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How does high DHA fish oil affect health? A systematic review of evidence

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ABSTRACT

The health benefits of fish oil, and its omega-3 long chain polyunsaturated fatty acid content, have attracted much scientific attention in the last four decades. Fish oils that contain higher amounts of eicosapentaenoic acid (EPA; 20:5n-3) than docosahexaenoic acid (DHA; 22:6n-3), in a distinctive ratio of 18/12, are typically the most abundantly available and are commonly studied. Although the two fatty acids have traditionally been considered together, as though they were one entity, different physiological effects of EPA and DHA have recently been reported. New oils containing a higher quantity of DHA compared with EPA, such as fractionated and concentrated fish oil, tuna oil, calamari oil and microalgae oil, are increasingly becoming available on the market, and other oils, including those extracted from genetically modified oilseed crops, soon to come. This systematic review focuses on the effects of high DHA fish oils on various human health conditions, such as the heart and cardiovascular system, the brain and visual function, inflammation and immune function and growth/Body Mass Index. Although inconclusive results were reported in several instances, and inconsistent outcomes observed in others, current data provides substantiated evidence in support of DHA being a beneficial bioactive compound for heart, cardiovascular and brain function, with different, and at times complementary, effects compared with EPA. DHA has also been reported to be effective in slowing the rate of cognitive decline, while its possible effects on depression disorders are still unclear. Interestingly, gender- and age- specific divergent roles for DHA have also been reported. This review provides a comprehensive collection of evidence and a critical summary of the documented physiological effects of high DHA fish oils for human health.

KEYWORDS

22:6n-3; Docosahexaenoic acid; Tuna oil; Omega-3; Seafood; Health benefits

1. Introduction

Fish oils contain high, but variable, levels of omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA), including eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), as well as small quantities of docosapentaenoic acid (DPA; 22:5n-3). Triggered by the pioneering study of Greenland Eskimos conducted by Bang et al. (1971) more than 40 years ago, both scientific and public interest surrounding the role of fish oil and, thus, n-3 LC-PUFA in nutrition and health, has increased over the past few decades. The original study by Bang et al. (1971) deduced that even though the Eskimos had a high consumption of fat, their incidence of ischemic heart disease was very low. These indications of the possible cardioprotective effect of dietary n-3 LC-PUFA present in oily fish has fuelled numerous studies examining the health effects of dietary fish oil (Dyerberg et al. 1978). Research in this area was also stimulated by the finding that mammalian brains were invariably rich in DHA (Crawford et al. 2009), which further induced research into maternal and early infant nutrition. There is a plethora of studies to date that demonstrate the positive effects of supplementation with fish oil containing EPA and DHA on human health and, in some circumstances, the prevention of certain diseases. Consequently, major health organizations globally have made

recommendations for increased dietary intakes of EPA, DPA, and DHA, all of which can be found primarily in fish oils, and the flesh of oily fish, in nutritionally meaningful quantities. The Food and Agriculture Organization of the United Nations (2010) recommends a dose of 250 mg/day of EPA plus DHA for adult males and non-pregnant/non-lactating adult females, while the American Heart Association recommends about 1 g/day of EPA plus DHA for patients with known coronary heart disease, and 2 to 4 g/day for patients requiring triacylglycerol (TAG) lowering (Kris-Etherton et al. 2002).

Most of the currently available studies investigating the effect of dietary n-3 LC-PUFA, on which dietary recommendations are based upon, used commercially available fish oil supplements, typically containing mixtures of EPA and DHA. The most abundantly produced and available fish oils globally are anchovy (*Engraulis ringens*) oil, capelin (*Mallotus villosus*) oil, menhaden (*Brevoortia tyrannus*) oil and herring (*Clupea harengus*) oil, all of which contain variable amounts of n-3 LC-PUFA, but are characterised by higher concentrations of EPA than DHA (Turchini et al. 2009). In human and animal studies, the most commonly studied fish oil is anchovy oil, which typically contains about 30% of EPA and DHA, in a 18/12 ratio (180 mg of EPA and 120 mg of DHA per g of oil). The EPA

and DHA content and ratio in the oil might vary depending on the kind of feed (microalgae, zooplankton, etc.) and the season. Accordingly, it is now a widely-accepted industry standard that the oil intended for human consumption should contain 30% of EPA plus DHA, where 18/12 ratio is the most preferred and valuable profile. Batches characterised by different quantities of EPA and DHA are instead commonly redirected towards feed applications” (Gustavo Ferreyros, TASA, personal communication).

From nutritional/practical viewpoint, EPA and DHA are generally considered a single entity derived from a consistent ratio of two oils, with EPA being 50% more abundant than DHA. Nevertheless, there is a growing body of evidence showing that EPA and DHA exert heterogeneous effects on health outcomes, especially those that are cardiometabolic related. Biologic pathways through which n-3 LC-PUFA play essential roles include: i) modulation of cell and organelle membrane structure and function; ii) modulation of ion channels and cellular electrophysiology; iii) regulation of nuclear receptors and transcription factors; and iv) production of n-3 LC-PUFA derived bioactive metabolites (Mozaffarian and Wu 2012). Compared to EPA, DHA contains a longer carbon chain (22 vs 20) and an additional double bond (6 vs 5) per molecule, which is deemed to be the reason for the different metabolic effects between the two molecules. For example, it has been reported that DHA has a greater influence on membrane fluidity and, thus, on the activity of membrane protein and ion channels (Hashimoto et al. 1999). There is also increasing evidence for the different roles and actions of the bioactive metabolites derived from EPA and DHA, such as eicosanoids, lipoxygenase metabolites and docosanoids (Serhan 2005). In addition, it has been shown that dietary DHA and EPA have a different metabolic fate in animal models, with DHA being preferentially retained over EPA, and EPA being β -oxidised more than DHA (Ghasemifard et al. 2015a). Further, a review paper by Cottin et al. (2011) explored the known differential effects of EPA and DHA in human subjects, and concluded that there is an apparent efficacy of DHA to improve several cardiovascular risk factors, and although both EPA and DHA can reduce TAG levels, the actual role for EPA has not yet been fully clarified. Hence, it appears to be plausible that fish oils with a higher amount of DHA than EPA might possess different health benefits compared to the high EPA fish oil traditionally used. In light of these considerations, and the ever-increasing availability of new fish oils, and n-3 LC-PUFA preparations containing higher concentrations of DHA, such as tuna oil, calamari oil, fractionated and concentrated fish oils, single cell oils from heterotrophic algae and genetically modified oilseed crops (Miller et al. 2011; Olsen et al. 2011), it is important to gain a better understanding of the potential metabolic and health effects of DHA, uncoupled by higher EPA content.

