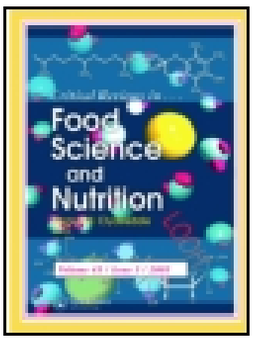

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REVIEW

Bioavailability and conversion of plant based sources of omega-3 fatty acids – a scoping review to update supplementation options for vegetarians and vegans

Katie E. Lane[a], Megan Wilson[a], Teuta G. Hellon[b], and Ian G. Davies[a]

[a]School of Sport and Exercise Sciences, Faculty of Science, Liverpool John Moores University, Research Institute for Sport and Exercise Sciences, Liverpool, UK; [b]School of Medicine, University of Central Lancashire, Liverpool, UK

ABSTRACT

Omega-3 (*n*-3) fatty acids offer a plethora of health benefits with the majority of evidence showing beneficial effects from marine sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Emerging research examines the effects of *n*-3 dietary intakes on blood markers of vegetarians and vegans, but official guidance for plant based marine alternatives is yet to reach consensus. This scoping review provides an overview of trials investigating bioavailability of plant *n*-3 oils including EPA and DHA conversion. Searches of MEDLINE, PubMed, CINAHL and clinical trial registers identified randomized controlled trials from January 2010 to September 2020. The 'Omega-3 index' (EPA + DHA (O3I)), was used to compare *n*-3 status, metabolic conversion and bioavailability. Two reviewers independently screened articles and extracted data on outcomes. From 639 identified articles, screening and eligibility checks gave 13 articles. High dose flaxseed or echium seed oil supplements, provided no increases to O3I and some studies showed reductions. However, microalgal oil supplementation increased O3I levels for all studies. Findings indicate preliminary advice for vegetarians and vegans is regular consumption of preformed EPA and DHA supplements may help maintain optimal O3I. Further studies should establish optimum EPA and DHA ratios and dosages in vegetarian and vegan populations.

KEYWORDS

Alpha-linolenic acid; linoleic acid; eicosapentaenoic acid; docosahexaenoic acid; omega-3 metabolic pathway; conversion

Introduction

Consumption and high biomarker concentrations of omega-3 (*n*-3) fatty acids, show convincing cardioprotective benefits and offer additional mental and physical health benefits in humans including decreased risk of chronic diseases and cognitive decline (Gillingham et al. 2013; Russell and Meital 2018). The most beneficial *n*-3 fatty acids, eicosapentaenoic acid (20:5*n*-3 (EPA)) and docosahexaenoic acid (22:6*n*-3 (DHA)) are mainly obtained from marine sources in the diet. Although recently debated, there is now convincing evidence for the reduction of cardiovascular events with high dose intake of the *n*-3 fatty acid, EPA (Mason, Libby, and Bhatt 2020). DHA is also important especially with respect to mental and cognitive effects (Ghasemi Fard et al. 2019). Therefore, intake of these marine *n*-3 fatty acids is important for leading a healthy lifestyle, presenting a potential challenge for vegetarians and vegans whose dietary intakes are low (Table 1) (Cholewski, Tomczykowa, and Tomczyk 2018; Russell and Meital 2018).

Despite the well evidenced health benefits, current European and US dietary reference values are quite generic in relation to *n*-3 fatty acid sources and few give recommendations for specific *n*-3 fatty acids suitable for vegetarians and vegans (Cuervo et al. 2009). The most up to date UK Scientific Advisory Committee on Nutrition (SACN) (2004) recommendations state at least one portion of oily fish per week should be consumed to provide 0.45 g/d EPA and DHA. Whilst the Committee recognized that groups of the population do not eat fish (e.g. vegetarians and vegans), the evidence base was considered insufficient to conduct a risk assessment on this issue and specific recommendations for these groups were not made. Also most European food based dietary guidelines are nonspecific in relation to the different *n*-3 fatty acids and give no details of alternative options to oily fish for vegetarians and vegans (Montagnese et al. 2015).

Further consideration to *n*-3 bioavailability deserves attention, a definition for this in the field of nutrition has not reached consensus. For the purpose of this review, bioavailability is defined as, 'the intestinal digestion, absorption, and appearance of alpha linolenic acid (18:3*n*-3 (ALA)), EPA and DHA in tissue' (Ghasemifard, Turchini, and Sinclair 2014), such bioavailability assessment is becoming common practice in *n-3* studies (Maki 2018). The essential fatty acid ALA is the most prevalent plant based source and must undergo conversion to the more beneficial EPA and DHA in the *n*-3 metabolic pathway. It is also possible for *n*-

CONTACT Katie E. Lane K.e.lane@ljmu.ac.uk Liverpool John Moores University, School of Sport and Exercise Sciences, Faculty of Science, IM Marsh, Barkhill Road, Aigburth, Liverpool, L17 6BD.

Table 1. Dietary *n*-3 intakes.

Author and participants	Study design	Fatty acid	Vegans	Vegetarians	Fish eaters	Omnivores	Significance
Kornsteiner, Singer, and Elmadfa (2008)	Data collection by 24hr food diary on dietary fat intake of omnivores, vegetarians, vegans and semi-omnivores as well as its impact on lipid profiles	18:3*n*-3g/d	2.63 ± 1.67	1.73 ± 1.38	–	1.19 ± 0.40	$p < 0.01$
		20:5*n*-3mg/d	6 ± 12	4 ± 12	–	48 ± 109	n.s.
Vegan *n*37		22:5*n*-3mg/d	15 ± 64	5 ± 10	–	84 ± 155	n.s.
Vegetarian *n*25		22:6*n*-3mg/d	25 ± 70	69 ± 199	–	234 ± 389	n.s.
Omnivore *n*23		Total *n*-3%	3.0 ± 1.4	1.9 ± 0.9	–	1.6 ± 0.5	$p < 0.001$
		n-6/*n*-3, g/g	9.4/1.0	10.2/1.0	–	6.8/1.0	n.s.
		18:2*n*-6g/d	22.52 ± 11.07	14.96 ± 12.03		7.86 ± 4.04	$p < 0.001$
Welch et al. (2010)	To determine intakes, food sources and *n*-3 status according to dietary habit as part of the EPIC study with 7 day food diaries	18:3*n*-3g/d (males)	1.02 ± 0.71	1.25 ± 0.54	1.23 ± 0.43	1.11 ± 0.55	$p < 0.001$
Males							
Vegan *n*12		20:5*n*-3g/d	0.01 ± 0.001	0.02 ± 0.02	0.11 ± 0.15	0.02 ± 0.02	$p < 0.001$
Vegetarians *n*96		22:5*n*-3g/d	–	–	–	–	
Fish eaters *n*5952		22:6*n*-3g/d	0 ± 0	0.0007 ± 0.004	0.16 ± 0.22	0.02 ± 0.02	$p < 0.001$
Omnivores *n*996		Total *n*-3%	1.04 ± 0.71	1.27 ± 0.56	1.57 ± 0.58	1.15 ± 0.55	$p < 0.001$
Females		18:2*n*-6g/d	12.79 ± 10.80	14.78 ± 6.9	12.41 ± 4.8	11.80 ± 5.95	$p = 0.135$
Vegan *n* 16		18:3*n*-3g/d (females)	0.86 ± 0.69	0.97 ± 0.45	1.01 ± 0.35	0.86 ± 0.33	$p < 0.001$
Vegetarians *n* 154							
Fish eaters *n* 6258		20:5*n*-3g/d	0.02 ± 0.08	0.01 ± 0.01	0.11 ± 0.13	0.02 ± 0.01	$p < 0.001$
Omnivores *n* 938		22:5*n*-3g/d	–	–	–	–	–
		22:6*n*-3g/d	0 ± 0	0.0004 ± 0.005	0.15 ± 0.19	0.01 ± 0.01	$p < 0.001$
		Total *n*-3%	0.91 ± 0.67	0.98 ± 0.45	1.27 ± 0.49	0.89 ± 0.33	$p < 0.001$
		18:2*n*-6g/d	11.91 ± 9.92	10.06 ± 6.02	9.52 ± 3.75	8.59 ± 4.15	$p < 0.001$
Pinto et al. (2017)	Heart rate variability and *n*-3 status (FFQ) in age and BMI matched middle aged vegans and omnivores	18:3*n*-3g/d	0.8: 0.5,1.2	–	–	0.7: 0.5,1.0	$p = 0.425$
Vegan *n*23		20:5*n*-3g/d	0.00: 0.00	–	–	0.14: 0.09,0.24	$p < 0.001$
Omnivores *n*24 (data shown as median and IQR)		22:5*n*-3g/d	–	–	–	–	–
		22:6*n*-3g/d	0.01: 0.01,0.01	–	–	0.45: 0.30,0.81	$p < 0.001$
		Total *n*-3%	–	–	–	–	–
		18:2*n*-6g/d	10.5: 7.3, 18.5	–	–	7.6: 5.9,10.2	$p = 0.025$

(Mean ± SD unless stated otherwise, – value not given) EPIC = European Prospective Investigation into Cancer and Nutrition (Norfolk cohort). FFQ = food frequency questionnaire. IQR = interquartile range. Total *n*-3% is % of total fatty acids

