo					
<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age/ Gender</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect</i>
<i>Woodcock et al. 1984 RCT</i>	<i>England</i>	<i>7 weeks</i>	<i>19;</i> <i>Mean age >60</i>	<i>1.8 g EPA</i>	<i>Serum lipid profile;</i>
<i>Gans et al. 1990 RCT</i>	<i>Netherlands</i>	<i>4 months</i>	<i>32;</i> <i>Mean age >60</i>	<i>1.8 g EPA + 1.2g DHA</i>	<i>Serum lipid profile; C-reactive protein</i>
<i>Mori et al. 1992 RCT</i>	<i>Australia</i>	<i>1 month</i>	<i>29;</i> <i>Mean age >60</i>	<i>2.8 g EPA + 1.8g DHA</i>	<i>Serum lipid profile;</i>
<i>Ramirez-Tortosa et al. 1999 RCT</i>	<i>Spain</i>	<i>3 months</i>	<i>25;</i> <i>Mean age >50</i>	<i>2.88 g/day EPA + 1.66g/ day DHA</i>	<i>Serum lipid profile;</i>
<i>Schiano et al. 2008 RCT</i>	<i>Italy</i>	<i>3 months</i>	<i>32;</i>	<i>2g/day (EPA:DHA 0.9:1.5)</i>	<i>Serum lipid profile; inflammatory marker</i>

			<i>Mean age >60</i>		
<i>Ishikawa et al. 2010 RCT</i>	<i>Japan</i>	<i>5 years</i>	<i>173;</i> <i>Mean age >60</i>	<i>EPA 1.8 g/day</i>	<i>Serum lipid profile;</i>
<i>Grenon et al. 2015 RCT</i>	<i>USA, California</i>	<i>1 month</i>	<i>72;</i> <i>Mean age >60</i>	<i>2.6 g EPA + 1.8g DHA</i>	<i>Serum lipid profile;</i>
<i>Hammer et al. 2019 RCT</i>	<i>Austria</i>	<i>3 months</i>	<i>70;</i> <i>Mean age >60</i>	<i>2.2 g EPA + 1.8g DHA</i>	<i>Serum lipid profile;</i> <i>proinflammatory,</i> <i>endothelial and platelet</i> <i>activation markers</i>
<i>Ramirez et al. 2019 RCT</i>	<i>USA, California</i>	<i>3 months</i>	<i>24;</i> <i>Mean age >60</i>	<i>2.6 g EPA + 1.8 g DHA</i>	<i>Serum lipid profile;</i> <i>Inflammatory marker</i>
Comments: Omega-3 PUFA supplementation had a significant effect on the mean change in triglycerides (Hedges'g =-0.34, 95%CI [-0.55–0.13], p<0.01, I ² ¼32.5%), while it had no significant effects on other lipid parameters such as total, low-density lipoprotein and high-density lipoprotein cholesterol; hs-CRP; or flow-mediated vasodilation. There was a triglyceride-lowering effect of omega-3 PUFAs.					

Watson and Stackhouse (2020)

Characteristics of the systematic review	
Title	Omega-3 fatty acid supplementation for cystic fibrosis (Review)
Author(s)	Watson H, Stackhouse C
Year of publication	2020
Journal	Cochrane Database of Systematic Reviews
Country of origin (corresponding author)	UK
Funding	Internal sources: Sheffield Children's Hospital Appeal, UK.

	- External sources: National Institute for Health Research, UK. This systematic review was supported by the National Institute for Health Research, via Cochrane Infrastructure funding to the Cochrane Cystic Fibrosis and Genetic Disorders Group.
Reported conflict of interest	None known
What is the main objective of the review?	To determine whether there is evidence that omega-3 polyunsaturated fatty acid supplementation reduces morbidity and mortality. To identify any adverse events associated with omega-3 polyunsaturated fatty acid supplementation in cystic fibrosis (CF).
Inclusion/Exclusion criteria	<p>Inclusion criteria</p> <p>Types of studies: RCTs, quasi-randomised trials, and cross-over trials.</p> <p>Types of participants: People with CF, of any age and severity, diagnosed clinically and by sweat or genetic testing.</p> <p>Types of interventions: Dietary supplementation of omega-3 essential fatty acids of any dosage, frequency and duration compared with placebo in people with CF. The supplements contain omega-3 fatty acids in the form of EPA or DHA, or both. Studies were included if they compared the effect of this intervention with a placebo with low omega-3 or omega-6 fatty acid content, such as olive oil.</p>
Start and ending dates of the literature search	Searches were not restricted by year. The search was performed 1 April 2020
Population/Subjects	Children and adults with cystic fibrosis. Details on age and sex for the included studies are not reported.
Intervention(s)	Oral omega-3 supplementation (EPA or DHA), or both. See inclusion criteria for more details.
Comparator	Placebo
Outcome(s) of relevance	Adverse effects: diarrhoea, stomach pain, steatorrhoea, inflammation (IL-8), asthma exacerbations, cytokines (IL-8)
Quality assessment tool(s)	The domain-based evaluation described in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011) were used.
Results of quality assessment	Low risk of bias: random sequence generation (n=3); blinding (n=3); incomplete outcome data (n=3); other bias (n=4).

	High risk of bias: Selective reporting (n=1); other bias (n=1).				
	All other was considered as unclear risk of bias.				
Assessment of confidence in evidence	GRADE was conducted for the outcome diarrhoea.				
Data synthesis methodology	A fixed-effect model was used.				
Total number of included studies	5 RCTs, all five studies reported on adverse events.				
Results (relevant) and the confidence in the evidence	<p>Adverse events: diarrhoea. Follow-up: 6 weeks. 1 study reported drop out due to diarrhoea. 2 out of 7 participants in the fish oil group dropped out and 2 out of 5 participants in the placebo group. There was no significant difference between the groups, OR 0.6 (CI 0.05 to 6.79). The quality of the evidence (GRADE) was very-low.</p> <p>One study reported an increase in steatorrhoea requiring participants to increase their daily dose of pancreatic enzymes, but three studies had already increased pancreatic enzyme dose at study begin so as to reduce the incidence of steatorrhoea.</p> <p>Other adverse events included stomach pains (5/35 participants, one study) but the intervention arm wasn't specified.</p> <p>A significant decrease of IL-8 in the supplemented group was reported, however, data for the placebo group was lacking.</p> <p>One six-week study (19 participants) reported three asthma exacerbations leading to exclusion of participants since corticosteroid treatment could affect essential fatty acid metabolism.</p>				
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo					
<i>Reference and study design</i>	<i>Country</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age/ Gender</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect</i>

<i>Hanssens 2016 Parallel</i>	<i>Belgium</i>	<i>12 months</i>	<p><i>15 participants with CF in total. N = 13 completed, 6 intervention, 7 placebo.</i></p> <p><i>Age, mean: intervention group 14 years (5.2 - 26.0 years); placebo group 17.5 years (4.6 - 30.4 years).</i></p> <p><i>Gender (M:F): intervention group (4:3); placebo group (7:1).</i></p>	<p><i>Intervention group (n = 7): oral supplement of omega-3, 60 mg/kg.</i></p> <p><i>Control group (n = 8): identical placebo.</i></p>	<i>Steatorrhoea,</i>
<i>Henderson 1994 Parallel with four arms</i>	<i>USA</i>	<i>6 weeks</i>	<p><i>12 children and young adults diagnosed with CF.</i></p> <p><i>Age, mean (SD): age 12.2 (5.4) years.</i></p> <p><i>Gender: 7 males, 5 females.</i></p> <p><i>13 gender and age-matched people without CF (mean (SD) age 13.4 (6.3) years), 7 males, 6 females.</i></p>	<p><i>Intervention group: 8 x 1g capsules fish oil (4 capsules twice daily) containing 3.19 g EPA and 2.21 g DHA.</i></p> <p><i>Control: olive oil placebo capsules flavoured to obtain a slight fish taste.</i></p>	<i>Diarrhoea, steatorrhoea</i>
<i>Keen 2010, Parallel</i>	<i>Sweden</i>	<i>3 months</i>	<i>45 children and adults with "severe" CF mutations randomised;</i>	<i>Intervention group A: 50 mg/kg per day fatty acid blend capsules</i>	<i>Stomach pain, steatorrhoea</i>

			<p>43 commenced study and 35 participants completed the study. Disease status: 20 participants were chronically infected with <i>Pseudomonas aeruginosa</i>.</p> <p>Age (range): 7 to 41 years.</p> <p>Gender: 17 males; 18 females.</p>	<p>containing predominantly EPA and DHA.</p> <p>Intervention group B: placebo capsules containing predominantly saturated fatty acids.</p> <p>Intervention group C: 50 mg/kg per day fatty acid blend capsules containing a high proportion of linoleic acid and AA.</p> <p>Actual dose of EPA and DHA administered not described.</p>	
Lawrence 1993, cross-over	Australia	6 weeks	<p>19 adolescents and adults diagnosed with CF on genotype, sweat test, or clinically.</p> <p>Age, median (range): 17 years (12 years - 26 years).</p> <p>Gender: 11 males, 5 females.</p> <p>3 participants excluded due to requiring a course of corticosteroids for asthma attacks.</p>	<p>Intervention group: capsules with 2.7 g EPA daily.</p> <p>Control group: identical olive oil placebo capsules.</p>	Asthma, steatorrhoea

<i>Panchaud 2006, cross-over trial with no washout period.</i>	<i>Switzerland</i>	<i>1 year (2 x 6-month periods).</i>	<p><i>17 children and young adults with CF; 1 participant discontinued study after 8 months for personal convenience.</i></p> <p><i>Age, mean (SD): 18 (9) years).</i></p> <p><i>Gender: 10 males, 7 females.</i></p>	<p><i>Intervention Group: liquid dietary supplement containing PUFA mixture (EPA, DHA, GLA and STA).</i></p> <p><i>Control Group: liquid dietary supplement without PUFA mixture.</i></p> <p><i>Volume of supplementation was determined according to participant's weight; intake ranged from 100mg - 300 mg DHA and 200 mg - 600 mg EPA per day.</i></p>	<i>No adverse effects of treatment</i>
Comments:					

Xin et al. (2013)

Characteristics of the systematic review	
Title	Fish Oil and Atrial Fibrillation after Cardiac Surgery: A Meta-Analysis of Randomized Controlled Trials
Author(s)	Xin W, Wei W, Zhang X, Yang H, Zhang T, Li B, Mi S.
Year of publication	2013
Journal	PLOS ONE
Country of origin (corresponding author)	China
Funding	The authors reported no support or funding.
Reported conflict of interest	None.