Therefore, it is the purpose of this review to systematically assess the role of high DHA fish oils in human health, with a focus on studies conducted with humans, but also considering relevant information from mammalian animal studies, using fish oils reported to have a higher DHA content than EPA. The ultimate objective of this review is to provide evidence, based on the currently available literature, for the clinical therapeutic potential of dietary administration of high DHA fish oil

preparations, and/or to highlight possible knowledge gaps and potential future research directions.

2. Literature search; methods and statistical considerations

Following on from a pioneering review paper on this topic, and currently the only one of its kind (Horrocks and Yeo 1999), we have systematically searched all scientific literature from the year 2000 to the present date, and retrieved all clinical and animal based studies where high DHA oils were used and tested.

To retrieve the relevant studies to be used in this review, a search strategy using Medline/PubMed, ScienceDirect, Web of Science and EMBASE was conducted using the following search terms: “long chain fatty acids”, “omega-3”, “DHA”, “EPA”, “fish oil”, “capsule”, and “heart”, “brain”, “body mass”, “asthma”, “growth”, “diabetes”, “visual” and “medical uses”. Subsequently all retrieved studies were analysed by reading the full-text articles.

The exclusion criteria were: thesis; patents; research protocol studies; DHA content lower than EPA; the use of fully purified DHA/ EPA; the use of omega-3 sources not from fish oil; the use of other interventions in addition to fish oil administration; any publication before 2000; more than one supplier during the study; and any language other than English.

In addition, some studies were excluded as they did not provide sufficient details such as EPA and DHA content (Haugard et al. 2006; Larter et al. 2008; Teague et al. 2013; Teague et al. 2014), or the source of supplement was not specified (Tokuda et al. 2015), or they used DHA/ EPA from algae (Corder et al. 2016; DiLorenzo et al. 2014; Guzmán et al. 2011; Quinn et al. 2010; Singhal et al. 2013; Yurko-Mauro et al. 2010), or they used fully purified DHA/ EPA (Egert et al. 2008; Woodman et al. 2003). Several studies tested the effects of fish oil with other interventions, and although their study design and sample size was acceptable, they were excluded from this analysis because of possible confounding effects of the secondary intervention. For example, in the Childhood Asthma Prevention Study, the effect of dietary omega-3 supplementation and/or house dust mite was studied (Brew et al. 2015; Hansell et al. 2014; Marks et al. 2006; Skilton et al. 2012), while in the Nutrition and Health Lifestyle Study, the effect of fish oil supplementation with or without folate was assessed (Campoy et al. 2011; Escolano-Margarit et al. 2011). In addition, two studies assessed the effects of DHA as one component of a multi-nutrient supplement on cognition function (Jackson et al. 2016; Strike et al. 2015); Micallef and Garg (2009) conducted a randomized, double-blind, placebo-controlled study where 60 hyperlipidemic participants were provided with either sunola oil (high-oleic sunflower oil) or n-3 LC-PUFA capsules with or without plant sterols for three weeks; Johnson et al. (2008) tested the low dietary intake of DHA and/or foods rich in lutein on cognitive decline in the elderly; Smuts et al. (2015) investigated the effects of iron and DHA supplementation, alone or in combination, on physical activity; Meyer et al. (2007) tested the benefits of high DHA fish oil supplementation as an adjunct to statin therapy for hyperlipidaemia; and Newens et al. (2011) investigated the effect of acute elevation of non-essential fatty acids enriched with either saturated fatty acids (SFA) or SFA

plus fish oil on vascular function. Further, a double-blind, randomized, controlled trial run by D'Vaz et al. (2012) studied the effect of early postnatal fish oil supplementation on infant cellular immune function at 6 months of age in the context of allergic disease examined. Although important and informative, this study was excluded from our analysis as two different fish oil suppliers, with different EPA and DHA contents and ratios, were used during the trials.

While the studies detailed above were all excluded from this review paper, intervention trials of omega-3 and regular exercise (Buckley et al. 2009; Hill et al. 2007a; Hill et al. 2007b; Marques et al. 2015; Ninio et al. 2008; O'Keefe Jr et al. 2006; Peoples et al. 2008), and omega-3 and weight-loss programs (Gunnarsdottir et al. 2008; Hill et al. 2007a; Munro and Garg 2013a) were included, with the exception of a study by Park et al. (2000) who studied the effect of high DHA with pectin on a variety of parameters in rats; this study was excluded because of the confounding effect of pectin.

Of the 375 human and animal studies retrieved and reviewed, a total of 113 studies, including human studies, follow-up studies and animal studies, met the inclusion criteria and were reported and analysed separately herein (Figure 1). The human and animal studies have been subdivided into three sub-sections according to overall metabolic aspects and the number of publications available: (i) heart and exercise; (ii) brain and visual system and (iii) other medical aspects. All follow-up studies were presented in a single, individual section.

In instances where the fatty acid composition of doses/diets was reported by authors with qualitative values (percentages) only, and quantitative data was not reported, the content of DHA and EPA delivered to subjects/animals was estimated. This estimate was based on a simple computation relative to percent values reported and the total volume of oil/lipid administered, by assuming that 1 g of oil would contain 1000 mg of fatty acids, and thus a overestimation is likely.

To characterise the populations of the examined human studies, the following age classes were defined and used: infants (0–1 years old), toddlers (1–3 years old), schoolers (3–12 years old), adolescents (12–18 years old) and adults (above 18 years old, including young adults, middle-age adults and older adults) (Figure 2). Majority of the studies investigated the effect of high DHA fish oils in adults (76%), followed by studies on infants and toddlers (11%), schoolers (10%) and adolescents (2%). One study did not specify the age range of subjects (1%).

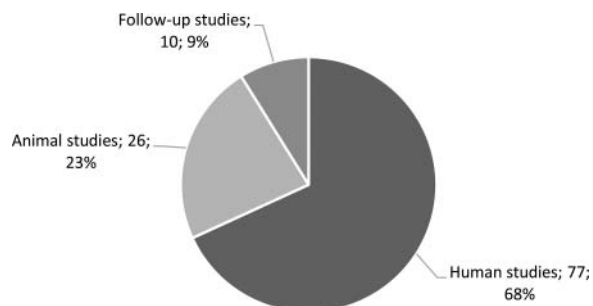


Figure 1. Summary and types of all studies retrieved, selected and used for the present analysis of high DHA fish oils (number of studies and percentage relative to entire dataset).

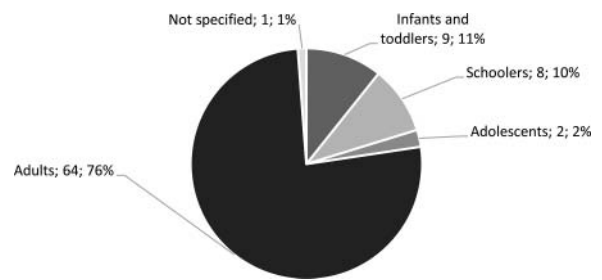


Figure 2. Number, and percentage relative to entire dataset, of retrieved and analysed studies per age classes used for the present analysis of high DHA fish oils.