3 fatty acids to undergo retro-conversion in the metabolic pathway; an example of this would be DHA converting to docosapentaenoic acid (22:5 *n*-3 DPA) or EPA (Park et al. 2016). The relationship between *n*-3 dietary intake, bioavailability and conversion is complex and influenced by many different factors such as genetics, smoking status, age, gender and consumption of omega-6 (*n*-6) rich food sources all of which can affect the processes (de Groot, Emmett, and Meyer 2019; Russell and Meital 2018). In addition to ALA, the other essential fatty acid, linoleic acid (18:2*n*-6 (LA)) is the most plentiful plant based *n*-6 in the human diet. Dietary sources of LA include nuts, seeds, certain vegetables; vegetable oils such as soybean oil, safflower oil, and corn oil, all of which form a staple of vegetarian and vegan diets (Burns-Whitmore et al. 2019). High dietary intakes of *n*-6 fatty acids can reduce *n*-3 metabolic conversion rates and vegetarian/vegan diets have significantly higher ratios of *n*-6:*n*-3 fatty acids than omnivorous diets (Table 1) (Kornsteiner, Singer, and Elmadfa 2008; Pinto et al. 2017; Welch et al. 2010). A large number of studies have shown high intake of *n*-6 fatty acids have been linked to various detrimental health effects when *n*-3 intake is low (Jang and Park 2020; Nindrea et al. 2019, Simopoulos 2016; Zhuang et al. 2019). Therefore several researchers have recommended vegetarians and vegans should aim for sufficient intake of ALA and limit LA intakes (Agnoli et al. 2017; Burns-Whitmore et al. 2019; Simopoulos 2000).

The 'Omega-3 index' (O3I) is a good biomarker of *n*-3 bioavailability, and acts as an indicator of high to low risk of death from coronary heart disease (Russell and Meital 2018; Stark et al. 2016). Optimum blood levels of *n*-3 are defined as an O3I of > 8% (Stark et al. 2016). Originally coined by Harris and Von Schacky (2004), and utilized by Stark et al. (2016) in their seminal global survey of EPA and DHA in the blood stream of healthy adults, O3I is characterized as EPA + DHA in erythrocytes or other equivalent blood fractions such as plasma or serum. The main population groups achieving optimal levels typically consume greater amounts of fish or marine oils, whilst those who adhere to a long term-vegan diet have a low corresponding O3I (<4%), with omnivores falling between the two extremes (Stark et al. 2016). A summary of recent studies (Tables 1 and 2) shows dietary *n*-3 intakes and total *n*-3 erythrocyte/plasma fatty acids are significantly lower in vegans and vegetarians in comparison to fish eaters and omnivores for the majority of participants ($p \leq 0.05$) (Kornsteiner, Singer, and Elmadfa 2008; Pinto et al. 2017; Welch et al. 2010).

Walnut, flax, chia, canola, hemp, echium and perilla seed oils are plant based sources of ALA (Table 3) and offer various health benefits. These include improvements to insulin sensitivity, inflammation, hepatic steatosis and cardiovascular disease (CVD) risk factors although the health benefits of ALA are not as well established as those attributed to EPA and DHA (Del Bo et al. 2019; Ghazani and Marangoni 2016; Kuhnt et al. 2012; Lenighan, McNulty, and Roche 2019; Shahidi and Ambigaipalan 2018). The majority of ALA rich oils are also abundant in LA, which leads to difficulty in consuming sufficient ALA without also increasing the amount of LA in the diet, unless specific foods/supplements high in ALA are consumed such as flaxseeds, flaxseed/linseed oil, hemp seeds, chia seeds or oils (Burns-Whitmore et al. 2019) (Table 3). Echium seed oil has been identified as a good natural plant based source of stearidonic acid (18:4*n*-3 (SDA)), which follows ALA in the *n*-3 metabolic conversion pathway. Whilst fewer studies have

Table 2. Erythrocyte and phospholipid *n*-3 status.

Author and participants	Fatty acid	Vegans	Vegetarians	Fish eaters	Omnivores	Significance
Kornsteiner, Singer, and Elmadfa (2008)	18:3*n*-3mol%	0.28 ± 0.21	0.24 ± 0.16	–	0.37 ± 0.25	n.s.
	20:5*n*-3mol%	0.16 ± 0.06	0.27 ± 0.10	–	0.35 ± 0.14	$p < 0.001$
Vegan *n*37	22:5*n*-3mol%	0.90 ± 0.27	1.25 ± 0.38	–	1.19 ± 0.31	$p < 0.001$
Vegetarian *n*25	22:6*n*-3mol%	0.87 ± 0.31	1.28 ± 0.37	–	1.81 ± 0.63	$p < 0.001$
Omnivore *n*23	Total *n*-3mol%	2.20 ± 0.60	3.04 ± 0.67	–	3.71 ± 0.89	$p < 0.001$
(erythrocyte	*n*-6/*n*-3mol%	11.62 ± 2.52	8.33 ± 1.86	–	6.62 ± 1.54	$p < 0.001$
sphingo- and	18:2*n*-6mol%	11.05 ± 1.46	9.90 ± 0.89	–	9.34 ± 1.04	$p < 0.001$
phospholipids)	EPA + DHA	1.03	1.55	–	2.16	–
Welch et al. (2010)	18:3*n*-3μmol/	15.8 ± 9.7	13.6 ± 10.1	10.9 ± 5.7	11.8 ± 7.0	$p < 0.001$
Males	L (males)					
Vegan *n*5	20:5*n*-3μmol/L	65.1 ± 45.5	55.9 ± 45.3	57.5 ± 43.2	47.4 ± 30.3	$p = 0.001$
Vegetarians *n*25	22:5*n*-3μmol/L	67.2 ± 26.8	77.5 ± 38.8	67.3 ± 29.4	70.0 ± 33.4	$p = 0.038$
Fish eaters *n*2257	22:6*n*-3μmol/L	195.0 ± 58.8	222.2 ± 138.4	239.7 ± 106.2	215.6 ± 96.4	$p < 0.001$
Omnivores *n*359	Total *n*-3μmol/L	327.4 ± 123.6	355.5 ± 211.1	364.5 ± 164.8	333 ± 147.7	$p = 0.002$
Females	18:2*n*-6μmol/L	1337.7 ± 414.1	1238.2 ± 421.6	1164.1 ± 329.5	1207.9 ± 333.3	$p < 0.001$
Vegan *n*5	EPA + DHA	2.60	2.78	2.98	2.62	-
Vegetarians *n*51	18:3*n*-3μmol/	13.71 ± 8.10	12.3 ± 4.8	12.4 ± 613	13.1 ± 7.3	$p = 0.22$
Fish eaters *n*1891	L (females)					
Omnivores *n*309	20:5*n*-3μmol/L	50.0 ± 29.4	55.1 ± 52.5	64.7 ± 43.4	57.1 ± 38.4	$p = 0.001$
(plasma	22:5*n*-3μmol/L	90.6 ± 54.0	75.0 ± 32.2	71.8 ± 29.6	74.7 ± 34.2	$p = 0.056$
phospholipid	22:6*n*-3μmol/L	286.4 ± 211.7	223.5 ± 137.8	271.2 ± 113.1	241.3 ± 109.6	$p < 0.001$
n-3 μmol/L)	Total *n*-3μmol/L	426.8 ± 284.0	353.5 ± 191.5	407.7 ± 169.3	373.1 ± 166.2	$p < 0.001$
	18:2*n*-6μmol/L	1406.0 ± 162.1	1325.9 ± 278.6	1236.9 ± 328.4	1271.2 ± 373.9	$p < 0.001$
	EPA + DHA	3.36	2.78	3.35	2.98	-
Pinto et al. (2017)	18:3*n*-3 (plasma)	0.71	–	–	0.53	$p = 0.006$
Vegan *n*23	20:5*n*-3	0.47	–	–	1.03	$p < 0.001$
Omnivores *n*24	22:5*n*-3	0.51	–	–	0.59	$p = 0.146$
(Plasma and	22:6*n*-3	0.91	–	–	2.23	$p = 0.113$
erythrocyte (E)	Total *n*-3	–	–	–	–	–
mean weight % of	18:2*n*-6	33.1	–	–	27.1	$p < 0.001$
fatty acids)	18:3*n*-3 (E)	0.32	–	–	0.34	$p = 0.610$
	20:5*n*-3	0.67	–	–	1.26	$p < 0.001$
	22:5*n*-3	2.15	–	–	2.62	$p = 0.005$
	22:6*n*-3	2.07	–	–	4.19	$p < 0.001$
	Total *n*-3	2.71	–	–	5.42	$p < 0.001$
	18:2*n*-6g	13.3	–	–	11.7	$p = 0.002$
	EPA + DHA	2.54	–	–	5.22	-

(Mean ± SD unless stated otherwise, - value not given)

Table 3. Vegetarian omega-3 source oils.