What is the main objective of the review?	To investigate the possible role of fish oil supplementation for the prevention of postoperative atrial fibrillation (POAF).
Inclusion/Exclusion criteria	Inclusion criteria: 1) published as full-length article or abstract in any language; 2) reported as a prospective, randomized, and controlled trial with a parallel design (regardless of sample size); 3) included adult human subjects (18 years of age or older) who underwent a cardiac surgery and assigned to perioperative fish oil supplementation (orally or intravenously) or a control group; 4) aimed to investigate the effect of fish oil supplementation on the prevention of POAF.
Start and ending dates of the literature search	The final literature search was performed on November 25th, 2012. There was no restriction on the start date of the search.
Population/Subjects	Patients undergoing cardiac surgery. Mean age for patients in the included studies reporting on bleeding were >60 years.
Intervention(s)	EPA and DHA in combination
Comparator	None or oil without EPA/DHA
Outcome(s) of relevance	Major bleeding following cardiac surgery, defined as bleeding more than 3L through chest tube drain, needing transfusion or needing reexploration or reoperation.
Quality assessment tool(s)	Jadad score (including allocation concealment) and Cochrane risk of bias tool.
Assessment of confidence in evidence	Not performed.
Results of quality assessment	Two studies had low RoB for all criteria (Mozaffarian 2012; Farquharson 2011). Summary risk of bias is not given for the three other studies, but they all lacked information on allocation concealment.
Data synthesis methodology	Dichotomous data were analyzed using risk ratio (RR) with 95% confidence intervals (CI), whereas continuous variables were analyzed using weighted mean differences (WMD).
Total number of included studies	Eight studies, whereof five assessed major bleeding.
Results (relevant) and the confidence in the evidence	By pooling these studies (5 in total), no significant influence of fish oil on the incidence of major bleeding ($I^2= 39\%$, fixed-effect model: RR = 0.82, 95% CI 0.59 to 1.55, $p = 0.26$; random-effect model: RR = 0.83, 95% CI 0.51 to 1.35, $p = 0.45$) were found.
Characteristics of the studies addressing potential adverse effects of n-3 PUFAs compared to placebo	

<i>Reference and study design</i>	<i>Study design</i>	<i>Follow-up period/ measurement time points.</i>	<i>Number of participants/ Age/ Gender</i>	<i>Substance tested (EPA and DHA in combination or separately)</i>	<i>Adverse effect</i>
<i>Calo 2005</i>	<i>Randomized, open-label</i>	<i>At least 5 days before surgery until discharge.</i>	<i>160 participants in total. Mean age was 65.5 years. 85% were males.</i>	<i>EPA: 0.58 g/day; DHA 1.16 g/day Control: no treatment</i>	<i>Major bleeding</i>
<i>Heidarsdottir 2010</i>	<i>Randomized, double-blind, placebo-controlled</i>	<i>6 days (median) before surgery until discharge or 14 days after surgery.</i>	<i>168 participants in total. Mean age was 67 years. 79.4% were males.</i>	<i>EPA: 1.24 g/day; DHA 1.0 g/day Control: olive oil</i>	<i>Major bleeding</i>
<i>Farquharson 2011</i>	<i>Randomized, double-blind, placebo-controlled</i>	<i>21 days before surgery until discharge or 6 days after surgery.</i>	<i>194 participants in total. Mean age was 64 years. 73.2% were males.</i>	<i>EPA: 2.7 g/day; DHA 1.9 g/day Control: sunola</i>	<i>Major bleeding</i>
<i>Sandesara 2012</i>	<i>Randomized, double-blind, placebo-controlled</i>	<i>2.5 days (median) before surgery until 14 days after surgery.</i>	<i>2430 participants in total.</i>	<i>EPA: 1.07 g/day; DHA 0.86 g/day Control: corn oil</i>	<i>Major bleeding</i>

			<p><i>Mean age was 62.7 years.</i></p> <p><i>80.7% were males.</i></p>		
<i>Mozaffarian 2012</i>	<i>Randomized, double-blind, placebo-controlled</i>	<i>2–5 days before surgery until discharge or 10 days after surgery.</i>	<p><i>1516 participants in total.</i></p> <p><i>Mean age was 63.7 years.</i></p> <p><i>72.2% were males.</i></p>	<p><i>EPA: 1.03 g/day; DHA 0.83 g/day</i></p> <p><i>Control: Olive oil</i></p>	<i>Major bleeding</i>
<i>Comments:</i>					

10.2 Literature search for RCTs

Database:

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to date of the search>

Embase 1974 to date of the search

Date of search: August 30, 2021.

Search strategy:

- Eicosapentaenoic acid" or Icosapent or Timnodonic acid or Icosapentaenoic acid or EPA or docosahexaenoic acid or DHA
- The search was limited to English language, children and adolescents, 2014-current, and randomised controlled trials.

Result: 1100; 387 (Medline) and 713 (Embase)

10.2.1 Studies excluded after full-text evaluation

An overview of the publications considered not to fulfil the eligibility criteria is given in Table 10.2.1-1.

Table 10.2.1-1. Publications considered not eligible.

Reference	Reason for exclusion
(Allaire et al., 2018)	Population
(Beatriz et al., 2019)	Publication type
(Crippa et al., 2018)	Publication type
(Haberling et al., 2019)	Publication type
(Huang et al., 2014)	Publication type
(Huang et al., 2015)	Publication type
(Jung et al., 2014)	Population
(Lopez-Frias et al., 2018)	Publication type
(Mazahery et al., 2016)	Publication type
(Mazahery et al., 2020)	Outcome
(McNamara et al., 2017)	Publication type
(McNamara et al., 2019)	Publication type
(McNamara et al., 2014a)	Publication type
(McNamara et al., 2021)	Outcome
(McNamara et al., 2014b)	Publication type
(McNamara et al., 2014c)	Outcome
(Meguid et al., 2016)	Publication type
(Meldrum et al., 2015)	Publication type
(Meldrum et al., 2018)	Outcome
(Montgomery et al., 2014)	Outcome

Reference	Reason for exclusion
(Moreno et al., 2014)	Publication type
(Mossaheb et al., 2018)	Outcome
(Myhrstad et al., 2014)	Population
(O'Connor et al., 2016)	Outcome
(Parellada et al., 2015)	Publication type
(Pawelczyk et al., 2016)	Population
(Pawelczyk et al., 2019)	Outcome
(Rodriguez et al., 2019)	Outcome
(Romieu et al., 2014)	Publication type
(Sabour et al., 2015)	Population
(Scaiola et al., 2018)	Population
(See et al., 2017)	Population
(Signorini et al., 2014)	Publication type
(Skae et al., 2014)	Publication type
(Skouroliakou et al., 2016)	Population
(Spahis et al., 2016)	Publication type
(Van Der Wurff et al., 2018)	Publication type
(Vericel et al., 2016)	Population
(Widenhorn-Muller et al., 2014)	Exposure
(Yang et al., 2021)	Outcome
(Zulyniak et al., 2016)	Outcome

10.2.2 Evaluation of risk of bias

An overview of the studies included for evaluation of RoB is given in table 10.2.2-1.

Table 10.2.2-1. RCTs included for RoB evaluation.

Reference
(Chang et al., 2019)
(Chase et al., 2015)
(Cornu et al., 2018)
(Crippa et al., 2019)
(de Ferranti et al., 2014)
(Engler et al., 2004)
(Hanssens et al., 2016)
(Hughbanks-Wheaton et al., 2014)
(Janczyk et al., 2015)
(Manos et al., 2018)
(Mazahery et al., 2019)
(Milte et al., 2015)
(Montgomery et al., 2018)
(Pacifico et al., 2015)
(Rodriguez-Cruz et al., 2019)

Reference
(Rodriguez-Cruz et al., 2018)
(Smuts et al., 2015)
(van der Wurff et al., 2019)
(Verduci et al., 2014)
(Voigt et al., 2014)

The response options and symbols used for the RoB rating were as follows:

- Definitely low risk of bias ++
- Probably low risk of bias +
- Probably high risk of bias -
- Definitely high risk of bias - -

The detailed RoB evaluations for the RCTs are shown below.

Chang et al. (2019)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	The randomisation numbers were generated by computer.	++
	2	Was allocation to study groups adequately concealed?	The investigators were blinded to the group allocation during the study, however, the method was not described.	+
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	The investigators were blinded during the study and when assessing the outcome measurements. Participants received Omega-3 or placebo (soybean oil), and it is reported that the study is double-blind. However, there was no information whether there were identical bottles etc.	+
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Reasons for drop-out were described (11 participants). Only 80 of the 86 included participants was included in the analysis for CRP. The discrepancy for this was not explained.	-
Detection bias	5	Can we be confident in the exposure characterisation?	The daily dose of EPA was 1.2 g. How compliance was checked or instructions to participants were not reported. No information on stability and purity.	-
	6	Can we be confident in the outcome assessment?	CRP was measured by ELISA. The investigators were blinded when assessing the outcome measurements. The blood samples were analysed in duplicate.	++
Selective reporting bias	7	Were all measured outcomes reported?	Yes	++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	No protocol was mentioned. A power analysis was performed and statistical methods used were appropriate.	+

Chase et al. (2015)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	The participants were randomized, but the method was not described.	+
	2	Was allocation to study groups adequately concealed?	Not reported.	-
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	The study is double-blind. Intervention (DHA/EPA or corn/soy oil) were given as capsules. Information on identical appearances was not described.	+
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Loss to follow-up and non-compliance was not described. From the results, there seemed to be more than 50% loss to follow-up at the end of the study.	-
Detection bias	5	Can we be confident in the exposure characterisation?	Concentration and derivation of EPA and DHA was reported. The intervention was taken as capsules.	++
	6	Can we be confident in the outcome assessment?	The outcomes were measured by acceptable methods.	++
Selective reporting bias	7	Were all measured outcomes reported?		++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	A priori protocol was published. No power analysis was performed. Statistical methods used were appropriate.	+

Cornu et al. (2018)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
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Selection bias	1	Was administered dose or exposure level adequately randomized?	Randomization was performed according to a pre-established blocked randomization list, stratified by centre. This list was generated by the study statistician.	++
	2	Was allocation to study groups adequately concealed?	Centralized allocation concealed from patients, investigators and the coordination centre. Patients, investigators, and the coordination centre were blinded to group allocation.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	The placebo capsules were indistinguishable from active capsules; this was assessed using panel testing. Participants, care providers, those assessing outcomes, study coordinators and monitors were blinded to the administered treatment.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	The number of participants experiencing adverse events were reported, however, the total number reporting on adverse effects were not reported.	+
Detection bias	5	Can we be confident in the exposure characterisation?	The concentration of EPA and DHA was reported, and the compliance was evaluated. No information about purity and stability.	+
	6	Can we be confident in the outcome assessment?	Joint, lumbar and muscle pain: method not reported	-
			Headache: method not reported	-
			Skin problem: method not reported	-
Gastrointestinal effects: method not reported			-	
Selective reporting bias	7	Were all measured outcomes reported?	Most were reported.	+
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	No statistics for adverse events. A protocol was available. A power analysis was performed for expected effect size of 0.5 SD (90% power) for continuous outcomes. No power calculation for adverse events.	-

Crippa et al. (2019)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Computer-generated randomization.	++
	2	Was allocation to study groups adequately concealed?	Children, parents, and study investigators were blinded to the randomization.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	DHA and placebo supplement matched in touch, smell and size and was unlabeled.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	NR	-
Detection bias	5	Can we be confident in the exposure characterisation?	No information on stability and purity. DHA dose and treatment adherence was reported.	+
	6	Can we be confident in the outcome assessment?	Parents visited the institute monthly to report any adverse events. No details on about how this assessment was made or what type of events were considered adverse.	-
Selective reporting bias	7	Were all measured outcomes reported?	NR	-
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	No statistics for adverse events.	-

de Ferranti et al. (2014)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Block randomization.	++
	2	Was allocation to study groups adequately concealed?	Pharmacy-controlled randomization.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Both groups received four 1 g capsules daily. Capsules were color-blinded.	++

Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	The dropout during the 6 months was above 20%. The number of analyses per outcome was not stated specifically.	3 months: - 6 months --
Detection bias	5	Can we be confident in the exposure characterisation?	The dose (EPA and DHA) was reported, and compliance was assessed. No information on purity and stability.	+
	6	Can we be confident in the outcome assessment?	Lipid profile: Measured using "standard methods", no details provided.	-
			Inflammatory markers: hs-CRP was measured by an immunoturbidimetric assay	++
			Glucose: NR	-
			Joint, lumbar and muscle pain: Asked specifically in questionnaire, no information about validation.	-
			Gastrointestinal effects: Asked specifically in questionnaire, no information about validation.	-
Skin problems: Asked specifically in questionnaire, no information about validation.	-			
Selective reporting bias	7	Were all measured outcomes reported?	CRP results were reported to be nonsignificant, but data was not shown. Differences in frequency of musculoskeletal outcomes, gastrointestinal effects and skin problems were reported by p-values only.	-
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Differences in mean changes of glucose and lipid profile (LDL, HDL, and total cholesterol) was shown accompanied by p-values from Student t-test. A protocol was approved. Power was calculated for TG levels only. Due to low enrollment rate and lower effect size than expected, post-hoc power calculations indicated less than 10% power for TG.	Inflammatory marker, glucose and lipid profile: + Joint, lumbar and muscle pain, gastrointesintal effects and skin problems: -