As shown in Figure 3, the majority of human studies focused on the effects of high DHA fish oils on the brain (41%), heart (32%), immunity (16%) and growth/body mass (8%). In animal studies (Figure 4), the focus was on the heart (38%), followed by the brain (23%), growth/body mass (19%) and then immunity (4%). In follow-up studies (Figure 5), 70% of studies focused on the brain and visual system, followed by growth/body mass (20%) and then immunity (10%).

3. Results of selected studies

3.1. Human studies

3.1.1. Heart and exercise

A total of 24 studies focusing on the effects of administering high DHA fish oils on heart functioning and exercise performance were analysed, and are summarised in Table 1.

Amongst these studies, there were three postprandial cross-over studies, two of which were specifically designed to test the

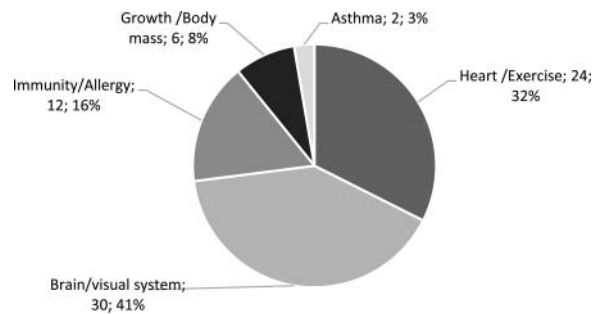


Figure 3. The physiological/medical application areas of human studies retrieved and used for the present analysis of high DHA fish oils (number of studies and percentage relative to human studies).

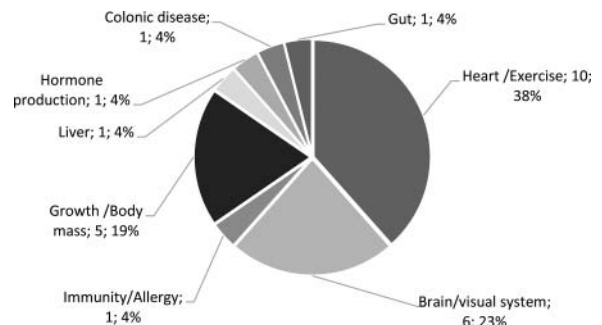


Figure 4. The physiological/medical application areas of animal studies retrieved and used for the present analysis of high DHA fish oils (number of studies and percentage relative to animal studies).

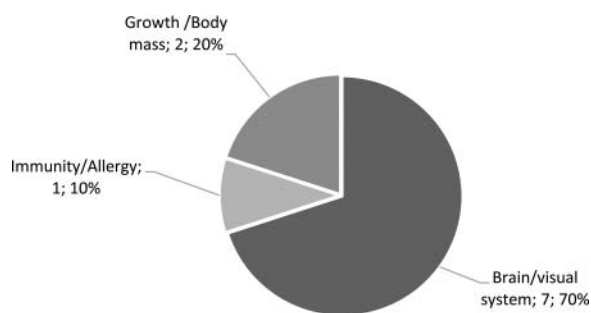


Figure 5. The physiological/medical application areas of follow-up studies retrieved and used for the present analysis of high DHA fish oils (number of studies and percentage relative to follow-up studies).

effect of high DHA fish oils on cardiovascular outcomes in healthy subjects (Armah et al. 2008; Chong et al. 2010), and one testing individuals with increased cardiovascular disease (CVD) risk (McManus et al. 2016). The remainder of the studies were long-term, intervention trials with a duration of 2 weeks to 8 months (the mean intervention trial duration was 12 weeks). The number of subjects recruited ranged from 16 to 312 (median of 41), while postprandial studies typically had a relatively small number of participants compared with longer-term studies. Most trials were conducted with male and female participants (62.5%), though one trial was conducted with females only (4.2%), and the remainder with males only (33.3%). A total of 62% of the studies were carried out with healthy subjects, and 37% with subjects that had a disorder, such as being overweight, hyperlipidemic, and hypertriglyceridaemic.

In general, the doses of EPA plus DHA given to subjects were < 1.0 g/day in four trials (Macartney et al. 2014; McEwen et al. 2013; McEwen et al. 2015; O'Keefe Jr et al. 2006), 1.0–1.9 g/day in ten trials (Buckley et al. 2009; Hill et al. 2007a; Khan et al. 2003; Kumar et al. 2011; Mann et al. 2010; Ninio et al. 2008; Ottestad et al. 2012; Pedersen et al. 2010; Phang et al. 2013; Poppitt et al. 2009), and 3.0–6 g/day in six trials (Armah et al. 2008; Buckley et al. 2004; Chong et al. 2010; Krebs et al. 2006; McManus et al. 2016; Peoples et al. 2008). Two different doses of EPA plus DHA were used in three studies; Sjoberg et al. (2010) trialled 0.6 and 1.9 g/day, Finnegan et al. (2003) trialled 0.8 and 1.7 g/day and Caslake et al. (2008) trialled 0.7 and 1.8 g/day. Milte et al. (2008) used three dosages (0.52, 1.04 and 1.56 g) of EPA plus DHA per day in their study, where the median dose was 1.6 g/day (range: 0.64 to 5.7 g/day). As described earlier, and according to selection criteria, all of these studies used a preparation where DHA was higher than EPA (DHA>EPA), and the actual DHA: EPA ratio in these studies varied from approximately 1.3: 1 (Ottestad et al. 2012) to 6.1: 1 (Buckley et al. 2004).

A variety of parameters were measured in these studies, including: i) plasma phospholipid fatty acids and other plasma measurements (fasting plasma lipid, apolipoprotein B, glucose, insulin) (Armah et al. 2008; Buckley et al. 2004; Caslake et al. 2008; Chong et al. 2010; Finnegan et al. 2003; Mann et al. 2010; Milte et al. 2008); ii) lipid and apolipoprotein concentrations, lipoprotein subclass distribution, and markers of oxidative status (Caslake et al. 2008; Ottestad et al. 2012; Pedersen et al. 2010; Poppitt et al. 2009); iii) erythrocyte n-3 fatty acid content (Buckley et al. 2009; Macartney et al. 2014); iv) haemostatic markers, platelet

aggregation, and thromboxane (Mann et al. 2010; McEwen et al. 2013; McEwen et al. 2015; Phang et al. 2013; Poppitt et al. 2009); v) blood pressure (McManus et al. 2016; O'Keefe Jr et al. 2006; Pedersen et al. 2010; Sjoberg et al. 2010); vi) heart rate (HR), resting HR, HR recovery, heart rate variability (HRV), and HR response to submaximal exercise (Buckley et al. 2009; Macartney et al. 2014; Ninio et al. 2008; O'Keefe Jr et al. 2006; Peoples et al. 2008; Sjoberg et al. 2010); and vii) endothelium-dependent and -independent vascular responses, and pulmonary vein and atrial electrophysiology (Khan et al. 2003; Kumar et al. 2011).