Source oil	ALA	SDA	EPA	DHA	LA	GLA	AA	DPA
	(18:3*n-3*)	(18:4n-3)	(20:5n-3)	(22:6n-3)	18:2n-6)	(18:3n-6)	(20:4n-6)	(22:5n-6)
Flaxseeds/oil	56.0	–	–	–	17.0		–	–
Chia seed oil	60.2	–	–	–	19.1	–	0.06	–
Canola oil	9.20	–	–	–	19.4	–	–	–
Echium seed	36.6	10.5	–	–	–	10.2	–	–
Hemp seed oil	23.0	1.10	–	–	48.3	3.1	–	–
Perilla seed	54.0	–	–	–	11	–	–	–
Walnut oil	11.4	–	–	–	37.0	–	–	–
Algae*	0.11	–	<0.10	39.4	1.0	0.20	0.90	16.7

(Typical representation of values g/100g of oil, * dependant on source – indicates FA not detected. (Asif 2011, Del Bo et al. 2019, Ghafoor et al. 2018, Ghazani and Marangoni 2016, Harper, Edwards, and Jacobson 2006, Kuhnt et al. 2016, Lane et al. 2020, Sabudak 2007).

evaluated the health benefits of echium seed oil, potential improvements to CVD risk markers including serum triglycerides (TG) and O3I have been demonstrated (Dittrich et al. 2015; Kuhnt et al. 2014). Microalgal oils offer a useful direct plant based source of *n*-3 suitable for consumption by vegetarians and vegans (Craddock et al. 2017). Commercial applications have recently successfully developed direct sources of EPA and DHA rich oil from microalgal sources, which provide a plant based bioequivalent source of *n*-3 to those found in fish oil (Arterburn et al. 2008; Craddock et al. 2017, Winwood 2013). Nutritional oil derived from marine algae containing EPA and DHA has been shown to be as effective as fish oil for lowering TG; however, microalgal sources of DHA have also been associated with increases to low-density lipoprotein cholesterol (LDL-C) in meta-analyses of supplement studies (Bernstein et al. 2012, Maki et al. 2014). There is debate as to whether this increases cardiovascular risk as it increases the larger LDL particles and decreases small, dense low density lipoproteins (LDL); a shift toward a less atherogenic LDL profile (Allaire et al. 2017, Diffenderfer and Schaefer 2014, Ramasamy 2018).

Preliminary literature searches show a high degree of variability for the randomized controlled trials (RCTs) conducted within this topic area. Previous studies are heterogeneous particularly in relation to comparators, study types, dosages and eligible participants with a high number of crossover trials. Existing studies include a variety of subjects ranging from healthy, overweight and obese, otherwise

healthy participants but with hypertriglyceridemia (HTG), metabolic syndrome (MetS) or MetS risk factors and type 2 diabetes (T2DM), all of which can affect fatty acid metabolism (Walle et al. 2017). Unlike systematic reviews and meta-analysis, scoping reviews have a broader scope and provide a description of current evidence, regardless of quality (Tricco et al. 2018). Further literature searches reveal no other published studies have evaluated the *n*-3 bioavailability of plant based sources since an initial review prepared by the authors over 10 years ago (Lane et al. 2014). Taking into account the number of vegans in the UK, which have quadrupled between 2014 and 2019 (The Vegan Society 2020) and the increasing numbers in Europe (Statista 2018) there is a pressing need for an update in this important area. The present scoping review maps the evidence with the aim to inform future research in relation to the most effective vegan/vegetarian alternative *n*-3 sources to oily fish by evaluating bioavailability and conversion to longer chain *n*-3 of plant based *n*-3 supplement sources suitable for vegetarians and vegans.

Objectives of the review

The aim of this scoping review was to explore interventions that evaluate plant based *n*-3 source oils using RCTs to highlight the most effective supplementation options for vegetarians and vegans. The main objective was to assess the bioavailability of plant based oils previously defined as good sources of *n*-3 fatty acids (typically >24% of total fatty acids (Asif 2011; Kuhnt et al. 2012)(Table 3)). The research objectives were: (1) To provide an overview of up to date literature relating to the bioavailability of *n*-3 from recognized plant based source oils, (2) To evaluate changes to O3I (baseline to endpoint) brought about by plant based *n*-3 oil supplementation, (3). To identify the most effective plant based source oils in terms of *n*-3 bioavailability.

Methods

The review followed the PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation (Tricco et al. 2018) and Chapter 11 of the Joanna Briggs Reviewers manual (Peters et al. 2017)

Search strategy

The following databases, MEDLINE, PubMed, CINAHL, Cochrane Central Register of Controlled Trials and the International Clinical Trials Registry Platform (ICTRP), which includes Cochranelibrary.com and ClinicalTrials.gov identified English-language, relevant peer reviewed RCTs published from January 2010 to September 2020. The date ranges were selected to give a comprehensive update to our previous review, which identified papers published over a 10 year period from 2001 to 2011 (Lane et al. 2014). Further studies were searched from reference lists of eligible studies and review articles. Included studies published after the date of the literature search were identified by publication alerts.

The search used the following keywords; 'Flax* oil' OR 'Linseed oil' OR 'Chia' OR 'Chia seed oil' OR 'Rapeseed oil' OR 'Echium seed oil' OR 'Hemp seed oil' OR 'Perilla seed oil' OR 'Alga* oil' in article title or abstract AND 'Omega-3 OR Omega 3′ 'Omega-3*omega-6 ratio' OR 'Alpha-linolenic acid' OR 'Stearidonic acid' OR 'Eicosapentaenoic acid' OR 'Docosahexaenoic acid' OR 'omega-3/omega-6 ratio' in title OR abstract OR text. 'Placebo' OR 'Control group' OR 'Intervention' OR 'Controlled clinical trial' OR 'Random* controlled trial' in article title, abstract OR text.

Inclusion criteria

Determination of included studies followed methods employed by Stark et al. (2016) in their global survey of *n*-3 fatty acids in the blood stream of healthy adults. Included studies were RCTs with adults ($\geq$ 18 years), reporting study baseline and endpoint, red blood cell, plasma or whole blood EPA and DHA biomarkers. Included studies used a control or placebo of a different source oil (plant or marine based), reported plasma and/or erythrocyte and/or whole blood *n*-3 concentrations, clearly stated the supplement source and dose and analyzed the differences in *n*-3 blood fatty acid status at baseline and at the endpoint of the intervention period following recommendations from the International Society for the Study of Fatty Acids and Lipids (ISSFAL) (de Groot and Meyer 2020).

Participants: To maximize the number of included studies, RCTs that used individuals with specific disease factors were included. Healthy normal weight and overweight/obese participants alongside those with established or at risk of MetS, CVD, HTG and T2DM were included as *n*-3 fatty acid supplementation is well evidenced to offer improvements to those with or at risk of these conditions (Bernstein et al. 2012; Brown et al. 2019; Egert et al. 2014; Hu, Hu, and Manson 2019).

Exclusion criteria

Studies of pregnant and breastfeeding women, infants and children or subjects with existing serious disease that may affect fatty acid metabolism such as cystic fibrosis, multiple sclerosis, cancer, kidney disease and inflammatory bowel disease were excluded in line with similar RCTs, systematic reviews and meta-analysis that have evaluated *n*-3 supplementation (Bernstein et al. 2012; Kuhnt et al. 2014; Stark et al. 2016). Studies that evaluated the bioavailability of plant based *n*-3 fortified animal products, did not use *n*-3 oils, only reported study endpoint bioavailability measurements or where the *n*-3 source was unclear were excluded from the review.

Selection of studies

To minimize potential bias during the selection procedure, duplicates of full articles retrieved for further assessment were independently read by 2 reviewers (KEL and MW). A third reviewer (TGH) then made a consensus decision for

inclusion. The articles were added to an independent Endnote database and grouped in accordance with the inclusion criteria separately by each reviewer.

Data extraction

The following data were collected: title; first author; year of publication; country; design of RCT (parallel, cross-over, factorial); blinding of participant and personnel (open, single, double); baseline characteristics of study participants, (age, sex, BMI (where available); total number of participants, randomization of participants; health status; baseline, endpoint and changes to *n*-3 markers; intervention comparisons and key findings. Data was categorized by blood fraction analyzed including plasma total lipids, plasma phospholipid, erythrocytes and whole blood in accordance with methods used by Stark et al. (2016). In order to compare *n*-3 status, metabolic conversion and bioavailability, O3I status was selected as a previously recognized marker of increased health benefits and reductions in risk of CVD and total mortality (Alexander, Justice, and Madden 1985; Armstrong, Metherel, and Stark 2008; Flock, Harris, and Kris-Etherton 2013; Harris et al. 2009; Stark et al. 2016). Endpoint EPA + DHA status was calculated by adding or subtracting the percentage change value from baseline markers for studies where percentage change only was reported. Erythrocyte measures were used for studies presenting both plasma and erythrocyte markers as the literature indicates this as the preferred blood fraction (Stark et al. 2016). Continuous data was tabulated and assigned to one of four discrete blood level groupings that corresponded to EPA + DHA weight percentage values associated with high to low risk of death from CHD identified by O3I in accordance with Harris and Von Schacky (2004). Categories were grouped by EPA + DHA status. Group 1: very low, indicated in dark red, group 2: low, indicted by red, group 3: moderate, indicated by amber, group 4: high, indicated by green. Groupings were adjusted in accordance with the selected blood fractions used by Stark et al. (2016), EPA + DHA erythrocytes levels of $\leq$ 4% (very low), $>$ 4–6% (low), $>$ 6–8% (moderate), $>$ 8% (high). Equivalent groupings for plasma total fatty acids [$\leq$ 2.9% (very low), $>$ 2.9–4.0% (low), $>$ 4.0–5.2% (moderate), $>$ 5.2% (high)], plasma phospholipids [$\leq$ 3.8% (very low), $>$ 3.8–5.7% (low), $>$ 5.7–7.6% (moderate), $>$ 7.6% (high)] and whole blood [$\leq$ 3.0% (very low), $>$ 3.0–4.4% (low), $>$ 4.4–5.9% (moderate), $>$ 5.9% (high)] (Stark et al. 2016).