Engler et al. (2004)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	The participants were randomised, but the method was not described.	+
	2	Was allocation to study groups adequately concealed?	Allocation concealment was not described, however, it is likely that this did not affect the results, since the study applied a crossover design.	+
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	It is stated that the study was double-blind. The capsules were identical.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	The number of samples was not reported	-
Detection bias	5	Can we be confident in the exposure characterisation?	Concentration and producer of the oils were described. Intervention was given by capsules and compliance was assessed by capsule counts. The washout period was 6 weeks, which is considered sufficient. No information on purity and stability.	+
	6	Can we be confident in the outcome assessment?	Inflammatory markers: acceptable method was used	++
			Lipid profile: acceptable methods were used	++
		Oxidative stress: acceptable method were used	++	
Selective reporting bias	7	Were all measured outcomes reported?	All outcomes were reported.	++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	No protocol was mentioned. Power analysis was not performed. Statistical methods used were appropriate.	-

Hanssens et al. (2016)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
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Selection bias	1	Was administered dose or exposure level adequately randomized?	Block randomization.	++
	2	Was allocation to study groups adequately concealed?	Pharmacy-controlled randomization.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Placebo capsules looked identical to active capsules. No information about taste or smell. No information about blinding during the study (i.e., box labeling).	+
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Two of 15 participants were dropouts.	+
Detection bias	5	Can we be confident in the exposure characterisation?	No information on purity. Poor compliance between 6 and 12 months follow-up. The dose (EPA and DHA) was reported.	-
	6	Can we be confident in the outcome assessment?	Inflammatory markers: White blood cell and neutrophil count, CRP, ESR, IgG were assessed by unspecified biochemical blood analyses.	-
			Liver enzymes: Laboratory test not specified.	-
			Platelets and coagulation: Laboratory test not specified.	-
		Safety: Data were collected in diaries at each visit.	-	
Selective reporting bias	7	Were all measured outcomes reported?	No data was shown for inflammatory markers, liver enzymes, platelets or other safety parameters, only a statement that there were no significant change between start and end values.	--
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Pilot study with only n=15 participants. No power analysis. The trial was registered prior to enrolment of the first patient.	+

Hughbanks-Wheaton et al. (2014)

Type of bias	No Question	Risk of bias evaluation	Risk of bias rating
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Selection bias	1	Was administered dose or exposure level adequately randomized?	A computer-generated, varied block size randomization schedule was stratified by age and vitamin A supplementation.	++
	2	Was allocation to study groups adequately concealed?	NR	-
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	NR	-
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Dropout above 20%.	-
Detection bias	5	Can we be confident in the exposure characterisation?	The DHA dose was reported, and the compliance was evaluated. No information on purity and stability.	+
	6	Can we be confident in the outcome assessment?	Lipid profile: fasting blood samples, method not reported.	-
			Glucose: fasting blood samples, method not reported.	-
			Platelet aggregation: fasting blood samples, method not reported.	-
			Liver and kidney function: fasting blood samples, method not reported.	-
		Gastrointestinal effects: self-reported in diaries.	-	
Selective reporting bias	7	Were all measured outcomes reported?	All were reported.	++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	A protocol was published, and a power analysis was performed. The statistics seemed appropriate.	++

Janczyk et al. (2015)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Computer-generated block randomization.	++
	2	Was allocation to study groups adequately concealed?	Central/external randomization and allocation.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Same dose, shape and color of capsules. No information about taste or smell. Blinded by supplier.	+
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	64 of 76 completed the trial. The reasons for the withdrawal were not reported.	+
Detection bias	5	Can we be confident in the exposure characterisation?	The dose (EPA and DHA) was reported. No information on compliance.	+
	6	Can we be confident in the outcome assessment?	Liver effects (enzymes): Laboratory test not specified.	-
			Lipid profile: Laboratory test not specified.	-
			Glucose: Laboratory test not specified.	-
Inflammatory markers: Laboratory test not specified.			-	
Selective reporting bias	7	Were all measured outcomes reported?		++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Comparisons were made between omega-3 and placebo at each timepoint, but the change between timepoints was not compared between groups. A protocol was prepared, and a power analysis was performed.	+

Manos et al. (2018)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
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Selection bias	1	Was administered dose or exposure level adequately randomized?	A block randomization scheme was electronically generated by NCH Investigational Drug Service Pharmacy (IDS) to randomize participants into blocks of 8 (Suresh, 2011). IDS assigned participants to a treatment arm sequentially from a prepared list.	++
	2	Was allocation to study groups adequately concealed?	The research personnel and subjects did not know what study group subjects were allocated to. Study staff were not involved with randomization and were unaware of upcoming allocation.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Participants, study staff, and statistician were blinded to drug assignment. Supplements contained lemon essential oil to mask potential fishy aftertaste. Placebo capsules were identical color, size, and flavor but contained predominantly soybean oil (3,960 mg total daily dose) and negligible omega-3 PUFA (40 mg total daily dose).	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Loss: 2 in the treatment group and 4 in the control group. Of the 24 participants initially randomised, 18 completed the study (above 20%).	-
Detection bias	5	Can we be confident in the exposure characterisation?	The dose (EPA and DHA) was reported. No information on compliance. No information on stability and purity.	-
	6	Can we be confident in the outcome assessment?	Medication tolerability was assessed via selfreport of nine potential side effects (e.g., diarrhea, burping). No information about the validation of the method. No list of the included side effects.	-
Selective reporting bias	7	Were all measured outcomes reported?	Side effects were reported as such, the specific effects were not specified.	++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Statistical methods seemed acceptable. The analyses outlined in the Methods section were performed. No protocol. Power analysis was conducted.	+

Mazahery et al. (2019)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	A third party not involved in any aspect of the study was responsible for generating the randomisation sequence. Randomisation was stratified by age (2.5–5.0 years old and 5–8.0 years old) and severity of ASD (mild, moderate, and severe) and the sequence was generated using the Website Randomisation.com and random block design in blocks of 4 and 8.	++
	2	Was allocation to study groups adequately concealed?	Researchers, children, and caregivers were blinded to treatment allocations.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	All study capsules were identical in appearance and were tasteless and colorless.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Loss of subjects was addressed. 73 of 117 completed the trial, above 35%.	-
Detection bias	5	Can we be confident in the exposure characterisation?	DHA dose reported, compliance addressed. No information on purity and stability.	+
	6	Can we be confident in the outcome assessment?	Caregivers completed weekly online surveys to collect information regarding adverse events. No information about validation of the online survey form.	-
Selective reporting bias	7	Were all measured outcomes reported?	All mentioned outcomes were reported	++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	A protocol was prepared. No statistics on the relevant outcomes. A power analysis was performed.	-

Milte et al. (2015)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Children were independently allocated to one of three treatment conditions using the process of randomization by minimization (Altman & Bland, 2005) on the basis of age and gender.	++
	2	Was allocation to study groups adequately concealed?	NR	-
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Parents and children were blinded to the randomization until completion of data collection and analysis. However, no information about the blinding of investigators.	+
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Loss of subjects was addressed. 90 of 96 completed the trial. The total n for each outcome was not reported in the result section. EPA treatment reported adverse effects: itchy skin (1), bad breath and gastrointestinal symptoms (2). DHA treatment reported adverse effects: five children reported one of the following: gastrointestinal symptoms, unpleasant taste, nose-bleed, skin rash and yellow teeth.	+
Detection bias	5	Can we be confident in the exposure characterisation?	The dose (EPA and DHA) was reported, and compliance was addressed. No information on purity and stability.	+
	6	Can we be confident in the outcome assessment?	Between each visit, parents were contacted via phone or email at least 3 times to report any adverse events. No information on validation of the questions used.	-
Selective reporting bias	7	Were all measured outcomes reported?	All mentioned outcomes were reported	++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods)	Statistical methods seemed acceptable. The analyses outlined in the Methods section were performed. No protocol. No statistics on the relevant outcomes.	-

		were appropriate and researchers adhered to the study protocol)?	
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Montgomery et al. (2018)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	A statistician at Sealed Envelope Ltd. independently performed the randomization with minimization via a 1:1 allocation ratio using minimization algorithm.	++
	2	Was allocation to study groups adequately concealed?	The randomisation sequence was independently concealed until after the initial two-group analyses were complete.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Investigators, participants and those assessing outcomes were all blind to treatment allocation.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Loss: 2 treatment group and 2 control group. Reasons for loss were given. Total n for the data on adverse effects was not reported.	+
Detection bias	5	Can we be confident in the exposure characterisation?	The dose was reported, and compliance was assessed. No information on purity and stability.	+
	6	Can we be confident in the outcome assessment?	Acceptable methods (Barkley Side Effects Rating Scale (SERS)) were used to assess side effects.	++
Selective reporting bias	7	Were all measured outcomes reported?	Relevant outcomes were reported	++

Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	A protocol was prepared. Power calculations were performed, however, the number of participants were lower than the calculated number. The statistics seemed appropriate.	+
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Pacifico et al. (2015)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	A randomization list (in a 1:1 ratio to treatment with DHA or placebo) was generated by an independent statistician who was blinded to participants' clinical data and did not perform the final analysis.	++
	2	Was allocation to study groups adequately concealed?	All participants and research staff were blind to the group assignment, however, no information on how concealment was handled.	+
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	All participants and research staff were blind to the group assignment. DHA and placebo pills were of similar appearance and taste.	++

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Loss to follow up was reported, less than 20% (14% in each group).	+
Detection bias	5	Can we be confident in the exposure characterisation?	Pills were stored at the hospital pharmacy and dispensed at the baseline visit and every month thereafter, not by parents. No information on purity. The placebo included omega 6 fatty acids (should have been short chain fatty acids). The DHA dose was reported, and compliance was assessed	+
	6	Can we be confident in the outcome assessment?	Biochemical measurements for BMI, blood glucose and insulin, triglycerides, ALT, cholesterol, CRP and cardiac function. Methods are referred to previous published studies.	++
			Parents were asked for adverse effects, but no data on this outcome was reported.	-
Selective reporting bias	7	Were all measured outcomes reported?	None of the relevant outcomes were excluded, however, no report at all about the adverse effects asked for at the consultation at follow-up.	Adverse effects: - Glucose/insulin; liver; inflammatory markers: +
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	A research protocol was prepared. A potential confounding factor was the use as placebo of germ oil, high in omega 6 fatty acids. Sound power calculation to detect a 20% difference in liver fat (small sized study for smaller effects).	Adverse effects: - Glucose/insulin; liver; inflammatory markers: +

Rodriguez-Cruz et al. (2018)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	The randomization scheme was prepared prior to trial onset using Random Allocation Software by the Staff Chemist utilizing (randomly selected) blocks of ten.	++
	2	Was allocation to study groups adequately concealed?	Yes, investigators were unaware of the boys' group assignment. When the study ended, the Staff Chemist communicated the research group assignments to subjects and investigators	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Double blinded study, identical capsules were the same in volume, appearance, and smell, and were strawberry flavored to mask the fish-oil taste	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Inflammatory markers: Yes, one participant was lost to follow-up	++
			Adverse effects: Yes, three participants were lost to follow-up	++
Detection bias	5	Can we be confident in the exposure characterisation?	Parents were instructed to administer the capsules three times a day (before each meal) to the study participants. They were also reminded of the importance of registering capsule intake in a logbook provided at study onset. No info on purity or degradation or actual intake (trust in parental follow-up). However, molecular grade oil. The dose (EPA and DHA) was reported, and compliance was assessed.	+