Hill et al. (2007a) reported that the daily intake of high DHA fish oil could be a useful adjunct to exercise programs aimed at improving body composition and decreasing CVD risk in overweight individuals. Findings from this trial suggest that regular exercise coupled with a moderate dose of high DHA fish oil improves multiple cardiovascular risk factors, including plasma TAGs, high-density lipoprotein (HDL) cholesterol, and flow-mediated dilatation. O'Keefe Jr et al. (2006) found that high DHA fish oil decreased HR at rest from 73 ± 13 to 68 ± 13 beats/min, and improved 1-minute HR recovery after exercise. Overall, HRV was unaffected by high DHA fish oil administration, though HRV in the high-frequency band increased significantly ($p < 0.02$). There were no significant effects on blood pressure, arterial compliance, lipids, or inflammatory markers reported between groups. Buckley et al. (2009) showed that the erythrocyte EPA, DHA and total omega-3 fatty acids content increased almost two-fold, while serum TAG and HR reduced during submaximal exercise in athletic subjects receiving a high DHA fish oil supplementation, compared to the placebo. Despite no effect on resting HR, HR during submaximal exercise decreased by ~ 8 beats/min with high DHA fish oil, compared to only ~ 2 beats/min with sunflower oil, suggesting that high DHA fish oil improved CV efficiency during submaximal exercise. In this study, high DHA fish oil did not affect endurance exercise performance or recovery. Ninio et al. (2008) reported that high DHA fish oil supplementation improved HRV in volunteers by increasing high-frequency power, and it reduced HR at rest and during submaximal exercise. The effects of high DHA fish oil supplementation on O_2 consumption during exercise was investigated in trained male cyclists (Peoples et al. 2008). No difference in O_2 consumption or peak workload after supplementation with fish oil was recorded. However, the high DHA fish oil supplementation, compared to control, lowered HR (including peak HR) during incremental workloads to exhaustion, lowered steady-state submaximal exercise HR, whole-body O_2 consumption, and rate pressure product. Armah et al. (2008) found that postprandial triacylglycerol-rich lipoproteins, isolated after a high DHA fish oil meal, increased endothelial nitric oxide synthase and decreased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase gene expression compared to the control meal. Chong et al. (2010) found that consumption of the high DHA meal had an attenuating effect on augmentation index and arterial stiffness index compared with the control meal. McManus et al. (2016) reported that a single dose of DHA, but not EPA, significantly improved postprandial arterial stiffness compared to the control, as assessed by augmentation index, which, if sustained, would be associated with a significant decrease in CVD risk. McEwen et al. (2013) showed that supplementation of high

Table 1. A summary of experimental design and results from human studies testing the effects of high DHA fish oil on heart functioning and exercise performance.

Reference & Journal	Aim of study	Type of study	Number of participants / Test formulation	Age range (years)	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Hill et al. 2007a) The American Journal of Clinical Nutrition	To examine the individual and combined effects of n-3 FA supplements and regular exercise on body composition and cardiovascular health.	A randomized trial.	81 overweight men and women with high blood pressure, cholesterol, or TAG were randomly assigned to 4 groups. Two groups took 6 × 1 g tuna fish oil (1.56 g DHA + 0.36 g EPA) per day and 2 groups took 6 × 1 g sunflower oil per day, over 12 weeks. One of the groups assigned to each oil treatment also exercised regularly.	25–65	4.3: 1	Fish oil supplementation lowered TAG, increased HDL cholesterol and improved endothelium-dependent arterial vasodilation. Exercise improved arterial compliance. Both fish oil and exercise independently reduced body fat.	+	Nu-Mega Ingredients (Clover), Australia
(O'Keefe Jr et al. 2006) The American Journal of Cardiology	To explore some of the possible mechanisms by which relatively low doses of n-3 fatty acids could decrease the risk of sudden cardiac death.	A randomized, double-blind, placebo-controlled, crossover trial.	18 males with a history of myocardial infarctions received either a placebo or an n-3 fatty acid supplement (0.58 g DHA + 0.22 g EPA), taken 3 times a day for two sequential 4-month periods.	67.8 ± 6.5	2.6: 1	High DHA fish oil decreased HR at rest from 73 ± 13 to 68 ± 13 beats/min and improved 1-minute HR recovery after exercise. Though HRV in the high-frequency band increased ($p < 0.02$), overall HRV was unaffected by the n-3 group.	No effect +	Ocean Nutrition Canada, Ltd., Canada
(Buckley et al. 2009) Journal of Science & Medicine in Sport	To test whether supplementation with a high DHA fish oil could improve endurance exercise performance, recovery, or CV responses to exercise.	A randomized double-blind, placebo-controlled, parallel matched-pairs design.	25 male Australian Rules football players randomly received either 6 g/d of high DHA fish oil (1.56 g DHA + 0.36 g EPA) or sunflower oil (placebo) during 5 weeks of training. Gender not specified.	Not specified	4.3: 1	Supplementation with fish oil improved CV function and reduced CV risk factors after 5 weeks, but did not improve endurance performance or recovery in elite Australian Rules footballers.	+	Nu-Mega Ingredients (Clover), Australia
(Ninio et al. 2008) British Journal of Nutrition	To determine whether fish oil supplementation improves HRV, a predictor of cardiac death.	A randomized, double-blind, parallel trial.	65 overweight men and women consumed 6 g/d of fish oil (DHA 1.56 g + EPA 0.36 g) or sunflower-seed oil (placebo) for 12 weeks with/without moderate physical activity.	25–65	4.3: 1	Fish oil supplementation improved HRV by increasing high-frequency power. It also reduced HR at rest and during submaximal exercise.	+	Nu-Mega Ingredients (Clover), Australia
(Peoples et al. 2008) Journal of Cardiovascular Pharmacology	To test the effects of fish oil supplementation on O ₂ consumption during exercise.	A double-blind, parallel design.	16 well-trained, male cyclists, randomly assigned to groups, consumed capsules containing 1 g olive oil (control) or fish oil (2.4 g DHA + 0.8 g EPA/day), 8 times a day for 8 weeks.	21–30	3.0: 1	No difference in O ₂ consumption or peak workload was observed after supplementation with fish oil.	No effect +	Nu-Mega Ingredients (Clover), Australia
(Armah et al. 2008) Clinical Science	To examine the impact of fish oil fatty acids, added to a standard test meal, on postprandial vascular reactivity.	A single-blind crossover trial.	25 healthy males received, at random, either a placebo oil meal (40 g placebo oil) or a fish oil meal (31 g placebo oil + 9 g fish oil, providing 3.2 g DHA + 2.2 g EPA) on two occasions, separated by one week.	46 (average)	1.5: 1	Fish oils significantly improved postprandial endothelium-independent vasodilation.	+	Ocean Nutrition Canada, Ltd., Canada
(Chong et al. 2010) Clinical Nutrition	To investigate the acute effects of a n-3 LC-PUFA rich meal on measures of arterial stiffness.	A n-3 postprandial single-blind, randomized, controlled crossover study.	25 healthy men and women received either a control meal or a n-3 LC-PUFA rich meal (23.2 g of control oil and 6.8 g fish oil, providing 2.0 g EPA + 2.7 g DHA) on two occasions, in a random order.	19–68	1.3: 1	Consumption of the n-3 LC-PUFA rich meal had an attenuating effect on augmentation index and stiffness index compared with the control meal.	+	Croda Europe Ltd, United Kingdom
(McManus et al. 2016) Journal of Lipid Research	To investigate the impact of EPA versus DHA on arterial stiffness and reactivity, and the underlying mechanisms (focusing on plasma oxylipins), in the postprandial state.	Three-arm, crossover, acute test meal trial.	26 males with an increased CVD risk received a high-fat test meal containing either high EPA fish oil (0.68 g DHA + 4.16 g EPA), high DHA fish oil (4.16 g DHA + 0.81 g EPA) or control oil.	35–55	5.1: 1	A single dose of DHA significantly improved postprandial arterial stiffness.	+	Epax, FMC Health and Nutrition, Norway