Results

Overview of identified studies

The database search identified 604 articles and 35 further studies were found from registers of clinical trials and other searches. Following removal of duplicates 522 articles were screened. Title and abstract screening resulted in the exclusion of 356 studies (Figure 1), leaving 166 articles for full text assessment. A further 153 articles were excluded with the main reasons including studies that did not evaluate *n*-3 bioavailability and studies using participants with existing serious diseases defined earlier. Screening and eligibility checks left a total of 13 articles that fitted the review criteria and are summarized in Table 4. The 13 studies were published between January 2010 and September 2020 and included a total of 668 participants, 267 of these were healthy although BMI status was not clearly indicated for all healthy participants, the remaining participants were diagnosed with or at risk of CVD, HTG, MetS, or T2DM but otherwise healthy in accordance with the selected study methods.

The majority of studies aimed to investigate the effect of plant based *n*-3 supplementation in participants with established or at risk of MetS, CVD, HTG and T2DM and were not designed to specifically evaluate *n*-3 bioavailability (Dewell et al. 2011; Dittrich et al. 2015; Kawakami et al. 2015; Kontogianni et al. 2013; Kuhnt et al. 2014; Maki et al. 2015; Neff et al. 2011; Nelson, Hokanson, and Hickey 2011; Pieters and Mensink 2015; Zheng et al. 2016). Only 2 of the 13 identified RCT's were designed to investigate the bioavailability of plant based *n*-3 source oils, the rest evaluated the effect of supplementation on other health related biomarkers but did include relevant bioavailability data. The search criteria identified *n*-3 bioavailability studies for flaxseed, echium seed and microalgal oils; suitable chia, hemp and perilla seed oil trials were not identified.

Bioavailability of n-3 from flaxseed oil

All but three of the identified studies (Maki et al. 2014; Neff et al. 2011; Ryan and Symington 2015) evaluated flaxseed oil as a source of *n*-3 with various comparators including sunflower oil, fish oil, corn oil, echium seed oil, safflower oil, soybean oil, olive oil and microalgal oil (Tables 5–7). Three of the flaxseed oil trials were crossover trials (Dittrich et al. 2015; Kawakami et al. 2015; Kontogianni et al. 2013), the remainder parallel design (Table 4). The fatty acid composition of flaxseed oil supplements varied leading to differences in ALA content as well as dosages. Overall, flaxseed oil supplementation did not offer improvements to baseline/endpoint O3I for the majority of the identified studies and DHA levels were not significantly different to the controls at endpoint.

Bioavailability of n-3 from echium seed oil

The echium seed oil studies by Dittrich et al. (2015) and Kuhnt et al. (2014) showed slight improvements in O3I but no changes in grouping levels for participants with low or moderate baseline O3I. For the Dittrich et al. (2015) study, erythrocyte DHA levels were significantly higher ($p \leq 0.001$) in the microalgal oil comparator group and significantly lower than the control for the echium seed oil supplementation group ($p \leq 0.001$). Plasma DHA percentage change levels were significantly higher than the control for microalgal oil ($p < 0.001$) but there were no significant differences for echium seed oil.

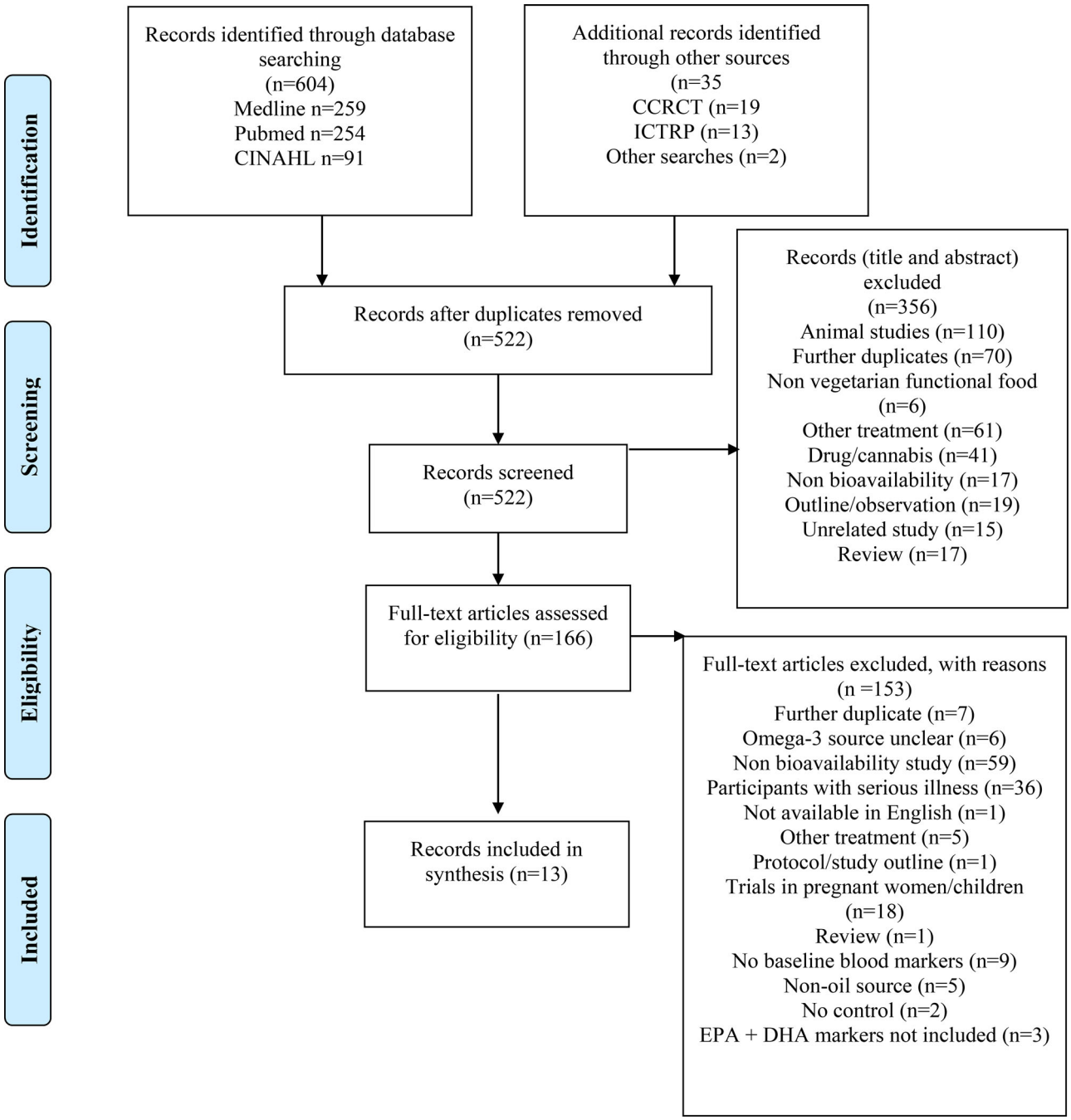

Figure 1. PRISMA Flow Diagram for the scoping review process.

Bioavailability of n-3 from microalgal oil

The remaining RCT's evaluated microalgal oil in comparison to controls of flaxseed and fish oil or a placebo of canola oil (Dittrich et al. 2015; Kuhnt et al. 2014; Maki et al. 2013; Ryan and Symington 2015). Improvements to O3I were noted in both of the high dose microalgal oil studies by Dittrich et al. (2015) (1.6 g/d DHA, 10 weeks) and Maki et al. (2013) (0.66 g/d EPA, 1.78 g/d DHA, 14 weeks) in participants with HTG and a low dose short term study (200 mg/d DHA, 2 weeks) by Ryan and Symington (2015) in healthy participants. The study by Ryan and Symington (2015) was the only trial to evaluate the use of plant based *n*-3 sources compared to fish oil using a small vegetarian/vegan cohort ($n = 10$) who received microalgal oil supplementation (200 mg/d DHA). The vegetarian/vegan cohort had a 'low' mean O3I at baseline, which increased to 'moderate' at endpoint after 2 weeks of microalgal oil supplementation. The endpoint DHA levels between all groups were not significantly different ($p > 0.05$) confirming microalgal oil was on par with fish oil for fish and non-fish eaters. The results of this pilot study indicate that microalgal oil supplements are a sufficient and viable source of DHA for both fish and non-fish eaters alike. The microalgal oil in all studies gave a direct source of DHA and this led to significant increases in baseline/endpoint DHA blood markers ($p \leq 0.05$) and significantly higher percentage change levels when compared to plant based controls.

Table 4. Summary of study design and findings.