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
	6	Can we be confident in the outcome assessment?	Inflammatory markers: RT-qPCR, circulating cytokines with multiplex MAGPIX System assay and CK (U/L) was determined by chemiluminescent immunometric assay by a commercial kit. No information about the method for the detection of adverse effects (non-specified).	++ -
Selective reporting bias	7	Were all measured outcomes reported?	All data for the inflammatory markers were reported. No specific data for adverse effects were reported.	Inflammatory markers: ++ Adverse effects: --
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Relatively small sized study. Methods and statistics seemed to be appropriate. No mention of a protocol. The trial was registered at clinicaltrials.gov before onset. Power analysis was performed.	Inflammatory markers: + Adverse effects: -

Rodriguez-Cruz et al. (2019)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure	Random Allocation Ver. 2.0 software, by a researcher who did not participate in the measurement of outcomes, using blocks of 10 subjects.	++

		level adequately randomized?		
	2	Was allocation to study groups adequately concealed?	Investigators were not informed of the group assignment. When the study was concluded, the research technician disclosed the research-group assignments to the researchers.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Double blind study with capsules that looked and smelled identical.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	40 participants were randomised, 4 was lost to follow-up, however, only data from 28 participants were analysed.	-
Detection bias	5	Can we be confident in the exposure characterisation?	Yes, diary and parental monitoring of pills, however no mention about purity (pharmaceutical grade). Dose (EPA and DHA) reported and compliance was assessed.	+
	6	Can we be confident in the outcome assessment?	Glucose and insulin: yes	++
Adverse effects: No information about the method.			-	
Selective reporting bias	7	Were all measured outcomes reported?	Data on glucose/insulin were reported. Data on adverse effects were not reported.	Glucose/insulin: + Adverse effects: --

Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Statistical power was 77.3 and 79.7 at 3 and 6 months respectively. No mention of a study protocol. The trial was registered at clinicaltrials.gov before the enrolment of participants. The statistics applied for glucose/insulin seemed appropriate. No statistics applied to the results on adverse effects.	Glucose/insulin:+ Adverse effects: -
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Smuts et al. (2015)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Random assignment was performed by using a computer-generated list, which was blocked by school, and enrolled subjects were assigned treatment codes and respective group colors, which were used on supplement containers and forms throughout the trial.	++
	2	Was allocation to study groups adequately concealed?	Group codes were held by a member of an independent safety monitoring board until data analyses were completed	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Participants, investigators, staff, and the sponsors were blinded to treatment assignment. Identical capsules.	++

Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	98 were randomised, 9 were dropouts. The reasons for dropout were explained. The mean adherence to treatment (observed capsules and tablets swallowed during the 105 d trial) was 90 % and did not differ between the treatment groups.	++
Detection bias	5	Can we be confident in the exposure characterisation?	Dose (EPA and DHA) was reported, and compliance was good. Purity and stability were not described.	+
	6	Can we be confident in the outcome assessment?	CRP was analysed in blood samples. Automated chemiluminescent immunoassay system (IMMULITE), previously described.	++
Selective reporting bias	7	Were all measured outcomes reported?	The CRP results were reported.	++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	A protocol was prepared, and power was analysed. The statistics (for CRP results) seemed appropriate.	+

van der Wurff et al. (2019)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level	The group allocation sequence was computer generated.	++

		adequately randomized?		
	2	Was allocation to study groups adequately concealed?	An independent researcher allocated participants to the groups.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Both researchers at the site, as well as the participants and parents, were blind to the treatment condition. Furthermore, capsules were coloured black, and a vanilla odour was added to ensure blinding. The packing of the boxes and placebo and krill oil capsules were, visually, exactly the same.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	General for all adverse effects is a relatively high loss to follow-up (above 20%).	-
Detection bias	5	Can we be confident in the exposure characterisation?	Participants were instructed to take four capsules per day, groups 2 8 capsules. Left over capsules were counted and resulted in 628.82 ± 395.23 left-over capsules. As such, on average, participants did not take the capsules 78.6 days of the approximately 180 days between T2 and T3. The dose (EPA and DHA) was reported.	-
	6	Can we be confident in the outcome assessment?	Headache: Information on all adverse effects is only descriptive, no data	-
			Gastrointestinal effects: 5 reported after krill and 3 after placebo	-
		Skin problems: No	-	
Selective reporting bias	7	Were all measured outcomes reported?	Probably yes.	+
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Power was analysed. No statistics applied to the adverse effect results. Protocol published previously.	-

Verduci et al. (2014)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias (address allocation concealment of personnel and subjects)	1	Was administered dose or exposure level adequately randomized?	Children were assigned to the supplementation groups based on a computer generated, blocked randomization list. A block size of six was used, stratified according to gender and age.	++
	2	Was allocation to study groups adequately concealed?	The randomization codes were revealed after completion of the data analysis.	++
Performance bias (address blinding during the study)	3	Were the research personnel and human subjects blinded to the study group during the study?	All individuals involved in the trial, including doctors, research staff and parents of children, were unaware of the specific product administered. The products were prepared as gelatin-soft gel capsules (AFR Advanced Food Research, Limbiate, Milan, Italy) and packaged in identical opaque, coded bottles, each containing 15 capsules. They were identical in aroma, taste, color and shape.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Lipid profile: Compliance to intervention estimated on the basis of non-consumed capsules was 96.5%, 96.9%, 96.7% in DHA, DHA plus EPA mixture and control groups, respectively. Results from all participants included.	++
			Adverse effects: No data on adverse effects, no difference in loss to follow-up	-
Detection bias	5	Can we be confident in the exposure characterisation?	Compliance to supplementation was estimated on the basis of non-consumed capsules. The doses were reported. No information on purity or stability.	+
	6		Lipid profile: Total plasma cholesterol, HDL-C and TGs, were measured using a dry multiplayer enzymatic method. Plasma lipids were analysed by gas chromatography.	++

		Can we be confident in the outcome assessment?	Adverse effects: No data available. The products administered were well tolerated by children throughout the entire supplementation period without any treatment-related adverse event. The method used to assess adverse effects was not reported.	-
Selective reporting bias	7	Were all measured outcomes reported?	Probably yes, no information on protocol and no data on adverse effects.	+
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	The study was able to detect a change of 10% in HDL-C in the DHA group with a power of 64.7%. Power calculations required more participant and the study was not adequately powered for tests of statistical significance. The statistics applied to the LDL/HDL results seemed appropriate. No statistics applied on the adverse effects results.	Lipid profile: + Adverse effects: -

Voigt et al. (2014)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	A randomization scheme was stratified by sex and generated using a block approach by the study statistician.	++
	2	Was allocation to study groups adequately concealed?	NR	-
Performance bias	3	Were the research personnel and human subjects blinded to the	All of the participants, families, and study personnel (including those performing baseline and outcome assessments) were blinded to group assignment for the entire	++

		study group during the study?	study. The oils were orange flavored, and the capsules were identical in appearance. All study capsules were provided by the same company.	
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Above 20% lost during the trial.	-
Detection bias	5	Can we be confident in the exposure characterisation?	There was no information about the purity of the used oil. Corn and soybean oil were used in the placebo capsules because they have a similar texture and are composed of fatty acids, but not longer-chain v-3 fatty acids. EHA dose was reported, and compliance was assessed and reported to be excellent.	+
	6	Can we be confident in the outcome assessment?	To monitor for safety of the supplementation, the Treatment Emergent Symptoms Scale (TESS) was completed via parent interview after 3 and 6 months of supplementation. The TESS is a standardized rating scale that measures the occurrence or non-occurrence of 24 potential adverse effects of medication in 6 categories (gastrointestinal, urinary, respiratory, skin, neurological, and psychological).	++
Selective reporting bias	7	Were all measured outcomes reported?	The reporting on the data from TESS was scarce.	-
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Although the study was designed to enrol 32 patients per group, there were only 24 subjects per group. No statistics were applied to the relevant results. No information about a protocol.	-

10.2.3 Data extraction forms

Chang et al. (2019)

Study characteristics	Title	High-dose eicosapentaenoic acid (EPA) improves attention and vigilance in children and adolescents with attention deficit hyperactivity disorder (ADHD) and low endogenous EPA levels
	Author(s)	Jane Pei-Chen Chang, Kuan-Pin Su, Valeria Mondelli, Senthil Kumaran Satyanarayanan, Hui-Ting Yang, Yi-Ju Chiang, Hui-Ting Chen, Carmine M. Pariante
	Year of publication	2019
	Country (corresponding author)	UK/Taiwan
	Funding	Dr. J.P.-C.C. and Dr. K.P.S. are supported by the following Grants: MOST 108-2320-B-039-048; 108-2314-B-039-016; and 107-2314-B-039-005 from the Ministry of Science and Technology, Taiwan; NHRI-EX108-10528NI from the National Health Research Institutes, Taiwan; and CMU106-S-33, CRS-106-063, DMR-107-202, DMR-107-204, DMR-107-091, DRM-107-097, DRM-108-091, CRS-108-048, CMU108-SR-106, DMR-108-216, CMRC-CMA-3 and Chinese Medicine Research Center from the China Medical University, Taichung, Taiwan. Dr. J.P.-C.C. is supported by a Federation for Women Graduates (FFWG) Main Foundation Grant (2018-2019), UK. Dr. C.M.P. and Dr. V.M. are also supported by the grants "Immunopsychiatry: a consortium to test the opportunity for immunotherapeutics in psychiatry"(MR/L014815/1) and 'Persistent Fatigue Induced by Interferon-alpha: A New Immunological Model for Chronic Fatigue Syndrome'(MR/J002739/1), from the Medical Research Council (UK), and by the National Institute for Health Research Mental Health Biomedical Research Centre in Mental Health at South London and Maudsley NHS Foundation Trust and King's College London.
	Reported conflict of interest	Dr. C.M.P. and Dr. V.M. have received research funding from Janssen Pharmaceutical NV/Janssen Pharmaceutical Companies of Johnson and Johnson. Dr. C.M.P. has also received speaker's fees from Lundbeck and consultation fees from Consultant to Eleusis Benefit Corporation. The remaining authors declare that they have no conflict of interest.
	Study design	RCT, parallel design

Methods/ intervention	Blinding	It is stated that the investigators were blinded during the study and when assessing the outcome measurements. Participants received Omega-3 or placebo (soybean oil), and it is reported that the study is double-blind. However, it is no information whether there were identical bottles etc.
	Randomisation	The randomisation numbers were generated by computer.
	Exposure (including duration of the study)	1.2 g/day EPA or placebo (1.2 g/day soybean oil). 12 weeks
Participants	Number of participants and completion rate	543 were contacted, 103 were enrolled, 103 were randomized and N=92 completed the trial. For the outcome CRP: intervention 43, placebo: 37
	Inclusion/exclusion criteria for participants	Inclusion: 6-18 years; ADHD diagnose; drug naïve or had no medication for the past 6 months. Exclusion: Intelligence quotient<70 based on a documented history of mental retardation; for those ages 6–12 years old, a Peabody Picture Vocabulary Test-Revised (PPVT-R) percentile scores less than 5% (indicating speech delay or intellectual disability); other comorbid psychiatric disorders, such as autism spectrum disorder, anxiety disorder, conduct disorder, and other major psychiatric disorders; comorbid physical disorders, such as thyroid dysfunction and cerebral palsy; currently using n-3 PUFAs supplements; allergy to n-3 PUFAs.
	Gender	85.9% of the subjects were male.
	Age	6-18 years, mean age of subjects: 9.49 + 3.05 years.
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Youth with Attention Deficit Hyperactivity Disorder.
Results	Parameters measured, methods used, and measurement time points	High sensitive (Hs)-CRP, measured by enzyme-linked immunosorbent assay (ELISA).
	Reported outcome (including measures of variance)	EPA (n=43, mean (SD)): Baseline = 2.00 (0.90), week 12 = 1.98 (0.81) Placebo (n=37): Baseline = 2.27 (1.39), week 12 = 2.19 (1.15) Mean change -0.04 (0.38), P=0.747
	Power analysis	A power analysis was performed