(Continued on next page)

Table 1. (Continued)

Reference & Journal	Aim of study	Type of study	Number of participants / Test formulation	Age range (years)	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(McEwen et al. 2013) Seminars in Thrombosis and Hemostasis	To investigate the effects of n-3 LC-PUFA on platelet function in healthy subjects and subjects with CVD.	A randomized study.	40 healthy men and women, and 16 with CVD, received n-3 LC-PUFA (0.52 g DHA + 0.12 g EPA/day) for 4 weeks. No placebo test group.	Healthy subjects: 21–64 Subjects with CVD: 50–83	4.3: 1	In healthy subjects, n-3 LC-PUFA significantly reduced adenosine diphosphate (ADP)-induced and adrenaline-induced platelet aggregation. n-3 LC-PUFA also reduced P-selectin expression on platelets and platelet–monocyte aggregates after activation with ADP 0.5 μ M. There were fewer changes in platelet aggregation and activation found in subjects with CVD.	+	Nu-Mega Ingredients (Clover), Australia
(McEwen et al. 2015) Seminars in Thrombosis and Hemostasis	To investigate the effects of n-3 on novel markers of global coagulation (the generation of fibrin and thrombin) in healthy subjects and subjects with CVD.	A randomized study.	40 healthy men and women, and 16 with CVD, received 0.52 g DHA + 0.12 g EPA per day for 4 weeks. No placebo test group.	21–64	4.3: 1	In subjects with CVD, the high DHA preparation reduced the fibrin generation parameter of overall haemostasis potential, a more global summary of coagulation, but did not affect fibrinolysis.	+	Nu-Mega Ingredients (Clover), Australia
(Macartney et al. 2014) British Journal of Nutrition	To test the effect of low-dose fish oil supplementation on HR response to intense exercise and recovery in physically fit males.	A double-blind, parallel design.	26 healthy males consumed either 2 \times 1 g soya bean oil (control) or tuna fish oil (0.56 g DHA + 0.14 g EPA) per day for 8 weeks.	18–40	4.0: 1	Peak HR was not affected by supplementation with fish oil, however, during submaximal exercise over 5 min, fewer total heart beats were recorded in the fish oil group compared to the control group. Supine HR recovery (half-time) after cycling was significantly faster after fish oil supplementation.	+	Nu-Mega Ingredients (Clover), Australia
(Poppitt et al. 2009) Stroke	To test whether treatment with a guideline-recommended moderate-dose fish oil supplement could improve cardiovascular biomarkers, mood- and health-related quality of life in patients with ischemic stroke.	A randomized, double-blind trial.	102 overweight men and women with CT-confirmed stroke were randomized to groups receiving either 3 g/d of placebo oil or fish oil (0.7 g DHA + 0.3 g EPA) for 12 weeks.	>45	2.3: 1	There was no effect on cardiovascular biomarkers or mood in patients with ischemic stroke following 12 weeks of treatment with a moderate-dose fish oil supplement.	No effect	Sea Dragon, New Zealand
(Mann et al. 2010) Lipid	To test and compare the effects of seal oil (DPA) and tuna fish oil (low DPA, 40% less EPA and double the DHA of seal oil) on blood lipids and platelet factors in healthy volunteers.	A randomized, parallel, double-blind, placebo-controlled, clinical trial.	30 men and women consumed, in capsule form, 1 g/d of either seal oil (0.45 g DHA + 0.34 g EPA + 0.23 g DPA), fish oil (0.81 g DHA + 0.21 g EPA + 0.030 g DPA) or sunola oil (placebo), for 2 weeks.	20–50	3.8: 1	Seal oil may be more efficient than fish oil at promoting healthy plasma lipid profiles and lowering thrombotic risk, possibly due to its high DPA and EPA content.	No effect	Nu-Mega Ingredients (Clover), Australia
(Phang et al. 2013) Journal of Nutrition	To determine whether there is a gender bias on platelet aggregation during long-term n-3 LC-PUFA supplementation.	A double-blind, randomized, placebo-controlled trial.	94 healthy men and women, assigned into groups at random, consumed sunola oil (placebo), EPA-rich fish oil (0.2 g DHA + 1 g EPA) or DHA-rich fish oil (1 g DHA + 0.2 g EPA) for 4 weeks.	40 (mean)	5.0: 1	Males are more likely to benefit from supplementation with EPA, whereas females are more responsive to DHA.	+	EPAX 1050 TG/N, Norway
(Khan et al. 2003) Cardiovascular Research	To evaluate the effects of dietary achievable supplementation of n-3 and n-6 fatty acids on microvascular blood flow and endothelial function in healthy subjects.	A double-blind, randomized, placebo-controlled study.	173 healthy men and women took 5 g/day of an oil supplement for 8 months. Supplements included either placebo oil, oleic acid-rich sunflower oil, evening primrose oil, soya bean oil, tuna fish oil (6% EPA + 27% DHA) or tuna/evening primrose oil mix.	45–61	4.5: 1	Fish oil supplementation has a beneficial effect on endothelial function, even in normal healthy subjects.	+	Not specified