Author, year, country and aim	Study design	Intervention source and dose	No of participants and intervention allocation	Participant health status	Time period	Main Findings
Dewell et al. (2011), USA. Determine effect of high and low plant and marine *n*-3 doses on plasma inflammatory markers	DB parallel RCT high and low dose of plant and marine *n*-3 compared to soy bean oil placebo, erythrocytes	Flaxseed high dose 12 capsules (6.6 g/d ALA (HFL)), low dose 4 capsules (2.2 g/d ALA (LFL)). Fish oil high dose 6 capsules (2.1 g/d EPA, 1.5 g/d DHA) low dose 2 capsules (0.7 g/d EPA, 0.5 g/d DHA). Placebo high (6 g/d), low (4 g/d) soy bean oil (composition not stated)	98 adults HFL ($n = 19$) LFL ($n = 20$) LFO ($n = 20$) HFO ($n = 19$) SBO ($n = 20$)	MetS, otherwise healthy, BMI $\leq$39	8 weeks per arm	No beneficial effects detected for inflammatory markers regardless of source or dose
Dittrich et al. (2015), Germany. Investigate effect of *n*-3 rich oil enriched foods on CVD risk factors in subjects with HTG	DB crossover RCT, flaxseed, echium oil, microalgal oil and sunflower oil (control) enriched foods, erythrocyte lipids	20g/d flaxseed oil (FL) (7 g/d ALA), 20 g/d echium oil (EO) (4.8 g/d ALA, 1.6 g/d SDA), 12 g/d microalgal oil (1.6 g/d DHA) 20 g/d sunflower oil (SO) (10 g/d LA)	46 adults SO ($n = 46$) FL ($n = 15$) EO ($n = 15$) AO ($n = 16$)	HTG otherwise healthy	10 weeks per arm, all groups crossed over to the SO control, 10 week washout	ALA and SDA rich oils increased *n*-3 content of erythrocytes. Improvements to blood lipids noted for sunflower, flaxseed and microalgal oils but not echium oil
Kawakami et al. (2015), Japan. Investigate the effect of 12 week flaxseed oil supplementation on serum sd- LDL-C	DB crossover RCT, plasma serum	10g/d flaxseed oil (FL) (5.49 g ALA), 10 g/d corn oil (CO) (0.09 g/d ALA)	15 adults	Healthy males	12 weeks per arm, 8 week washout	Flaxseed oil gives lower sd-LDL-C concentrations
Kontogianni et al. (2013), Greece. Investigate effect of 12-week supplementation of flaxseed oil on CVD risk factors	DB crossover RCT, olive oil and flaxseed oil, erythrocyte membrane	13.8g/d flaxseed oil (FL) (8 g/d ALA) or olive oil (OO) (0.13 g/d ALA)	37 males and females (18–35 yrs)	Healthy normal weight	6 weeks per arm, 6 week washout	Flaxseed oil supplementation leads to lower sd-LDL concentrations
Kuhnt et al. (2014), Germany. Investigate the effect of echium oil on n-3 accumulation and blood biochemical markers in age, sex and MetS	DB parallel arm RCT, plasma serum	Echium seed oil (EO) 17 g/d (2 g/d SDA, 5 g/d ALA). Fish oil mixed 9:1 in olive oil (FO) 17 g/d (1.9 g/d EPA, 0.2 g/d DHA)	78 adults EO1 ($n = 20$) healthy weight NTG 20–35yrs EO11 ($n = 20$), 49–69yr, NTG EO111 ($n = 19$) o/wt MetS or obesity FO1 ($n = 10$) healthy weight 20–35yrs NTG FO11 ($n = 9$) 49–69yrs healthy weight NTG	59, healthy weight NTG 19 o/wt MetS or obesity	8 weeks	EO increases *n*-3 in blood fractions and is beneficial for those with MetS, but cannot replace dietary DHA
Lefort et al. (2016), Canada. Investigate the safety and dietary efficacy of Ahiflower oil in comparison to flaxseed oil.	Parallel group DB RCT, plasma serum	Ahiflower oil extracted from *Buglossoides arvensis* seeds (AH) 9.1 g/d (4.19 g/d ALA, 1.82 g/d SDA) or 9.1 g/d flaxseed oil (FL) (5.369 g/d ALA)	40 ($n = 20$ each arm),	Healthy men and women 18-65yrs, BMI 18.5-39.9, NTG	28 days	AH is safe and more effective for the enrichment of tissues with EPA and DHA than flaxseed oil
Maki et al. (2014), USA. Investigate the safety and efficacy of microalgal oil containing EPA and	Parallel arm DB RCT, plasma serum	Microalgal oil DHA-O (AO) 0.66 g/d EPA 1.78 g/d DHA, fish oil (FO) 1.16 g/d EPA	93 adults CS control ($n = 36$) AO ($n = 37$) FO ($n = 20$)	Normally active and generally healthy men and non-pregnant/lactating women	14 weeks per arm	Microalgal oil supplementation was safe, significantly reduced serum

(*continued*)

Table 4. Continued.

Author, year, country and aim	Study design	Intervention source and dose	No of participants and intervention allocation	Participant health status	Time period	Main Findings
DHA on cardiovascular risk.		0.82 g/d DHA corn-soy bean oil (CS) 50 g/d (ALA content not stated)		with mild to mod HTG (18-79yrs)		TG and increased LDL-C compared to CO control.
Neff et al. (2011), USA. Examone effects of DHA supplementation on plasma lipid and lipoprotein concentration and other biomarkers of CVD risk in absence of weight loss.	DB RCT, parallel arm, plasma phospholipids	Microalgal oil (AO) 5 mL/d (2 g/d DHA) or corn-soy bean oil (CS) 5 mL/d	33 adults AO ($n = 18$) CS ($n = 15$)	Overweight and obese otherwise healthy adults not taking medication for hypertension, dyslipidaemia, diabetes or weight control and not using fish oil supplements	4.5 months	DHA supplementation gave beneficial changes to TC, VLDL TG, VLDL, LDL and HDL particle sizes.
Nelson, Hokanson, and Hickey (2011), USA. Determine if 8 week isocaloric diet supplemented with flaxseed or fish oil alters phospholipase A_2	SB RCT parallel arm, erythrocyte membranes	Flaxseed oil (FL) 11 g/d (6.27 g/d ALA), fish oil (FO) 2 g/d and 7 g/d (360 g/d EPA, 240 g/d DHA) Iso-caloric control- olive oil (OO) 11 g/d (composition not stated)	59 adults FL ($n = 20$) FO ($n = 20$) OO ($n = 19$)	Healthy over 50, including those taking aspirin, statins, ibuprofen	8 weeks	Flaxseed and fish oil supplementation did not influence phospholipase $_{A2}$ in older adults
Neukam et al. (2011), Germany. Evaluate the effect of supplementation with flaxseed oil and safflowerseed oil on healthy volunteers with sensitive skin	DB parallel arm RCT, plasma	Flaxseed (FL) 4 capsules 555.32 mg (1.08 g/d ALA). Safflower oil (SfO) 4 capsules 560 mg/capsule (0.0143 g/d ALA)	26 females FL ($n = 13$) SfO ($n = 13$)	Healthy females 18-65yrs, not pregnant or lactating, BMI 18– 25	12 weeks	Lower inflammatory response of the skin after flaxseed oil intake.
Pieters et al. (2019), Netherlands. Examine the effects of ALA on 24 h ambulatory blood pressure (ABP) in subjects with hypertension	Parallel DB RCT, parallel arm, plasma phospholipids	Flaxseed oil (FL) 10 g/d (4.7 g/d ALA), high oleic sun flower oil control (SO) 10 g/d (0.0 g/d ALA)	56 participants, 40–70yrs, ($n = 28$ per arm)	Healthy overweight/ obese with hypertension	12 weeks	Higher intakes of ALA 3-5 times the recommended daily amount for 12 weeks does not affect 24 hr ABP or BP in subjects with hypertension
Ryan and Symington (2015), UK. Investigate microalgal oil as a viable source of DHA and determine if microalgal derived DHA is bioequivalent to DHA from fish oil	Open-label partially randomized, partially allocated parallel group, plasma phospholipids	Microalgal oil 3 capsules/d (600 mg/d DHA) Compared to fish oil 1 capsule (200 mg/d DHA, 300 mg/d EPA, 100 mg/d SDA & ALA)	31 adults AOv ($n = 10$) vegetarian/ vegans AO ($n = 9$) omnivore microalgal group FO ($n = 10$) omnivore fish oil group	Healthy adults	2 weeks	Algal oil supplements are a sufficient and viable source of DHA for both fish and non-fisheaters
Zheng et al. (2016), China. Investigate the change of serum metabolites in response to n-3 supplementation in patients with T2DM	DB parallel arm RCT, plasma serum	Flaxseed oil (FL) 2.52 g/d ALA Fish oil (FO) 4 g/d (1.2 g/d EPA, 800 mg/d DHA). Corn oil (CO) control 4 g/d 0.00 *n*-3	56 adults FL ($n = 20$) FO ($n = 18$) CO ($n = 18$)	Chinese T2DM otherwise healthy TG <4.56 mmol/L	180 days	No significant change in erythrocyte ALA composition between FO and CO. Fish oil gave the strongest improvements to serum TG

DB = double blinded. SB = single blinded. RCT = randomized controlled trial. MetS = metabolic syndrome. o/wt = overweight. HTG = hypertriglyceridemia. LDL-C = low density lipoprotein cholesterol. NTG = normlipidaemic. sd-LDL-C = small dense low density lipoprotein cholesterol. T2DM = type 2 diabetes mellitus. TG = triglycerides. VLDL = very low density lipoproteins. Source oils: AH = Ahiflower oil. AO=(micro) algal oil. CA = canola oil. CO = corn oil. CS = corn soy bean oil. EO = echium seed. oil FL = flaxseed oil. FO = fish oil. OO = olive oil. SBO-soy bean oil. SfO = safflower oil. SO = sunflower oil.