Statistical analysis	Statistical test	Independent-sample t-test
Comments		

Chase et al. (2015)

Study characteristics	Title	Effect of docosahexaenoic acid supplementation on inflammatory cytokine levels in infants at high genetic risk for type 1 diabetes
	Author(s)	Chase HP, Boulware D, Rodriguez H, Donaldson D, Chritton S, Rafkin-Mervis L, Krischer J, Skyler JS, Clare-Salzler M.
	Year of publication	2015
	Country (corresponding author)	USA
	Funding	The study was conducted by the Type 1 Diabetes TrialNet Study Group, a clinical trials network funded by NIH through the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Allergy and Infectious Diseases, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, and the National Center for Research Resources with support of the Juvenile Diabetes Research Foundation International, the American Diabetes Association, and the Children with Diabetes Foundation. Appreciation is expressed to Martek Biosciences Corporation (Columbia, MD for the DHA (DHASCO-S) and to Mead Johnson Nutrition (Evansville, IN) for the two infant formulas.
Reported conflict of interest	None reported.	
Methods/ intervention	Study design	Nine-center, two-arm, randomized, double-masked controlled clinical pilot trial
	Blinding	It is stated that the study is double-blind. Intervention (DHA/EPA or corn/soy oil) were given as capsules. It is not stated whether they had identical appearances.
	Randomisation	It is stated that the participants were randomized, but the method is not described.
	Exposure (including duration of the study)	Group A mothers were randomized to receive DHA (800 mg/d) or corn/soy oil (800 mg/d) in the last trimester of pregnancy and continued on this same dose after delivery if breast-feeding.

		<p>Formula fed infants received formula with 10.2 mg DHA/ounce (treatment) or 3.4 mg DHA/ounce (control). Formula-fed infants and infants of breast-feeding mothers in Group B (57 infants) were randomized in the first five postnatal months to receive similar dosages of DHA or corn/soy oil as their counterparts in Group A.</p> <p>At the age of 18 – 36 months, all infants received DHA or placebo capsules.</p>
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	Breastfed: n=57 infants; formula fed: n=41 infants.
	Inclusion/exclusion criteria for participants	All infants were required to have a first-degree relative with type 1 diabetes and to have human leukocyte antigen DR3and/or DR4 (91 infants) or to have multiple first-degree relatives with type 1 diabetes (7 infants). It was predetermined that infants would be removed from the study if there was persistent detection of \geq two positive islet autoantibodies.
	Gender	46% males in entry A and 51% males in entry B.
	Age	Infants
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Infants with a high genetic risk for type 1 diabetes (T1D).
Results	Parameters measured, methods used, and measurement time points	<p>Inflammatory markers were measured at 6, 12, 18, 24, 30 and 36 months.</p> <p>Levels of stimulated IL-1β, IL-6, IL-12p40, and TNFα cytokine production were assayed by Luminex multiplex assay. Hs-CRP was measured using the Nephelometric method.</p>
	Reported outcome (including measures of variance)	<p>IL-1β (pg/ml):</p> <p>6 months: control=907 (95 CI: 664, 1239); intervention=751 (560, 1005). P=0.38</p> <p>12 months: control=861 (95 CI: 626, 1183); intervention=763 (577, 1008). P=0.57</p> <p>18 months: control=756 (95 CI: 569, 1028); intervention=653 (488, 873). P=0.43</p>

		<p>24 months: control=634 (95 CI: 466, 862); intervention=589 (442, 784). P=0.72 30 months: control=636 (95 CI: 460, 879); intervention=503 (370, 684). P=0.27 36 months: control=569 (95 CI: 390, 830); intervention=514 (350, 756). P=0.69</p> <p>TNFa (pg/ml): 6 months: control=624 (95 CI: 458, 786); intervention=588 (440, 786). P=0.78 12 months: control= 838 (95 CI: 602, 1168); intervention=585 (438, 783). P=0.11 18 months: control= 1088(95 CI: 817, 1449); intervention=1009 (760, 1340). P=0.7 24 months: control=1095 (95 CI: 809, 1481); intervention=813 (613, 1078). P=0.15 30 months: control=903 (95 CI: 617, 1321); intervention=735 (542, 996). P=0.32 36 months: control=903 (95 CI: 617, 1321); intervention=680 (459, 1007). P=0.27</p> <p>IL-12p40 (pg/ml): 6 months: control=666 (95 CI: 545, 815); intervention=755 (623, 914). P=0.37 12 months: control=786 (95 CI: 657, 939); intervention=641 (548, 749). P=0.09 18 months: control=240 (95 CI: 197, 292); intervention=216 (178, 262). P=0.43 24 months: control=195 (95 CI: 159, 240); intervention=171 (141, 207). P=0.35 30 months: control=160 (95 CI: 129, 199); intervention=183 (149, 225). P=0.34 36 months: control=189 (95 CI: 146, 244); intervention=140 (108, 183). P=0.16</p> <p>The mean hs-CRP level at 12 months for the breast-fed treatment infants was 0.012 [0.003, 0.048]. Breast-fed infants of all control mothers (Group A or B), who were not receiving any DHA had a mean hsCRP level at 12 months of 0.0024 [0.002, 0.315] ($p>0.05$) in comparison to the breast-fed treatment infants. The mean hs-CRP level for all formula-fed infants (control and treatment) at 12 months was 0.086 [$p=0.007$ in comparison to breast-fed treatment infants]. Formula-fed control infants had a mean hsCRP level of 0.130 [0.053, 0.319]. The differences in hsCRP levels between infants formerly nursed vs. formula-fed were no longer significant (0.007</p>
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		vs. 0.033, respectively; p=0.16) at 18 months and thereafter when breast feeding had been discontinued. IL-6 and IL-10 were also measured in all control and treated infants. There were no consistent differences between groups for either cytokine (data not shown).
Statistical analysis	Power analysis	No power analysis was performed.
	Statistical test	The data were examined for normality prior to testing, and log transformations were made on the values where appropriate and noted. Statistical comparisons between groups were performed using Analysis of Covariance, Mixed linear model adjusting for kit effect, t-tests, and Pearson's correlation coefficient.
Comments		

Cornu et al. (2018)

Study characteristics	Title	A double-blind placebo-controlled randomised trial of omega-3 supplementation in children with moderate ADHD symptoms
	Author(s)	Catherine Cornu, Catherine Mercier, Tiphonie Ginhoux, Sandrine Masson, Julie Mouchet, Patrice Nony, Behrouz Kassai, Valérie Laudy, Patrick Berquin, Nathalie Franc, Marie-France Le Heuzey, Hugues Desombre, Olivier Revol
	Year of publication	2018
	Country	France
	Funding	The study was sponsored by the URGO laboratories. The sponsor had a role in the study design. The study was conducted, analysed and the article was written independently from the funding entity.
	Reported conflict of interest	None reported.
	Study design	A randomised (1:1), double-blind, placebo-controlled clinical trial.

Methods/ intervention	Blinding	The placebo capsules were indistinguishable from active capsules; this was assessed using panel testing. Participants, care providers, those assessing outcomes, study coordinators and monitors were blinded to the administered treatment.
	Randomisation	Randomization was performed according to a pre-established blocked randomization list, stratified by centre. This list was generated by the study statistician.
	Exposure (including duration of the study)	Children aged 6–8 years, EPA 336 mg and DHA 84 mg; for children aged 9–11 years, EPA 504 mg and DHA 126 mg, and for children aged 12–15 years EPA 672 mg and DHA 168 mg. 3 months.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	162 randomised, 148 analysed.
	Inclusion/exclusion criteria for participants	Inclusion: children and adolescents aged 6–15 years referred for hyperactivity symptoms to five reference centres for learning disabilities in France. Briefly, children had to have at least six hyperactivity–impulsivity symptoms for six months or more, and/or at least one of six inattention symptoms for six months or more; certain symptoms had to be present before the age of 7 years, and there was a functional impairment in two or more environment (school, home), with a clinically significant alteration in the social, school, or family functioning. Symptoms had not to be part of another psychiatric disorder. Exclusion: known intolerance to omega-3 fatty acids, intake of fatty acid/fish oil dietary supplements for more than 1 week during the 3 months preceding inclusion, or MPH or other ADHD drug during the month preceding inclusion. Children who required MPH treatment were also excluded to ensure equipoise.
	Gender	DHA+EPA: 61% males; placebo: 66% males.
	Age	6-15 years.
	Confounders and other variables as reported	

	Health and socioeconomic status of participants	Children and adolescents with established diagnosis of ADHD.
Results	Parameters measured, methods used, and measurement time points	Side effects, method not reported.
	Reported outcome (including measures of variance)	<p>Eleven (14.9%) children in the DHA–EPA group experienced 13 adverse events, 2 of which were judged to be related to the study treatment by the study authors: hip pain, fatigue, headache, fever and cough (n = 2), dermatitis, allergic reaction (n = 2), abdominal pain, diarrhoea (n = 3), and depression.</p> <p>Eight children (10.7%) in the placebo group experienced 10 adverse events: fatigue, influenza (n = 2), abdominal pain, dermatitis, swollen eyes, vomiting, and diarrhoea (n = 3).</p> <p>Two patients in the DHA–EPA group and none in the placebo group experienced a severe adverse event (hospitalisation for worsening ADHD symptoms)</p>
Statistical analysis	Power analysis	A power analysis was performed for expected effect size of 0.5 SD (90% power) for continuous outcomes. No power calculation for adverse events.
	Statistical test	No statistics for adverse events.
Comments		

Crippa et al. (2019)

Study characteristics	Title	Behavioral and cognitive effects of docosahexaenoic acid in drug-naïve children with attention-deficit/hyperactivity disorder: a randomized, placebo-controlled clinical trial
	Author(s)	Alessandro Crippa, Alessandra Tesei, Federica Sangiorgio, Antonio Salandi, Sara Trabattoni, Silvia Grazioli, Carlo Agostoni, Massimo Molteni, Maria Nobile

	Year of publication	2019
	Country	Italy
	Funding	Research grant from Dietetic Metabolic Food srl., which further provided the investigational product and respective placebo. Funders have not been involved in study design, data collection or analysis, or publication decisions.
	Reported conflict of interest	None reported.
Methods/ intervention	Study design	A randomized, placebo-controlled, double-blind intervention trial.
	Blinding	DHA and placebo supplement matched in touch, smell and size and was unlabeled. Children, parents and study investigators were blinded to the randomization until completion of data collection and analysis.
	Randomisation	Computer-generated randomization. Participants were assigned a study number and randomly allocated by an independent third person.
	Exposure (including duration of the study)	500 mg DHA per day. 6 months.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	N=50 randomised to treatment, 48 completed the study. DHA: n=25 Placebo: n=23 (4 lost to follow-up)
	Inclusion/exclusion criteria for participants	Inclusion: 7-14 years, ADHD diagnosis, Full Scale Intelligence Quotient (FSIQ) or estimated FSIQ scores higher than 80. Moreover, all children were required to be drug-naïve and not have consumed omega-3/omega-6 supplements during the 3 months prior to the recruitment. Exclusion: a history of seizures, other neurological disorders, or diagnosed genetic disorders.
	Gender	DHA: 2 females, 23 males; placebo: 2 females, 23 males.
	Age	7-14 years.
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Drug-naïve children with attention-deficit/hyperactivity disorder.