(Ottestad et al. 2012) Plos One	To investigate the effect of fish oil supplementation on the plasma lipid profile in healthy subjects.	A double-blind, randomized, parallel-group study.	37 healthy men and women consumed a capsule containing either 8 g of oleic acid-rich sunflower oil or 8 g fish oil (0.9 g DHA + 0.7 g EPA) per day for 7 weeks.	18–50	1.3: 1 In healthy subjects, fish oil supplementation alters lipid metabolism which increases the proportion of phospholipids and TAGs containing long-chain polyunsaturated fatty acids.	+	TINE SA, Norway
(Kumar et al. 2011) American Journal of Cardiology	To investigate the effects of chronic fish oil supplementation on human pulmonary vein (PV) and left atrial electrophysiology in paroxysmal atrial fibrillation (PAF).	A randomized trial.	36 males with PAF were randomized into a control group or a fish oil group (6 g/d of fish oil, providing 1.5 g DHA + 0.3 g EPA) for a mean of 40±12 days.	18–75	5.0: 1 Patients with PAF chronically supplemented with fish oil exhibited distinctive electrophysiologic properties including prolonged pulmonary venous and left atrial ERPs, and decreased susceptibility to initiation AF from within PVs.	+	Nu-Mega Ingredients (Clover), Australia
(Pedersen et al. 2010) The Journal of Pediatrics	To investigate the effect of fish oil on body composition and cardiovascular risk markers in boys during the pubertal growth spurt.	A randomized trial.	78 healthy, but slightly overweight, boys received bread containing High DHA fish oil (0.90 g DHA + 0.2 g EPA) or vegetable oil (placebo). 3 pieces of bread were consumed per day for 16 weeks.	13–15	4.6: 1 Fish oil improves blood pressure in normotensive and normolipidemic adolescent boys that are slightly overweight.	+	Nu-Mega Ingredients (Clover), Australia
(Buckley et al. 2004) British Journal of Nutrition	To establish the differential impact of EPA and DHA on plasma lipids and apolipoprotein B in adults.	A double-blind, placebo-controlled, parallel study.	42 healthy men and women were randomized to control oil, EPA-rich fish oil (0.7 g DHA + 4.8 g EPA/day), or DHA-rich fish oil (4.9 g DHA + 0.81 g EPA/day) for 4 weeks.	20–70	6.1: 1 DHA may be more efficacious than EPA in improving the plasma lipid profile.	No effect	Ocean Nutrition Canada, Ltd., Canada
(Krebs et al. 2006) International Journal of Obesity	To test whether the addition of n-3 LC-PUFA to a low fat, high carbohydrate weight-loss programme results in greater improvements in inflammation, insulin sensitivity and CVD risk, other than weight-loss alone.	A randomized, double-blind trial.	93 overweight, insulin-resistant females (BMI >27kg/m ²) were randomized to groups consuming, in capsule form, 5× 1 g of either fish oil (2.9 g DHA + 1.3 g EPA) or placebo oil per day for 12 weeks.	21–69	2.2: 1 Relative to the control group, TAG and adipose tissue were significantly lower and higher, respectively, in the high DHA fish oil group at 24 weeks, but not for LDL cholesterol, total cholesterol, systolic or diastolic blood pressure.	No effect	Pronova, Norway
(Sjoberg et al. 2010) British Journal of Nutrition	To investigate the dose-response effects of n-3 LC-PUFA on HR and HRV to better understand its effects and benefits.	A randomized, double-blind, placebo-controlled study.	67 overweight or obese men and women were randomly allocated to consume either 6 g/d of sunola oil (placebo), or fish oil (0.26 g DHA + 0.06 g EPA per g) at doses of 2, 4 or 6 g/d for 12 weeks.	52 (mean)	4.3: 1 Fish oil supplementation had no effect on blood pressure, small artery compliance or HR. However, the low to high frequency ratio of HRV decreased with increasing doses of fish oil.	No effect	Nu-Mega Ingredients (Clover), Australia
(Finnegan et al. 2003) American Journal of Clinical Nutrition	To compare the effects of increased dietary intakes of ALA and EPA + DHA on a range of atherogenic risk factors.	A double-blind, placebo-controlled, parallel study.	150 moderately hyperlipidemic men and women were randomized into five groups, receiving either a n-6 control, 0.28 g DHA + 0.18 g EPA, 0.76 g DHA + 0.49 g EPA, 3.7 g ALA or 8.7 g ALA per day, for 6 months.	25–72	1.5: 1 Long-term consumption of modest amounts of dietary ALA does not reproduce the effects of preformed EPA + DHA on TAG concentrations, tissue DHA concentrations, or in vitro susceptibility to oxidation of LDL.	No effect	Roche Vitamins Ltd, Switzerland
(Casiak et al. 2008) American Journal of Clinical Nutrition	To determine the effect of moderate EPA and DHA intakes on the plasma fatty acid profile, lipid and apolipoprotein concentrations, lipoprotein subclass distribution, and markers of oxidative status.	A double-blind, placebo-controlled, dose-response, crossover study.	312 men and women consumed capsules containing control oil or fish oil (either 0.57 g DHA + 0.13 g EPA or 1.48 g DHA + 0.32 g EPA), in random order, over an 8-week duration.	20–70	1.4: 1 Supplements providing EPA + DHA at doses as low as 0.7 g/day have a significant effect on the plasma lipid profile.	+	Ocean Nutrition Canada, Ltd., Canada
(Milte et al. 2008) British Journal of Nutrition	To test a dose-response relationship between moderate levels of high DHA fish oil and changes in both erythrocyte DHA content and blood lipids.	A randomized, double-blind, placebo-controlled, parallel study.	67 men and women consumed 2, 4 or 6 g/d of high DHA fish oil (0.52 g DHA + 0.12 g EPA), or a placebo, for 12 weeks.	53 (mean)	4.3: 1 For every 1 g increase in DHA intake per day, there was a 23% reduction in TAG, 4.4% increase in HDL cholesterol and 7.1% increase in LDL cholesterol.	+	Nu-Mega Ingredients (Clover), Australia

*Personal communication; the DHA: EPA ratio was not specified in the published paper, but was provided by authors of the study upon request.