Table 5. Relative percentage change to erythrocytes by *n*-3 source oil.

					n-3 changes after intervention expressed as relative percentages						
Author, year and country	Study design	Dose	No./type of participants	Time period	18:3*n*-3 ALA	18:4*n*-3 SDA	20:5*n*-3 EPA	22:5*n*-3 DPA	22:6*n*-3 DHA	*n*-6/*n*-3	*n*-3 total
Flaxseed/linseed											
Dewell et al. (2011), USA	DB RCT high (HFx) and low dose (LFx)	6.6 g/d ALA (HFx) 2.2 g/d ALA (LFx)	20 (HFx) 20 (LFx)	8 weeks	0.90* 0.38*	- -	0.45* 0.21	- -	−0.58(NS) −0.34(NS)	- -	- -
Dittrich et al. (2015), Germany.	DB crossover RCT, **flaxseed**, echium seed and microalgal oil enriched foods	7 g/d ALA	15 HTG otherwise healthy	10 weeks per arm	0.34***	–	0.30**	0.41***	−0.09(NS)	−0.64***	0.21(NS)
Kontogianni et al. (2013), Greece.	DB Crossover RCT, olive oil and flaxseed oil	13.8 g/d oil (8 g/d ALA)	37 healthy normal weight males and females aged 18-35	6 weeks per arm	0.22**	–	0.25**	0.40(NS)	0.03(NS)	−0.08(NS) -	- -
Lefort et al. (2016), Canada.	Parallel group DB RCT	9.1 g/d (5.369 g/d ALA)	40 healthy 18-65yrs, BMI 18.5-39.9	28 days	0.18**	0.00(NS)	0.05(NS)	0.13**	0.06(NS)	0.10(NS)	
Nelson, Hokanson, and Hickey (2011), USA.	SB RCT	11 g/d (6.27 g/d ALA)	20 healthy over 50	8 weeks	0.00(NS)	–	0.30(NS)	–	−0.20(NS)	0.10(NS)	
Echium seed											
Dittrich et al. (2015), Germany.	DB crossover RCT, flaxseed, **echium** seed and microalgal oil	2 g/d SDA	15 HTG otherwise healthy	10 weeks per arm	0.19***	–	0.60***	0.76***	−0.43(NS)	−0.71**	0.17(NS)
Kuhnt et al. (2014), Germany.	DB parallel arm RCT	17 g/d (2 g/d SDA)	20, healthy weight 20-35yrs (EO1), 20, 49-69yrs (EO11) 19 o/wt MetS (EO111)	8 weeks	0.19* 0.16* 0.23*	0.05* 0.03* 0.04*	0.26* 0.40* 0.24*	0.63* 0.76* 0.52*	−0.38* −0.76* −0.52*		
Microalgal oil											
Dittrich et al. (2015)	DB crossover RCT, flaxseed, echium seed and micro**algal oil**	2 g/d DHA	16 HTG otherwise healthy	10 weeks per arm	−0.02**	–	0.09(NS)	−0.62***	2.48***	−1.28***	2.93***

DB = double blinded. SB = single blinded. RCT = randomized controlled trial. MetS = metabolic syndrome. o/wt = overweight. HTG = hypertriglyceridemia. T2DM = type 2 diabetes mellitus. -=not measured. NS = not significant. * $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. % changes with no indicator are where significance was not measured.

Table 6. Relative percentage *n*-3 fatty acid change to plasma lipids by *n*-3 source oil.

Author, year and country	Study design	Source and dose	No./type of participants	Time period	*n*-3 changes after intervention expressed as relative percentages						
					18:3*n*-3 ALA	18:4*n*-3 SDA	20:5*n*-3 EPA	22:5*n*-3 DPA	22:6*n*-3 DHA	*n*-6/*n*-3	*n*-3 total
Flaxseed/linseed											
Dittrich et al. (2015), Germany.	DB crossover RCT, **flaxseed**, echium seed and microalgal oil enriched foods	Flaxseed oil 7 g/d ALA	15 HTG otherwise healthy	10 weeks per arm	1.67***		0.24(NS)	0.05*	−0.11(NS)	−2.97**	–
Kawakami et al. (2015), Japan.	DB crossover RCT	10 g/d flaxseed oil (5.49 g ALA)	15 healthy males	12 weeks per arm	–	–	–	–	–	–	3.90*
Lefort et al. (2016), Canada	Parallel group DB RCT	9.1 g/d (5.369 g/d ALA)	40 healthy 18-65yrs, BMI 18.5-39.9	28 days	1.44**	−0.01(NS)	0.08(NS)	0.18*	−0.02(NS)		
Neukam et al. (2011), France and Germany.	DB RCT	4 capsules 555.32 mg (2.7 g/d ALA)	13 healthy females 18-65yrs	12 weeks	0.62*	–	0.25(NS)	0.01(NS)	−0.08(NS)	−1.30(NS)	0.81(NS)
Pieters et al. (2019), Netherlands.	Parallel DB RCT	10 g/d flaxseed oil (4.7 g/d ALA)	28 healthy o/wt/obese, 40-70yrs	12 weeks	0.30	–	0.50	–	0.00	–	1.10
Zheng et al. (2016), China	DB parallel RCT	Flaxseed oil 2.52 g/d ALA	20 Chinese T2DM otherwise healthy	180 days	–	–	0.14	0.03	−0.02	–	–
Echium seed oil											
Dittrich et al. (2015), Germany	DB crossover RCT, flaxseed, **echium** seed and microalgal oil	Echium seed oil 2 g/d SDA	15 HTG otherwise healthy	10 weeks per arm	0.87***	–	0.88***	0.26***	−0.05(NS)	−5.11***	–
Kuhnt et al. (2014)	DB parallel arm RCT	Echium seed oil 17 g/d (2 g/d SDA)	20, healthy weight 20-35yrs (EO1), 20, 49-69yrs (EO11) 19 o/wt MetS (EO111)	8 weeks	1.17* 1.03* 1.07*	0.28* 0.25* 0.28*	0.88* 1.19* 0.90*	0.29* 0.30* 0.24*	0.00(NS) −0.09* −0.17*	- - - -	- - - -
Microalgal oil											
Dittrich et al. (2015), Germany.	DB crossover RCT, flaxseed, echium seed and micro**algal oil**	microalgal oil 2 g/d DHA	16 HTG otherwise healthy	10 weeks per arm			−0.044(NS)	–	0.08(NS)	0.12***	1.64*** −3.52** –
Maki et al. (2015), USA	DB parallel RCT	Microalgal oil 0.66 g/d EPA 1.78 g/d DHA	37 HTG otherwise healthy and active	14 weeks			–	–	0.11*	–	0.37* – –
Neff et al. (2011), USA	DB RCT, parallel arm. Micro**algal oil** and corn-soybean oil (1:1) mixture	Microalgal oil (AO) 5 mL/d (2 g/d DHA) or corn-soy bean oil (CS) 5 mL/d	O/wt and obese otherwise healthy adults not using fish oil supplements AO (n = 18) CS (n = 15)	4.5 months			0.00 ± 0.10(NS)	–	0.30 ± 0.20***	–	5.80 ± 1.10*** – –
Ryan and Symington (2015), UK	Open-label partially randomized, partially allocated parallel group	**Microalgal oil** 3 capsules/d (600 mg/d DHA) Compared to fish oil 1 capsule (200 mg/d DHA, 300 mg/d EPA, 100 mg/d SDA & ALA)	31 healthy adults, including 10 vegetarian/vegans (AOv)) Omnivore microalgal group (AO n = 9) and fish oil group (FO n = 10)	2 weeks AOv AO			−0.02(NS) 0.02(NS)	- -	−0.02(NS) 0.18(NS)	−0.37(NS) −0.24(NS)	2.32* 1.79*

DB = double blinded. SB = single blinded. RCT = randomized controlled trial. MetS = metabolic syndrome. o/wt = overweight. HTG = hypertriglyceridemia. NTG = normlipidaemic T2DM = type 2 diabetes mellitus. - =not measured. NS = not significant. * $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. % changes with no indicator are where significance was not measured.