Results	Parameters measured, methods used, and measurement time points	Parents visited the institute monthly to report any adverse events. No details on about how this assessment was made or what type of events were considered adverse.
	Reported outcome (including measures of variance)	No instances of either major or minor adverse events were reported.
Statistical analysis	Power analysis	No power analysis.
	Statistical test	No statistics for adverse events.
Comments		

de Ferranti et al. (2014)

Study characteristics	Title	Using high dose omega-3 fatty acid supplements to lower triglyceride levels in 10–19 year-olds
	Author(s)	Sarah D. de Ferranti, Carly E. Milliren, Erica R. Denhoff, Sarah K. Steltz, Elif Seda Selamet Tierney, Henry A. Feldman, Stavroula K. Osganian
	Year of publication	2014
	Country	USA
	Funding	Study drug and additional research support was provided by GlaxoSmithKline. Additional support was supplied by the Boston Children’s Heart Foundation and Harvard Catalyst. Dr. de Ferranti is supported by a National Institutes of Health Grant K23 HL 085308-03, Bethesda, MD and by the Boston Children’s Heart Foundation. Dr. Feldman was supported by Harvard Catalyst The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award #UL1 RR 025758 and financial contributions from Harvard University and its affiliated academic health care centers). The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, or the National Institutes of Health.
Reported conflict of interest	None reported.	

Methods/ intervention	Study design	A double-blind placebo controlled randomized trial.
	Blinding	It is stated that the study is double-blind. Both groups received four 1 g capsules daily. Capsules were color-blinded.
	Randomisation	Block randomisation. The randomisation was conducted and assigned by the research pharmacy of Boston Children`s Hospital using a list generated by the Clinical Research Center.
	Exposure (including duration of the study)	~3360 mg docosahexaenoic acid + eicosapentaenoic acid/day vs placebo. 1860 mg EPA + 1500 mg DHA 6 months.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	25 randomised, 24 analysed at 3 months.
	Inclusion/exclusion criteria for participants	Healthy youth ages 10–19 years with moderate to severe hypertriglyceridemia (TG). They were eligible to participate if they had a fasting TG level that was 150 to 1000 mg/dL and met all other study inclusion and exclusion criteria. Inclusion criteria included the ability to swallow pills and speak English. Patients were excluded if they reported allergy to fish, corn or other components of the pills, pregnancy or breast feeding, alcohol use, bleeding disorder or coagulopathy, thyroid disorder, diabetes or fasting glucose at or above 126 mg/dL, liver disease or alanine aminotransferase (ALT) greater than 2 times the upper limit of normal, treatment with medications affecting TG levels including oral hypoglycemic agents, insulin, non-statin lipid lowering medications, or omega-3 FA pills.
	Gender	42% males.
	Age	10-19 years.
	Confounders and other variables as reported	

	Health and socioeconomic status of participants	Hypertriglyceridemic children and adolescents.
Results	Parameters measured, methods used, and measurement time points	<p>Lipid profile: Measured using "standard methods", no details provided.</p> <p>Inflammatory markers: hsCRP was measured by an immunoturbidimetric assay.</p> <p>Glucose: method not reported.</p> <p>Joint, lumbar and muscle pain: Asked specifically in questionnaire, no information about validation.</p> <p>Gastrointestinal effects: Asked specifically in questionnaire, no information about validation.</p> <p>Skin problems: Asked specifically in questionnaire, no information about validation.</p>
	Reported outcome (including measures of variance)	<p>Total cholesterol (mg/dL). Baseline: Placebo=186±12, omega-3=181±12; 3 months: placebo=180±12, omega-3=189±12; 6 months: placebo=177±12, omega-3=178±12.</p> <p>HDL (mg/dL). Baseline: Placebo=34.1±1.72, omega-3=34.0±1.7; 3 months: placebo=33.6±1.7, omega-3=35.8±1.7; 6 months: placebo=35.0±1.9, omega-3=34.2±1.7.</p> <p>LDL (mg/dL). Baseline: Placebo=107±11, omega-3=110±11; 3 months: placebo=104±1, omega-3=116±11; 6 months: placebo=104±11, omega-3=109±11.</p> <p>LDL levels increased significantly in the omega-3 FA group by 14 ± 6 mg/dL (p=0.02) at 3 months but not at 6 months (7 ± 8 mg/dl; p=0.37) and were unchanged in the placebo group at both timepoints. Again, when the change from baseline to 3 months or 6 months was compared between groups, there was no significant difference in LDL change. VLDL cholesterol decreased significantly in the treatment group at 6 months (p=0.04). No significant changes were seen in any of the other lipid parameters within or between groups at any other time points.</p> <p>Fasting glucose and insulin: no effect.</p> <p>Glucose (mg/dL). Baseline: Placebo=89.5±1.8, omega-3=88.5±1.8; 3 months: placebo=90.8±1.8, omega-3=90.2±1.8; 6 months: placebo=89.8±2.0, omega-3=92.1±1.7.</p>

		<p>Insulin (mg/dL). Baseline: Placebo=22.6±3.1, omega-3=18.1±2.5; 3 months: placebo=28.0±3.8, omega-3=20.8±2.9; 6 months: placebo=27.0±4.0, omega-3=22.7±3.1. Insulin resistance HOMA-IR: (mg/dL×mcIU/mL). Baseline: Placebo=5.0±0.7, omega-3=4.1±0.6; 3 months: placebo=6.3±0.9, omega-3=4.6±0.7; 6 months: placebo=6.0±0.9, omega-3=5.1±0.7.</p> <ul style="list-style-type: none"> • Inflammatory markers: The majority of CRP levels were at the lower limit of reporting (0.1 mg/dL), and the prevalence of detectable CRP showed no change (p=0.28) or difference in time course between treatment groups (p=0.62) (data not shown). • Joint, lumbar and muscle pain: no effect • Skin problem: no effect • Gastrointestinal effects; no effect
Statistical analysis	Power analysis	Power was calculated for triglyceride levels only. Due to low enrollment rate and lower effect size than expected, post-hoc power calculations indicated less than 10% power for TG.
	Statistical test	Differences in mean changes of glucose and lipid profile (LDL, HDL, and total cholesterol) was shown accompanied by p-values from Student t-test.
Comments		

Engler et al. (2004)

Study characteristics	Title	Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY study
	Author(s)	M.M. Engler, M.B. Engler, M. Malloy, E. Chiu, D. Besio, S. Paul, M. Stuehlinger, J. Morrow, P. Ridker, N. Rifai, M. Mietus-Snyder
	Year of publication	2004

	Country	USA
	Funding	NIH grants and gifts from the Valentine Foundation.
	Reported conflict of interest	Not reported.
Methods/ intervention	Study design	RCT, crossover.
	Blinding	It was stated that the study was double-blind. The capsules were identical.
	Randomisation	The participants were randomised, but the method is not described.
	Exposure (including duration of the study)	DHA 1.2 g/day. 6 weeks.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	20 completed the trial
	Inclusion/exclusion criteria for participants	Inclusion: 8-21 years, familial hypercholesterolemia or familial combined hyperlipidemia. Exclusion: chronic systemic illness with or without secondary hyperlipidemia and current smoking.
	Gender	Not reported.
	Age	9-19 years.
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Children with familial combined hyperlipidemia.
Results	Parameters measured, methods used, and measurement time points	CRP, LDL, HDL and total cholesterol, plasma F2 isoprostanes and urinary 8-OH-2-dG. Acceptable methods were used.

	Reported outcome (including measures of variance)	<p>CRP (mg/dl): Baseline=0.070±0.078, NCEP-II diet=0.065±0.071, DHA+NCEP II diet=0.047±0.042. No effect on CRP.</p> <p>Total cholesterol (mg/dl): Baseline=282±85, NCEP-II diet=263±79, DHA+NCEP II diet=297±81. LDL (mg/dl): Baseline=213±89, NCEP-II diet=201±82, DHA+NCEP II diet=229±85. HDL (mg/dl): Baseline=48±9, NCEP-II diet=44±8, DHA+NCEP II diet=51±13. DHA supplementation was associated with increased levels of total cholesterol, LDL- and HDL cholesterol concentrations.</p> <p>F2 isoprostanes (ng/ml): Baseline=0.080±0.042, NCEP-II diet=0.053±0.017, DHA+NCEP II diet=0.060±0.030. 8-OH-2'dG (µg/g creatinine): Baseline=1.6±1.3, NCEP-II diet=1.8±2.0, DHA+NCEP II diet=1.8±1.8. No effect on biomarkers for oxidative stress (F2 isoprostanes or 8-OH-2-dG).</p>
Statistical analysis	Power analysis	No
	Statistical test	2-way repeated measures analysis of variance.
Comments		

Janczyk et al. (2015)

Study characteristics	Title	Omega-3 Fatty Acids Therapy in Children with Nonalcoholic Fatty Liver Disease: A Randomized Controlled Trial
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	Author(s)	Wojciech Janczyk, Dariusz Lebensztejn, Aldona Wierzbicka-Rucinska, Artur Mazur, Joanna Neuhoff-Murawska, Pawel Matusik, Piotr Socha
	Year of publication	2015
	Country	Poland
	Funding	Supported by the Polish Ministry of Science and Higher Education (3180/B/P01/2007/33).
	Reported conflict of interest	None reported.
Methods/ intervention	Study design	Multicenter, randomized, double-blind, placebo-controlled clinical trial.
	Blinding	Same dose, shape and color of capsules. No information about taste or smell. Blinded by supplier.
	Randomisation	Computer-generated block randomization.
	Exposure (including duration of the study)	Dose: <ul style="list-style-type: none"> - Body weight <40 kg: 267 mg DHA and 177.5 mg EPA per day - Body weight 40-60 kg: 534 mg DHA and 355 mg EPA per day - Body weight >60 kg: 800 mg DHA and 532.5 mg EPA per day. Study duration was 24 weeks.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	76 overweight/obese (54 overweight, 22 obese) children (65 boys, 11 girls) were recruited, 64 completed the trial.
	Inclusion/exclusion criteria for participants	Inclusion: age >5 and <19 years; overweight or obesity (according to International Obesity Task Force body mass index (BMI) charts); ALT activity ≥ 1.3 times the upper limit of normal; presence of hyperechogenic liver on ultrasound or liver histology consistent with NAFLD/nonalcoholic steatohepatitis; written consent obtained from the patient and/or a legal representative. Exclusion: age <5 or >19 years; history of significant alcohol consumption; any known pathological conditions affecting the liver eg, hepatitis B or C virus infection, chronic or acute liver failure, cholestasis, metabolic diseases, such as $\alpha 1$ -antitrypsin deficiency, Wilson disease, diabetes mellitus, and hypothyroidism; treatment with vitamin E, statins, antihypertensives,

		ursodeoxycholic acid, probiotics, or metformin within 3 months before randomization; history of parenteral nutrition; unlikely to cooperate with the study regime.
	Gender	65 boys, 11 girls were recruited.
	Age	Median age = 13 years.
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Children with nonalcoholic fatty liver disease.
Results	Parameters measured, methods used, and measurement time points	Liver effects (enzymes): Laboratory test not specified. Lipid profile: Laboratory test not specified. Glucose: Laboratory test not specified. Inflammatory markers: Laboratory test not specified.
	Reported outcome (including measures of variance)	<p>End-of-treatment characteristics after 24 weeks</p> <p>Liver effects: no differences in intervention and placebo in effect on ALT. AST levels was significantly lower in the intervention group. ALT (U/L, median (IQR)): placebo=53.5 (39-99), omega-3=48.5 (31-62). AST (U/L, median (IQR)): placebo=39.0 (27-55), omega-3=28.0 (25-36).</p> <p>Lipid profile: no effect Total cholesterol (mg/dL, median (IQR)): placebo=167.5 (159-193.5), omega-3=183.0 (149-202.5). HDL-C (mg/dL, median (IQR)): placebo=39.5 (35.5-44.5), omega-3=44.0 (36.5-48.5). LDL-C (mg/dL, median (IQR)): placebo=109.5 (94-136), omega-3=122.0 (90.5-144.5).</p> <p>Inflammatory markers: no effect hs CRP (µg/ml, median (IQR)): placebo=0.3 (0.3-0.5), omega-3=0.34 (0.3-0.4).</p> <p>Fasting glucose and insulin: no effect Glucose (mg/dL, median (IQR)): placebo=85 (79-89), omega-3=85.0 (79-91).</p>