DHA fish oil reduced measures of platelet aggregation and activation in healthy subjects, but induced few changes in patients with existing CVD, suggesting that the n-3 LC-PUFA dose should be higher in those suffering from CVD, than among healthy subjects. The latter randomized, intervention trial (McEwen et al. 2013) was also used to test the effects of n-3 LC-PUFA on novel markers of global coagulation by McEwen et al. (2015). Results of this study indicated a decrease in fibrin generation and an increase in fibrinolysis after supplementation with a high DHA preparation in healthy subjects. In subjects with CVD, the high DHA preparation reduced the fibrin generation parameter of overall haemostasis potential, which is a more global summary of coagulation, but did not affect fibrinolysis. Macartney et al. (2014) found that a low intake of high DHA tuna oil increased the omega-3 index (EPA plus DHA percent, relative to total fatty acids, in red blood cells), reduced the mean exercise HR, and improved HR recovery without compromising the peak HR. Poppitt et al. (2009) showed no significant differences in lipid, inflammatory, hemostatic, or composite mood parameters between high DHA fish oil supplementation and the placebo in patients with ischemic stroke. Mann et al. (2010) reported that both high DHA fish oil and seal oil (an oil characterised by higher DPA content, compared to traditional fish oil) elevated platelet DHA levels, while seal oil also raised platelet DPA and EPA levels. In addition, plasma TAG decreased and high-density lipoprotein (HDL) cholesterol levels increased following supplementation with seal oil. Comparing daily doses of high DHA oil versus EPA-rich oil it was reported that, although platelet aggregation was different for males and females, EPA and DHA reduced platelet aggregation in both sexes compared to the placebo (−11.8% and −14.8%, respectively) (Phang et al. 2013). The EPA treatment reduced platelet aggregation in males by −18.4%, compared to the placebo and females, and, in contrast, the DHA treatment reduced platelet aggregation in females by −18.9%, compared to the placebo and males. Khan et al. (2003) found that acetylcholine, but not sodium nitroprusside, response was significantly improved after tuna oil supplementation compared with other oil treatments, suggesting that high DHA fish oil supplementation has a beneficial effect on endothelial function. Total serum TAG, total low-density lipoprotein (LDL), and HDL cholesterol were unchanged by a daily supplementation with high DHA fish oil or high oleic acid sunflower oil for seven week (Ottestad et al. 2012). These authors also found that high DHA fish oil supplementation altered lipid metabolism: 23 lipids significantly decreased and 51 lipids, including, selected phospholipids and TAGs of n-3 LC-PUFA, increased. Kumar et al. (2011) showed that patients with paroxysmal atrial fibrillation supplemented with high DHA fish oil had distinctive electrophysiologic properties including prolonged pulmonary venous and left atrial effective refractory periods, and decreased susceptibility to initiation atrial fibrillation from within pulmonary venous. These results endorse the antifibrillatory effect of chronic n-3 LC-PUFA supplementation. Pedersen et al. (2010) reported that systolic blood pressure and diastolic blood pressure were lowered in the high DHA fish oil group compared with the control group. Plasma TAG concentration and insulin sensitivity were unaffected by either of the treatments. In the fish oil group, plasma HDL and non-HDL cholesterol were

increased by 5% and 7%, respectively. Buckley et al. (2004) reported that EPA and DHA treatments had no significant effects on circulating total cholesterol, LDL cholesterol or HDL cholesterol levels. There was a significant 22% reduction in TAG levels relative to the control following the DHA treatment ($p = 0.032$), with a 15% decrease in the EPA group, which was not significant. Krebs et al. (2006) showed that TAG and adipose tissue were significantly lower and higher, respectively, in the high DHA fish oil group at 24 weeks, relative to the control group. This trend was not exhibited for LDL cholesterol, total cholesterol, systolic or diastolic blood pressure. Compared to a sunola oil control, no effect of high DHA fish oil on blood pressure, small artery compliance or HR was observed (Sjoberg et al. 2010). However, the low: high frequency ratio of HRV decreased with increasing doses of high DHA fish oil. Finnegan et al. (2003) observed no significant effects on the postprandial TAG response, glucose, or insulin concentrations amongst the tested treatments consisting of an n-6 control or a series of treatments with different DHA, EPA and ALA supplementations. Caslake et al. (2008) found that plasma TAG concentrations decreased by 8% and 11% after low and high doses of high DHA fish oil, respectively. Furthermore, lower VLDL cholesterol, and higher LDL cholesterol, HDL cholesterol, and HDL2 concentrations were evident after fish-oil intervention. Milte et al. (2008) showed that for any 1 g/day increase in DHA intake, there was a 23% reduction in TAG, a 7.1% increase in LDL and a 4.4% increase in HDL.

3.1.2. Brain and visual system

Details of the 30 high DHA fish oil trials focusing on the brain and visual system that were analysed in this review are reported in Table 2. These were long-term studies, with an intervention duration ranging from 3 weeks to 3 years. The number of subjects per group within these studies varied from 7 to 1202. A total of 73% of these trials were conducted with male and female participants, 23% with females only, and one trial was conducted with males only. In majority (65%) of the trials participants were healthy, while in the remaining 35% participants suffered and/or were being treated for Alzheimer's disease, memory complaints, mild cognitive impairment, stress, or depression.

Doses of DHA plus EPA given to subjects were <1.0 g/day in thirteen trials (Barbadoro et al. 2013; Dangour et al. 2010; Freund-Levi et al. 2008; Hamazaki et al. 2008; Hirayama et al. 2004; Hurtado et al. 2015; Itomura et al. 2005; Jackson et al. 2012a; Jackson et al. 2012b; Makrides et al. 2010; Meldrum et al. 2012; Stough et al. 2012; van Goor et al. 2010), 1.0–1.9 g/day in twelve trials (Andrieu et al. 2017; Bradbury et al. 2004; Clayton et al. 2009; Lauritzen et al. 2004; Lee et al. 2013; Makrides et al. 2009; Mozurkewich et al. 2013; Phillips et al. 2015; Rogers et al. 2008; Smithers et al. 2008; Souied et al. 2013; Stonehouse et al. 2013), 2.0–2.9 g/day in three trials (Grenyer et al. 2007; Rees et al. 2008; Sinn et al. 2012), and 3–6 g/day in one trial (Silvers et al. 2005). Jackson et al. (2012c) used two different dosages (1 and 2 g/day) of DHA + EPA in their study, with a median dose of 0.98 g/day (range: 0.25 to 3 g/day). The DHA: EPA ratios in the trials analysed here were from approximately 1: 1 (Phillips et al. 2015) to 8: 1 (Makrides et al. 2010).

Table 2. A summary of experimental design and results from human studies testing the effects of high DHA fish oil on the brain and visual system.