Table 7 – Summary 'omega 3 index' at baseline and endpoints

			Plasma/erythrocyte percentage fatty acids					
Author, year and country	Intervention	Trial arm	Baseline EPA	Endpoint EPA	Baseline DHA	Endpoint DHA	Baseline EPA + DHA	Endpoint EPA + DHA
Dewell, et al. (2011), USA	Comparison of high and low flaxseed and fish oil doses. Soy bean oil (placebo)	HFL	0.69 ± 0.19	1.14 ± 0.10	4.13 ± 0.43	3.55 ± 0.10	4.82	4.69
		LFL	0.68 ± 0.12	0.89 ± 0.17	4.30 ± 0.24	3.96 ± 0.13	4.98	4.85
		LFO	0.98 ± 0.29	2.24 ± 0.26	3.94 ± 0.39	5.50 ± 0.26	4.92	7.74
		HFO	1.31 ± 0.38	5.81 ± 1.23	5.46 ± 0.40	7.69 ± 0.30	6.77	13.50
		SbO	1.02 ± 0.20	1.01 ± 0.06	4.76 ± 0.42	4.35 ± 0.12	5.78	5.36
Dittrich, et al. (2015), Germany	Comparison of sunflower oil (control), flaxseed oil, echium oil and microalgal oil	SO	0.97 ± 0.32	0.78 ± 0.23	4.30 ± 0.97	4.08 ± 0.72	5.27	4.86
		FL	0.96 ± 0.35	1.26 ± 0.35	4.31 ± 0.97	4.22 ± 0.94	5.27	5.48
		EO	0.95 ± 1.55	1.55 ± 0.56	4.02 ± 1.02	3.59 ± 0.55	4.97	5.14
		AO	0.88 ± 0.0.25	0.97 ± 0.25	3.73 ± 0.80	6.57 ± 1.71	4.61	7.54
Kawakami, et al. (2015), Japan	Flaxseed oil compared to corn oil	FL	0.78 ± 0.17	0.71 ± 0.01	1.32 ± 0.11	1.03 ± 0.98	2.1	1.74
		CO	0.78 ± 0.16	0.68 ± 0.01	1.38 ± 0.17	1.07 ± 0.13	2.16	1.75
Kontogianni, et al. (2013), Greece	Comparison of flaxseed oil and olive oil	FL	0.53 ± 0.25	0.78 ± 0.38	2.31 ± 0.41	2.71 ± 0.43	2.84	3.49
		OO	0.57 ± 0.30	0.58 ± 0.28	2.43 ± 0.60	2.52 ± 0.45	3.00	3.10
Kuhnt, et al. (2014), Germany	Comparison of echium oil and fish oil	EO1	0.48 ± 0.17	1.36 ± 0.41	1.40 ± 0.39	1.40 ± 0.35	1.88	2.76
		EO11	0.72 ± 0.28	1.91 ± 0.64	1.60 ± 0.44	1.54 ± 0.47	2.32	3.45
		EO111	0.73 ± 0.18	1.63 ± 0.27	1.70 ± 0.49	1.53 ± 0.42	2.43	3.16
		FO1	0.43 ± 0.10	3.87 ± 1.19	1.30 ± 0.46	1.89 ± 0.50	1.83	5.76
		FO11	0.85 ± 0.32	3.95 ± 0.74	1.71 ± 0.67	1.98 ± 0.63	2.56	5.93
Lefort, et al. (2016), Canada	Comparison of Ahiflower oil and flaxseed oil	AH	0.45(0.01)	0.74(0.01)	3.68(0.12)	3.27(0.16)	4.13	4.01
		FL	0.43(0.00)	0.55(0.02)	2.99(0.11)	2.89(0.13)	3.42	3.44
Maki et al (2014), USA.	Comparison of microalgal oil, fish oil and corn/soy oil control	AO	0.64 (0.49, 0.81)	1.97(1.57, 2.41)	2.38(1.86, 2.98)	4.99 (4.66, 6.52)	3.02	6.96
		FO	0.78(0.56,0.91)	3.15 (2.18, 3.62)	2.69 (2.16, 3.62)	4.99 (4.66, 6.52)	3.47	8.14
		CS	0.67(0.49,0.90)	0.65 (0.40, 0.93)	2.56 (1.89, 3.46)	2.63 (2.07, 3.69)	3.23	3.28
Neff, et al. (2011), USA	Comparison of microalgal oil and corn/soy oil control	AO	0.60 ± 0.20	1.00 ± 0.30	3.00 ± 0.80	8.80 ± 1.10	3.60	9.80
		CS	0.60 ± 0.20	0.60 ± 0.20	2.70 ± 0.70	2.70 ± 0.70	3.30	3.30
Nelson, et al. (2011), USA.	Comparison of flaxseed oil, fish oil and olive oil control	FL	0.10 ± 0.3	0.40 ± 0.60	4.40 ± 1.0	4.20 ± 1.10	5.50	4.60
		FO	0.00(ND)	0.60 ± 0.70	4.40 ± 1.10	5.30 ± 1.00	4.40	5.90
		OO	0.00(ND)	0.00(ND)	3.76 ± 1.30	3.81 ± 1.00	3.76	3.81
Neukam, et al. (2011), Germany.	Comparison of flaxseed oil and safflower seed oil	FL	1.61	1.86	3.48	3.40	5.09	5.26
		SfO	2.14	1.53	3.27	2.72	5.41	4.25
Pieters, et al. (2019), Netherlands.	Comparison of flaxseed oil and sunflower oil	FL	1.20 ± 0.60	1.70 ± 0.60	3.5 ± 0.90	3.5 ± 0.80	4.70	5.20
		SO	1.20 ± 0.70	1.10 ± 0.05	3.20 ± 0.80	3.30 ± 1.1	5.40	4.40
Ryan and Symington (2015), UK	Comparison of microalgal oil and fish oil	AOv	0.76 ± 0.48	2.76 ± 1.13	0.74 ± 0.19	5.08 ± 0.45	3.52	5.82
		AO	0.92 ± 0.40	4.01 ± 0.87	1.10 ± 0.50	5.8 ± 1.16	4.93	6.9
		FO	1.56 ± 0.99	4.57 ± 1.11	3.36 ± 1.25	5.12 ± 1.11	6.13	8.48
Zheng, et al. (2016), China	Comparison of flaxseed, fish oil and corn oil	FL	0.73 ± 0.10	0.87 ± 0.09	0.88 ± 0.08	0.86 ± 0.08	1.61	1.73
		FO	1.07 ± 0.08	3.05 ± 0.40	1.01 ± 0.06	1.55 ± 0.14	2.08	4.60
		CO	0.97 ± 0.06	0.73 ± 0.07	0.97 ± 0.08	0.83 ± 0.07	1.94	1.56

Equivalent groupings for plasma total lipids [≤2.9% (very low (dark red),N2.9–4.0%(low (red)),N4.0–5.2% (moderate (yellow)),N5.2% (high (green))]. Equivalent groupings for whole blood/erythrocyte [≤3.0% (very low (dark red)),N3.0–4.4% (low (red)),N4.4–5.9% (moderate (yellow)),N5.9% (high (green))]. Plasma phospholipids [≤3.8%(very low (dark red)),N3.8–5.7% (low (red)),N5.7–7.6% (moderate (yellow)),N7.6% (high (green))] Stark et al (2016).

DB = double blinded. SB = single blinded. RCT = randomised controlled trial. MetS = metabolic syndrome. o/wt = overweight. HTG = hypertriglyceridemia. NTG = normlipidaemic T2DM = type 2 diabetes mellitus. AH = Ahiflower oil. AO=(micro) algal oil. CA = canola oil. CO = corn oil. CS = corn soybean oil. EO = echium seed oil FL = flaxseed oil. FO = fish oil. OO = olive oil. SbO = soybean oil. SfO = safflower oil. SO = sunflower oil.

Discussion

The results of this scoping review indicate a lack of recent research into *n*-3 dietary intakes and blood markers of vegetarians and vegans. The RCTs that have evaluated plant based *n*-3 supplementation options are varied and seldom utilize appropriate vegan and vegetarian populations. Consequently, there is very little official guidance available in relation to appropriate plant based *n*-3 alternatives to oily fish.

Vegans eliminate all animal products from their diet and represent a sizable and increasing number within the worldwide population (Statista 2018). The number of vegans in the UK quadrupled between 2014 and 2019, with vegans accounting for 1.16% (600,000) of the population in 2019 (The Vegan Society 2020). Younger people are more likely to follow a vegan diet, European surveys suggest up to 12% followed this dietary pattern in 2017 and prevalence is increasing within this group (Statista 2018). The numbers of vegetarians remain consistent and relatively substantial; worldwide data suggest Asia Pacific region has the highest prevalence of people following a vegetarian diet standing at 19% of the population, 16% Africa/Middle East, 8% Latin America, 6% North America and 5% Europe (Statista 2018). There is strong evidence that a well-planned vegetarian or vegan diet with careful consideration to supplementation can provide all the necessary nutrients (Appleby and Key 2016; Melina, Craig, and Levin 2016). However, concerns have been raised in relation to *n*-3 status and some other vitamin and minerals levels where vegetarian/vegan diets are poorly planned (Appleby and Key 2016; Craig 2009; Melina, Craig, and Levin 2016). Whilst overall, data suggest that the long-term health of vegetarians is good, there is insufficient data to draw strong conclusions on the long term health of vegans (Appleby and Key 2016). In relation to *n*-3 status a

small number of recent studies have specifically analyzed the dietary intakes and O3I of 70 vegans in total (Tables 1 and 2). In combination the three studies found plasma/erythrocyte levels of DHA and EPA were significantly lower for the majority of vegans and *n*-6 levels were significantly higher for all vegan groups, although participant numbers were as low as 5 in some of the cohorts.

Further multicentre study research in larger cohorts of vegetarians and vegans is necessary and this should now be possible due to the increasing numbers of people following a vegan lifestyle and the relative consistency in numbers of vegetarians. In the present study, the participants, timeframes, sources and *n*-3 doses for the identified articles were highly varied. There was a high degree of heterogeneity and only one study examined a small cohort of vegetarians/vegans ($n = 10$) highlighting the difficulty in assessing differences in responses for groups in need of plant based *n*-3 sources.