		Insulin (mg/dL, median (IQR)): placebo=18.2 (12.9-22.4), omega-3=16.4 (12.9-26.2). HOMA-IR: placebo=3.8 (2.9-4.9), omega-3=3.7 (2.6-5.8).
Statistical analysis	Power analysis	Yes
	Statistical test	Comparisons were made between omega-3 and placebo at each timepoint, but the change between timepoints was not compared between groups. The final results were compared using the χ^2 test or Mann-Whitney U test. Changes in variables during the course of the study were tested with the Wilcoxon matched-pairs test. Statistica for Windows version 10 (Statsoft, Tulsa, Oklahoma) was used for descriptive statistics, the Mann-Whitney U test, and the Wilcoxon matched-pairs test; the χ^2 test was performed using StatsDirect version 2.7.9.
Comments		

Mazahery et al. (2019)

Study characteristics	Title	A Randomised-Controlled Trial of Vitamin D and Omega-3 Long Chain Polyunsaturated Fatty Acids in the Treatment of Core Symptoms of Autism Spectrum Disorder in Children
	Author(s)	Hajar Mazahery, Cathryn A. Conlon, Kathryn L. Beck, Owen Mugridge, Marlena C. Kruger, Welma Stonehouse, Carlos A. Camargo Jr., Barbara J. Meyer, Bobby Tsang, Beatrix Jones, Pamela R. von Hurst
	Year of publication	2019
	Country	New Zealand
	Funding	Partial funding for the study was provided by Massey University Strategic Innovation Fund, Massey University, New Zealand. Additional support was provided by Douglas Nutrition, Pty. Ltd., NZ who were supplying the active supplement and identical-appearing placebo, but who had no input into study design, implementation, data management, statistical analysis or reporting of results.
	Reported conflict of interest	None reported.
	Study design	A randomised placebo controlled double-blind study.

Methods/ intervention	Blinding	All study capsules were identical in appearance and were tasteless and colourless.
	Randomisation	A third party not involved in any aspect of the study was responsible for generating the randomisation sequence. Randomisation was stratified by age (2.5–5.0 years old and 5–8.0 years old) and severity of ASD (mild, moderate, and severe) and the sequence was generated using the Website Randomisation.com and random block design in blocks of 4 and 8.
	Exposure (including duration of the study)	722 mg docosahexaenoic acid. Study duration = 12 months.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	117 enrolled, 73 analysed.
	Inclusion/exclusion criteria for participants	Inclusion: 2.5 to 8 years, between 2.5 and 8 years, had a medical diagnosis of ASD and onset of symptoms after 18 months of age. Exclusion: they were diagnosed as having developmental delay since birth; they failed to take corrective action for nutritional deficiencies; they had serum 25(OH)D $\geq 75 + 10$ nmol/L (≥ 85 nmol/L) if they entered the trial in winter and ≥ 105 nmol/L + 10 nmol/L (≥ 115 nmol/L) if they entered the trial in summer.
	Gender	100 of the 117 enrolled were males.
	Age	2.5 to 8 years.
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Children with autism spectrum disorder.
Results	Parameters measured, methods used, and measurement time points	Relevant outcome was adverse effects. Adverse outcome was reported using an online survey, no information about validation of the survey.
	Reported outcome (including measures of variance)	Events reported over the study period by the OM and the placebo group:

		<ul style="list-style-type: none"> • Headache: 2 (OM); 0 (placebo) • Allergic reactions: 5 (OM); placebo (0) • Gastrointestinal effects: 10 (OM); 8 (placebo) • Nose-bleeding: 1 (OM); 0 (placebo) • Other side effects: 5 (OM); 3 (placebo)
Statistical analysis	Power analysis	Yes
	Statistical test	No statistics on the relevant outcomes.
Comments		

Montgomery et al. (2018)

Study characteristics	Title	Docosahexaenoic acid for reading, working memory and behaviour in UK children aged 7-9: A randomized controlled trial for replication (the DOLAB II study)
	Author(s)	Paul Montgomery, Thees F. Spreckelsen, Alice Burton, Jennifer R. Burton, Alexandra J. Richardson.
	Year of publication	2018
	Country	UK
	Funding	The study was funded by DSM Nutritional Products (http://www.lifesdha.com/). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
	Reported conflict of interest	Not reported.
Methods/ intervention	Study design	Parallel group, fixed-dose, randomized (minimization, 30% random element), double-blind, placebo-controlled trial.
	Blinding	Investigators, participants and those assessing outcomes were all blind to treatment allocation.

	Randomisation	A statistician at Sealed Envelope Ltd. independently performed the randomization with minimization via a 1:1 allocation ratio using minimization algorithm.
	Exposure (including duration of the study)	DHA: 600 mg/day for 16 weeks
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	376 randomised, n=185 (intervention) and 187 (placebo) completed.
	Inclusion/exclusion criteria for participants	Inclusion: children had to be below the 20 th centile on a standardized word reading test, "The British Ability Scales" (BASII) but with no other significant special educational needs. Exclusion: Children with specific medical disorders (e.g. visual or hearing impairment), or who were taking medications expected to affect behavior and learning.
	Gender	Of the 376 randomized, 235 were males and 141 were females.
	Age	7 to 9 years.
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Healthy school children.
	Results	Parameters measured, methods used, and measurement time points
Reported outcome (including measures of variance)		Gastrointestinal effects: no effect Headache: no effect, insomnia: no effect
Statistical analysis	Power analysis	Yes, however larger participant number needed for small effect size
	Statistical test	T-test and Wilcoxon test for association
Comments		

Pacifico et al. (2015)

Study characteristics	Title	Docosahexaenoic acid for reading, working memory and behaviour in UK children aged 7-9: A randomized controlled trial for replication (the DOLAB II study)
	Author(s)	Paul Montgomery, Thees F. Spreckelsen, Alice Burton, Jennifer R. Burton, Alexandra J. Richardson.
	Year of publication	2018
	Country	UK
	Funding	The study was funded by DSM Nutritional Products (http://www.lifesdha.com/). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
	Reported conflict of interest	Not reported.
Methods/ intervention	Study design	Parallel group, fixed-dose, randomized (minimization, 30% random element), double-blind, placebo-controlled trial.
	Blinding	Investigators, participants and those assessing outcomes were all blind to treatment allocation.
	Randomisation	A statistician at Sealed Envelope Ltd. independently performed the randomization with minimization via a 1:1 allocation ratio using minimization algorithm.
	Exposure (including duration of the study)	DHA: 600 mg/day for 16 weeks
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	376 randomised, n=185 (intervention) and 187 (placebo) completed.
	Inclusion/exclusion criteria for participants	Inclusion: children had to be below the 20 th centile on a standardized word reading test, "The British Ability Scales" (BASII) but with no other significant special educational needs.

		Exclusion: Children with specific medical disorders (e.g. visual or hearing impairment), or who were taking medications expected to affect behavior and learning.
	Gender	Of the 376 randomized, 235 were males and 141 were females.
	Age	7 to 9 years.
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Healthy school children.
Results	Parameters measured, methods used, and measurement time points	Barkley Side Effects Rating Scale (SERS) was used to assess side effects.
	Reported outcome (including measures of variance)	Gastrointestinal effects: no effect Headache: no effect, insomnia: no effect
Statistical analysis	Power analysis	Yes, however larger participant number needed for small effect size
	Statistical test	T-test and Wilcoxon test for association
Comments		

Rodriguez et al. (2018)

Study characteristics	Title	Potential therapeutic impact of omega-3 long chain-polyunsaturated fatty acids on inflammation markers in Duchenne muscular dystrophy: A double-blind, controlled randomized trial
	Author(s)	Maricela Rodríguez-Cruz, Oriana del Rocío Cruz-Guzman, Tomas Almeida-Becerril, Alan Donovan Solís-Serna, Salvador Atilano-Miguel, Juan Raúl Sanchez-Gonzalez, Lourdes Barbosa-Cortes, Eugenia Dolores Ruíz-Cruz, Juan Carlos Huicochea, Alan Cardenas-Conejo, Rosa Elena Escobar-Cedillo, Carlos Alberto Yam-Ontiveros, Edgar F. Ricardez-Marcial
	Year of publication	2018

	Country	Mexico
	Funding	ONACYT (Consejo Nacional de Ciencia y Tecnología) de Mexico (Grant #SALUD-2012-01-180058).
	Reported conflict of interest	None reported.
Methods/ intervention	Study design	A placebo-controlled, double-blind, randomized trial.
	Blinding	Double blinded study, identical capsules were the same in volume, appearance, and smell, and were strawberry flavored to mask the fish-oil taste.
	Randomisation	The randomization scheme was prepared prior to trial onset using Random Allocation Software by the Staff Chemist utilizing (randomly selected) blocks of ten.
	Exposure (including duration of the study)	450 mg EPA + 2250 mg DHA + 200 mg of additional omega 3 fatty acids per day. 6 months.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	66 recruited, 40 who fulfilled the inclusion criteria decided to participate, 36 completed.
	Inclusion/exclusion criteria for participants	Inclusion: boys, deletion in the DMD gene or its promoter (detected by Multiplex Polymerase Chain Reaction, (MPCR)), over the age of 3 and under the age of 18 years, and their parents or guardians had given their informed consent, along with an authorization letter for subject participation. Exclusion: Patients were not included if they had previously received glucocorticoids, consumed supplements with omega-3 long chain-PUFA, or had hypersensitivity to fish oil.
	Gender	Males
	Age	3-18 years (inclusion criteria).
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Boys with Duchenne Muscular Dystrophy.