A shortage of larger scale, consistent studies examining *n*-3 status and blood markers in vegans and fewer still that have evaluated the effectiveness of plant based *n*-3 sources has led to a lack of specific official recommendations for plant based alternatives to oily fish consumption and marine based supplementation. Guidelines from international organizations remain generic, are based on omnivorous diets and mainly focus on the shorter chain essential fatty acids ALA and LA as these must be obtained from the diet in all populations (Davis and Kris-Etherton 2003; Scientific Advisory Committee on Nutrition (SACN) 2004). The most recent UK recommendations state two portions of fish per week (one of oily fish) should be consumed, equating to a 140 g portion providing 0.2 g/d *n*-3 (Scientific Advisory Committee on Nutrition (SACN) 2004). Current UK recommendations are broad and do not give specific advice on the intakes of diverse population groups. The European Food Safety Authority (EFSA) recommend 250 mg/d EPA and DHA for adults (EFSA Panel on Dietetic Products and Allergies 2010). The International Society for the Study of Fatty Acids and Lipids (ISSFAL)) (2004) recommends 2% and 0.7% of energy per day should come from essential fatty acids LA and ALA respectively in healthy adults. For the US, the Food and Nutrition Board of the Institute of Medicine (2002) states the recommended nutrient intake (RNI) for ALA is 1.6 and 1.1 g/d for men and women aged 19 to > 70 years respectively (Trumbo et al. 2002). None of these organizations has been able to provide specific recommendations that relate to the more beneficial longer chain EPA and DHA for non-fish eaters due to the lack of scientific evidence in this area. Interestingly the studies identified in this review show consumption of high doses of ALA from flaxseed oil does not improve mean O3I and in some groups of participants led to overall decreases despite significant increases in plasma or erythrocyte ALA levels. In contrast to this the low dose flaxseed oil study by Neukam et al. (2011) gave a small increase to mean O3I for healthy female participants, which moved from moderate levels to high after 12 weeks. This surprising result shows potential conversion in the *n*-3 metabolic pathway which has also been previously demonstrated in the literature for females under 50 and may be due to increased adipose tissue and estrogen in women (de Groot, Emmett, and Meyer 2019). However, there may be some study design issues as whilst participants were asked to refrain from taking nutrition supplements during the trial, *n*-3 dietary intakes were not considered so direct sources of EPA and DHA may have been consumed. Previous research suggests there are differences in conversion of ALA to EPA and DHA in males and females, however more studies are needed to examine these differences in vegetarians and vegans (Burdge, Jones, and Wootton 2002; Burdge and Wootton 2002; Burns-Whitmore et al. 2019).

Whilst there are a lack of official vegan specific *n*-3 dietary reference values, Davis and Kris-Etherton (2003) recommend 2–4 g of ALA per day, and 100–300 mg/d of DHA for vegans. The conversion of ALA to EPA and DHA in the metabolic pathway relies on a series of desaturase and elongase enzymes. The enzyme delta-6-desaturase catalyzes both the metabolism of LA to arachidonic acid (20:4*n*-6 (AA)) and ALA to EPA and DHA. As levels of ALA are generally lower than LA in the human diet LA is converted to AA more readily meaning plasma and cellular levels of *n*-6 tend to be higher than *n*-3 (Russell and Meital 2018; Schuchardt and Hahn 2013). The ALA recommendation assumes a conversion rate of < 5–10% for EPA and 2–5% for DHA in the metabolic pathway, although a direct source of DHA is preferable. This is confirmed by the findings of the two high dose (Dittrich et al. 2015; Maki et al. 2014) and one low dose microalgal oil study (Ryan and Symington 2015) in the current review, which showed increases to O3I groupings by at least one level and improvements from low and modest levels to high levels for the majority participants. The low dose microalgal oil study by Ryan and Symington (2015) provided 200 mg/d DHA administered over 2 weeks which showed significant improvements in DHA plasma levels for the micoralgal oil study arms ($p \leq 0.05$) and bioequivalence to the comparator fish oil group. A subgroup of vegetarian/vegans (n = 6 of each) were recruited in this study, however only 10 of these participants completed the trial and the final ratio of vegetarians/vegans was not reported. Craddock et al. (2017) also found direct microalgal sources of DHA significantly improved DHA concentrations (including plasma, serum, platelet and red blood cell fractions), as well as O3I, in vegetarian populations in a systematic review and meta-analysis of 4 RCTs and 2 prospective cohort studies. Sarter et al. (2015) found four months of microalgal oil supplementation (172 mg/d DHA and 82 mg/d EPA) offered significant improvements to O3I in a subset of 46 vegans with a low O3I ($p = 0.009$) in their non RCT supplementation study. The evidence identified in this review shows microalgal oil offers a bioequivalent source of DHA to fish oil in omnivorous study groups, however it is not possible to make direct microalgal/fish oil comparisons in vegetarian/vegan population groups due to the nature of these diets. Our findings show strong evidence of the need for essential fatty acid status for EPA and DHA in vegetarians and vegans. To address EPA and DHA shortfalls, food based dietary guidelines should also include references to direct

plant based alternative sources to oily fish. Further research should ratify this in the form of large, powered, well designed (to reduce confounding) RCTs to establish optimum EPA and DHA ratios and daily dosage for vegetarian and vegan populations

Study limitations

When interpreting the results of this scoping review, it is important to note that although all of the included studies fitted with the inclusion criteria, some studies may have exhibited stronger methodological approaches than others, however this falls outside the scope of the review. The use of participants diagnosed with or at risk of non-communicable diseases creates limitations due to changes in fatty acid metabolism within these groups (Walle et al. 2017). There was also a large variability of dosages and study intervention time periods ranging from minimum 2 weeks and maximum of 6 months. Based on the full text publications, only one of the identified studies examined the bioavailability of plant based *n*-3 sources using a small undisclosed number of vegans (Ryan and Symington 2015), which confirms a lack of research in the target study population.

Whilst the O3I represents a well evidenced marker of health it may be questionable how relevant its use is where supplements do not provide a direct source of EPA + DHA. Increases in other beneficial to health *n*-3 fatty acids such as ALA and SDA are not directly measured as the index relies on their conversion in the *n*-3 metabolic pathway. In addition the use of percentage fatty acid measures in whole blood or plasma is a limitation as it can obscure and potentially mask changes in the size of blood lipid pools (Stark et al. 2016). In relation to O3I, erythrocytes have previously been identified as the potential 'gold standard' measure of the future (Harris and Von Schacky 2004), however there has not been a widespread shift to erythrocyte fatty acid analysis (Stark et al. 2016). This was evident in the identified studies as only 5 of them used erythrocyte measurements (Dewell et al. 2011; Dittrich et al. 2015; Kontogianni et al. 2013; Lefort et al. 2016; Nelson, Hokanson, and Hickey 2011). The study methods did not account for different analyses methods to establish *n*-3 blood markers, which were varied and did not always follow recommended ISSFAL standards for measuring blood *n*-3 long chain fatty acid levels in research (de Groot and Meyer 2020; von Schacky 2020).

Authors' conclusions

Plant based vegan diets that eliminate all animal products are a rapidly increasing lifestyle choice particularly in younger people whilst population levels of vegetarians remain constant (Statista 2018). Consumption of *n*-3 in the form of EPA and DHA predominantly found in oily fish offers numerous health benefits but there remains a lack of clear recommendations for specific plant based alternatives suitable for vegetarians and vegans. The main findings of this updated review are in agreement with our previous article. There have been very few attempts to directly study this important issue over the last 10 years despite the increased number of people choosing to consume vegetarian and vegan diets.

Based on the studies identified in this review and in agreement with our previous work, consumption of high doses of ALA from flaxseed oil and echium oil does not increase the O3I and may lead to overall decreases despite significant increases in blood ALA levels, which confirms previous recommendations that a direct source of EPA and DHA is most beneficial. All but one of the identified studies assessed the bioavailability of plant based *n*-3 supplementation in omnivorous participants, which highlights the need for larger long term plant based supplement RCTs with vegetarian and vegan participants to provide more specific and concise guidance for these groups. The three EPA and DHA microalgal oil supplementation studies identified in this review varied in terms of supplement dosages, timeframes and used small numbers of participants. All of them demonstrated ingestion of a direct EPA and DHA source offered improvements to the O3I. Further research in the form of large, powered, well designed (to reduce confounding) RCTs, in a variety of populations both normal healthy and at risk/with NCDs are needed to establish optimum EPA and DHA ratios and daily dosage for vegetarian and vegan populations to achieve this.

Considering the findings presented in the current review, and the observational evidence of low O3I status in vegans, this scoping review indicates the need for essential fatty acid status for EPA and DHA for populations that do not consume marine *n*-3 sources. The preliminary advice for vegetarians and vegans is to reduce levels of *n*-6 in the diet particularly if ALA is the main *n*-3 dietary source and regular consumption of a preformed EPA and DHA supplement is crucial to maintain an optimal O3I.

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Authorship

KEL designed and conducted the scoping review, conducted the initial database searches and drafted the manuscript. MW conducted title, abstract and full text article screening. TGH contributed to methodological approaches and manuscript writing and scrutiny. IGD contributed to interpretation of findings and to manuscript writing and scrutiny.

ORCID

Katie E. Lane http://orcid.org/0000-0002-9092-2927
Teuta G. Hellon http://orcid.org/0000-0001-6475-9428
Ian G. Davies http://orcid.org/0000-0003-3722-8466

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