Results	Parameters measured, methods used, and measurement time points	RT-qPCR, circulating cytokines with multiplex MAGPIX System assay and CK (U/L) was determined by chemiluminescent immunometric assay by a commercial kit. No information about the method for the detection of adverse effects (non-specified).
	Reported outcome (including measures of variance)	Inflammatory markers: Omega-3 LC-PUFA intake decreased the serum IL-1 β (-59.5%; p -0.011) and IL-6 (-54.8%; p -0.041), and increased the serum IL-10 (99.9%, p<0.005), in relation to those with placebo treatment.
Statistical analysis	Power analysis	Fourteen patients in each group were estimated to provide 80% study power to identify a difference of 2.3 pg/mL in IL-6 for omega-3 long chain-PUFA treatment compared to placebo with an assumption of a Standard Deviation (SD) of 2.9 pg/mL.
	Statistical test	Comparisons between treatment groups (placebo vs.omega-3 LC-PUFA) were conducted with the Mann-Whitney U test for nonparametric data or with the Student t test for normally distributed data. The General Linear Model approach was employed to assess the impact of supplementation of omega-3 long chain-PUFA on cytokine response. Percentages of changes of serum cytokines were introduced as dependent variables, and time of follow-up (baseline, and months 1, 2, 3, and 6) and treatments (placebo and omega-3 longchain-PUFA) were considered as factors.
Comments		Relatively small group size (22 and 18)

Rodriguez et al. (2019)

Study characteristics	Title	Evidence of muscle loss delay and improvement of hyperinsulinemia and insulin resistance in Duchenne muscular dystrophy supplemented with omega-3 fatty acids: A randomized study
	Author(s)	Maricela Rodríguez-Cruz, Salvador Atilano-Miguel, Lourdes Barbosa-Cortes, Mariela Bernabe-Garcia, Thomas Almeida-Becerril, Alan Cardenas-Conejo, Oriana del Rocío Cruz-Guzman, Jorge Maldonado-Hernandez
	Year of publication	2019

	Country	Mexico
	Funding	Consejo Nacional de Ciencia y Tecnología-Mexico (Grant#SALUD-2012-01-180058).
	Reported conflict of interest	None reported.
Methods/ intervention	Study design	Placebo-controlled, double-blind, randomized stud
	Blinding	Double blind study with capsules that looked and smelled identical.
	Randomisation	Random Allocation Ver. 2.0 software, by a researcher who did not participate in the measurement of outcomes, using blocks of 10 subjects.
	Exposure (including duration of the study)	450 mg EPA + 2250 mg DHA + 200 mg of additional omega 3 fatty acids per day. 6 months.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	40 participants were randomised, 4 was lost to follow-up, however, only data from 28 participants were analysed.
	Inclusion/exclusion criteria for participants	Inclusion: deletion(s) in the Duchenne Muscular Dystrophy gene, 3-18 years, and parents gave their written informed consent. Exclusion: previously pharmacological treatment (for instance, glucocorticoids), had ingested supplements with omega-3 LCPUFA, or had an allergic reaction to fish oil.
	Gender	Males
	Age	8.4±2.3 years (placebo) and 6.96±2.5 years (intervention).
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Children with Duchenne Muscular Dystrophy.
Results	Parameters measured, methods used, and measurement time points	Adverse effects: No information about the method. Glucose was quantified by the glucose-oxidase enzymatic method (Glucose-LQ, SpinReact, S.A., Girona, Spain). Insulin was measured using a commercial kit (Linco Research, St.Louis, MO, USA) based on radioimmunoanalysis.

	Reported outcome (including measures of variance)	<p>Fasting insulin, percentage of boys with hyperinsulinemia, and IR were similar between the placebo and n-3 LCPUFA groups during the 6 months of supplementation. The percentage of boys with IR was significantly (p =0.045) lower at month 6 of supplementation in the n-3 LCPUFA group than in the placebo group.</p> <p>Fasting insulin (µU/mL): Control baseline=14.8 (3.0, 34.7), 3 months=15.7 (2.3, 82.3), 6 months=17.9 (1.5, 28.9). Omega-3 LCPUFA baseline=7.9 (2.4, 28.4), 3 months=5.9 (3.2, 34.1), 6 months=6.9 (1.5, 28.9).</p> <p>Fasting hyperinsulinemia (% of boys) Control baseline=8 (57.1), 3 months=9 (64.3), 6 months=8 (66.7). Omega-3 LCPUFA baseline=5 (35.7), 3 months=5 (38.5), 6 months=4 (28.6).</p> <p>Fasting HOMA-IR Control baseline=2.9 (0.7, 9.2), 3 months=3.2 (0.5, 15.2), 6 months=3.9 (0.3, 15.3). Omega-3 LCPUFA baseline=1.6 (0.5, 6.1), 3 months=1.3 (0.7, 6.8), 6 months=1.4 (0.3, 5.7).</p> <p>Insulin resistance (% of boys) Control baseline=7 (50), 3 months=7 (50), 6 months=6 (66.7). Omega-3 LCPUFA baseline=4 (28.6), 3 months=5 (38.5), 6 months=3 (21.4).</p> <p>No adverse effects reported in any group.</p>
Statistical analysis	Power analysis	Yes
	Statistical test	<p>Comparisons between treatment groups were conducted by means of the Mann-Whitney U test, and statistical analysis along time, by means of the Friedman Ranges Test.</p> <p>No statistics applied to the results on adverse effects.</p>
Comments		Small group size, data from 28 participants

Smuts et al. (2014)

Study characteristics	Title	Long-chain n-3 PUFA supplementation decreases physical activity during class time in iron-deficient South African school children
	Author(s)	Cornelius M. Smuts, Jani Greeff, Jane Kvalsvig, Michael B. Zimmermann, Jeannine Baumgartner
	Year of publication	2014
	Country	South Africa/Switzerland
	Funding	Unilever Research and Development (Vlaardingen, The Netherlands), the North-West University (Potchefstroom, South Africa) and the Medicor Foundation (Vaduz, Principality of Liechtenstein). Paul Lohmann GmbH (Lomapharm, Emmertal, Germany) provided the Fe supplements and Burgerstein AG (Rapperswil, Switzerland) provided the DHA+EPA capsules. The European – South African Partnership in Nutrition Research provided travel support.
	Reported conflict of interest	Reported no conflict of interest, except two co-authors received speaking honoraria from Unilever.
Methods/ intervention	Study design	Randomised, placebo-controlled, double-blind trial.
	Blinding	Double-blind. Identical pills.
	Randomisation	Random assignment was performed by using a computer-generated list, which was blocked by school, and enrolled subjects were assigned treatment codes and respective group colors, which were used on supplement containers and forms throughout the trial.
	Exposure (including duration of the study)	420 mg DHA + 80 mg EPA. 8.5 months
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	98 were randomised, 9 were dropouts, and 89 completed the trial.
	Inclusion/exclusion criteria for participants	Inclusion criteria for the study were as follows: 6 – 11 years of age; Hb concentration >80 g/l; Fe deficiency; apparently healthy, with no chronic illness; no consumption of Fe- or omega-3 FA-containing supplements.
	Gender	

	Age	6-11 years.
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Healthy Fe-deficient school children.
Results	Parameters measured, methods used, and measurement time points	CRP was analysed in blood samples.
	Reported outcome (including measures of variance)	Inflammatory markers: no effects on CRP (baseline: 0.57 (0.00-10.30); completed study: 0.70 (0.00-7.32).
Statistical analysis	Power analysis	Yes
	Statistical test	All data were checked for normal distribution. Skewed variables were transformed before data analysis. Estimated intervention effects of and of DH+EPA and their interactions were analysed by using two-factor ANCOVA on the endpoint measurement by using the respective baseline values, sex, age and compliance (only for biochemical variables) as individual covariates.
Comments		

Verduci et al. (2014)

Study characteristics	Title	Blood lipids profile in hyperlipidemic children undergoing different dietary long chain polyunsaturated supplementations: a preliminary clinical trial
	Author(s)	Elvira Verduci, Carlo Agostoni, Giovanni Radaelli, Giuseppe Banderali, Enrica Riva, and Marcello Giovannini
	Year of publication	2014
	Country	Italy
	Funding	No financial support was provided from any sponsor.
	Reported conflict of interest	None declared.

Methods/ intervention	Study design	Three-arms double blind, randomized, clinical trial.
	Blinding	The products were prepared as gelatine-soft gel capsules (AFR Advanced Food Research, Limbiate, Milan, Italy) and packaged in identical opaque, coded bottles, each containing 15 capsules. They were identical in aroma, taste, color and shape.
	Randomisation	Children were assigned to the supplementation groups based on a computer generated, blocked randomization list. A block size of six was used, stratified according to gender and age.
	Exposure (including duration of the study)	After an 8-week stabilization period on the Step I diet, participants were randomized to additionally receive for a 16-week period one capsule (500 mg) daily of docosahexaenoic acid (DHA) alone or a DHA plus eicosapentaenoic acid (EPA) mixture (45.6% DHA; 41.6% EPA) or wheat germ oil (control).
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	36 were recruited and completed.
	Inclusion/exclusion criteria for participants	Inclusion: normal-weight according to the International Obesity Task Force, having primary hyperlipidemia. Exclusion: having any chronic systemic disease, secondary hyperlipidemia, having received dietary supplement of n-3 LCPUFA in the past 12 months, being under dietary fat restriction at recruitment and being participant in another study.
	Gender	
	Age	8-13 years.
	Confounders and other variables as reported	
	Health and socioeconomic status of participants	Children with primary hyperlipidemia.
Results	Parameters measured, methods used, and measurement time points	Total plasma cholesterol, HDL-C and TGs, were measured using a dry multiplayer enzymatic method. Plasma lipids were analysed by gas chromatography.

		The method used to assess adverse effects was not reported.
	Reported outcome (including measures of variance)	<p>An effect size (as percentage change from baseline) of +8%, -12% and -16% for high-density lipoprotein cholesterol (HDL-C), total cholesterol/HDL-C ratio and triglycerides was observed in children supplemented with DHA, compared to +2%, -8% and -12%, respectively, in children supplemented with DHA plus EPA.</p> <p>Total cholesterol (mg/dl, mean (SD)) Placebo: start=245.3 (43.3), end=230.7 (45.2). DHA: start=243.6 (47.2), end=231.5 (65.5). DHA+EPA: start=242.2 (71.3), end=232.0 (83.8).</p> <p>LDL (mg/dl, mean (SD)) Placebo: start=172.9 (48.2), end=163.8 (48.2). DHA: start=168.2 (51.7), end=158.9 (67.5). DHA+EPA: start=164.8 (71.2), end=155.1 (83.1).</p> <p>HDL (mg/dl, mean (SD)) Placebo: start=59.4 (9.4), end=62.0 (9.0). DHA: start=59.9 (7.2), end=64.7 (6.9). DHA+EPA: start=62.2 (8.2), end=63.5 (7.4).</p> <p>No treatment-related adverse events reported.</p>
	Power analysis	Yes
Statistical analysis	Statistical test	<p>Crude comparison among groups was performed by the one-way analysis of variance (ANOVA) or the Kruskal–Wallis test, as appropriate, and longitudinal variation within-group by ANOVA for repeated measures or the Friedman test. Overall longitudinal and among groups difference in lipid profile was additionally evaluated by two-way ANOVA with time as a repeated measure and supplementation as fixed factor.</p> <p>No statistics applied on the adverse effects results.</p>
Comments		Small sized study n=36

10.2.4 Evaluation of certainty in the evidence

The rating of certainty in evidence was performed according to the “Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration” (OHAT, 2019).

- For each study, an initial certainty rating was made to determine the ability of the study design to ensure that exposure preceded and was associated with the outcome. According to the method suggested by OHAT (2019), the following parameters are evaluated: Whether 1) the exposure was experimentally controlled, 2) the exposure occurred prior to the development of the outcome, 3) the outcome is assessed on the individual level (i.e., not through population aggregate data) and 4) an appropriate comparison group is included in the study. Fulfilment of all features will receive an initial rating of high certainty (++++). Lower ratings, i.e. moderate (+++), low (++) or very-low (+), correspond to the number of features fulfilled. Studies rated high or moderate will be included for further analysis.
- Factors that may downgrade the initial level of certainty in evidence are evaluated for each outcome, and we include internal validity/risk of bias, unexplained inconsistency, indirectness and imprecision.
- Factors that may upgrade the initial level of certainty in evidence are evaluated for each study, and we included large magnitude of effect (e.g. incidence, degrees of severity), the presence of a dose-response relationship, and consistency across study design type/dissimilar populations for the relevant studies combined.
- Following downgrading and upgrading, for each study the certainty in the evidence for a given effect will be determined using the following terms (OHAT, 2019):
 - “High certainty (++++) in the association between exposure to the substance and the outcome. The true effect is highly likely to be reflected in the apparent relationship.
 - Moderate certainty (+++) in the association between exposure to the substance and the outcome. The true effect may be reflected in the apparent relationship.
 - Low certainty (++) in the association between exposure to the substance and the outcome. The true effect may be different from the apparent relationship.
 - Very-low certainty (+) in the association between exposure to the substance and the outcome. The true effect is highly likely to be different from the apparent relationship.