Reference & Journal	Aim of study	Type of study	Number of participants / Test formulation	Age range (years)	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Hirayama et al., 2004) European Journal of Clinical Nutrition	To investigate whether docosahexaenoic acid (DHA) supplementation could ameliorate attention-deficit/hyperactivity disorder (AD/HD) symptoms in AD/HD children.	A placebo-controlled, double-blind study.	40 AD/HD (including eight AD/HD-suspected) boys and girls were randomized to groups receiving either high DHA fish oil (3.60 g DHA + 0.70 g EPA/week) or olive oil (placebo) for 2 months.	6-12	5.1:1	DHA supplementation did not improve AD/HD-related symptoms.	No effect	Not specified
(Tomura et al., 2005) Journal of Nutritional Biochemistry	To investigate whether fish oil supplementation affected Japanese schoolchildren's behaviour, with changes in aggression over time as the primary endpoint.	A randomized, double-blind, placebo-controlled trial.	166 boys and girls were randomized to receive control supplements or fish oil (3.60 g DHA + 0.84 g EPA/week) for 3 months.	9-12	4.3:1	Physical aggression might be affected by fatty acid nutrition, especially in girls. There were no significant changes in physical aggression in boys.	No effect +	Not specified
(Freund-Levi et al., 2008) International Journal of Geriatric Psychiatry	To determine effects of dietary n-3 supplementation to AD patients with a mild to moderate disease on psychiatric and behavioral symptoms, daily functions and a possible relation to APOE genotype.	A randomized, double-blind, placebo-controlled, clinical trial.	204 AD men and women were randomized into groups consuming placebo oil (1 g of corn oil, including 0.6 g of linoleic acid) or 4 fish oil (1.72 g DHA + 0.60 g EPA/day) for 6 months.	74 (mean)	2.8:1	Supplementation with n-3 in patients with mild to moderate AD did not result in marked effects on neuropsychiatric symptoms, except for possible positive effects on depressive symptoms in non-APOEε4 carriers and agitation symptoms in APOEε4 carriers.	No effect	Pronova Biocare A/S, Norway
(Hamazaki et al., 2008) Asia Pacific Journal of clinical nutrition	To observe the effects of fish oil on behavior and school attendance rates in school children.	A randomized, double-blind, placebo-controlled, clinical trial.	233 fourth to sixth year school boys and girls were randomized into groups receiving soybean oil (placebo) or fish oil (containing 0.65 g DHA + 0.10 g EPA/day) for 3 months.	9-12	6.5:1	There were no differences in behaviour between groups, however, children in the DHA group had a significantly higher attendance rate.	No effect +	Not specified
(van Goor et al., 2010) British Journal of Nutrition	To test whether supplementation of DHA during pregnancy and lactation influences the infant's brain development and whether additional 20:4n-6 modulates this effect.	A double-blind, placebo-controlled, randomized trial from about week 17 (range 14-20 weeks) of pregnancy until 12 weeks postpartum.	183 healthy females were randomized into groups receiving either a control, DHA (0.22 g DHA + 0.03 g EPA) or DHA + 20:4n-6 (0.22 g 20:4n-6 + 0.22 g DHA + 0.04 g EPA).	32-33 (mean)	6.1:1	Neither DHA alone nor DHA + 20:4n-6 influenced outcomes in the traditional examination. General movement quality of infants in the DHA group was lower than that of infants in the other two groups.	No effect -	Marinol D40, Lipid Nutrition B.V., The Netherlands
(Makrides et al., 2010) The Journal of the American Medical Association	To determine whether increasing DHA during the last half of pregnancy will result in fewer women with high levels of depressive symptoms and enhance the neurodevelopmental outcome of their children.	A double-blind, multicenter, randomized controlled trial (DOMInO trial).	2399 pregnant women who were less than 21 weeks' gestation were randomized into groups and consumed, in capsule form, either vegetable oil without DHA, or high DHA fish oil (0.80 g DHA + 0.10 g EPA/day), for ~10 weeks.	28.9 (mean)	8.0:1	The use of high DHA fish oil capsules compared with vegetable oil capsules during pregnancy did not result in lower levels of postpartum depression in mothers or improved cognitive and language development in their offspring during early childhood.	No effect	Incromega 500 TG, Croda Chemicals, England

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Table 2. (Continued)

Reference & Journal	Aim of study	Type of study	Number of participants / Test formulation	Age range (years)	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Dangour et al., 2010) American Journal of Clinical Nutrition	To test if n-3 LC-PUFA supplementation would benefit cognitive function in cognitively healthy older people.	A randomized, double-blind, controlled trial.	867 cognitively healthy men and women were randomized to olive oil or fish oil (0.50 g DHA + 0.20 g EPA/day) capsules for 24 months.	70-79	2.5:1	There was no change in cognitive function scores over 24 months, and intention-to-treat analysis showed no significant differences between trial arms at 24 months in the verbal learning test or any secondary cognitive outcome.	No effect	Ocean Nutrition Canada Ltd, Canada
(Jackson et al., 2012a) British Journal of Nutrition	To examine the effects of 12 weeks' dietary supplementation with high DHA fish oil or high EPA fish oil on cognitive function.	A double-blind, placebo-controlled, randomized trial.	159 healthy young men and women were randomized to either 1 g/d of olive oil (placebo), high DHA fish oil (0.45 g DHA + 0.09 g EPA) or EPA-rich fish oil (0.20 g DHA + 0.30 g EPA) for 12 weeks.	18-35	5.0:1	Dietary supplementation with n-3 LC-PUFA in healthy, normally developing, and impairment-free populations is unlikely to result in cognitive enhancement.	No effect	EPAX AS (Aalesund, Norway)
(Jackson et al., 2012b) British Journal of Nutrition	To investigate any treatment-related effects of supplementation with DHA- and EPA-rich fish oil in healthy volunteers on the cerebral haemodynamic response to cognitive tasks in the prefrontal cortex.	A double-blind, placebo-controlled trial.	22 healthy men and women were randomized to either 1 g/d placebo oil (olive oil), high DHA fish oil (0.45 g DHA + 0.09 EPA) or EPA-rich fish oil (0.20 g DHA + 0.30 g EPA) for 12 weeks.	22 (mean)	5.0:1	Supplementation with high DHA fish oil, in comparison with the placebo, resulted in a significant increase in the concentrations of oxy-Hb and total levels of Hb, indicative of increased cerebral blood flow (CBF), during the cognitive tasks. In comparison, no effect on CBF was observed following supplementation with EPA-rich fish oil.	+	EPAX AS (Aalesund, Norway)
(Stough et al., 2012) Neurobiology of Aging	To test the effects of supplementation with DHA on cognitive function and visual acuity in a healthy aging population.	A triple-blind, placebo-controlled, randomized, repeated-measures trial.	74 healthy men and women received either 1g of tuna oil (0.252 g DHA + 0.06 g EPA) or soybean oil (placebo) for 3 months.	45-77	4.2:1	DHA supplementation had no significant effects on cognitive functioning. For participants with corrected vision, the group receiving DHA were found to have significantly better right eye visual acuity post-treatment in comparison with the control. The results suggest that improved postnatal n-3 LC-PUFA intake in the first 6 months of life using high dose infant fish oil supplementation was not beneficial to global infant neurodevelopment. However, some indication of benefits to early communicative development was observed.	Brain: No effect Eye: +	Nu-Mega Ingredients (Clover), Australia
(Meldrum et al., 2012) British Journal of Nutrition	To test the effects of direct supplementation with high dose fish oil on infant neurodevelopmental outcomes and language.	A randomized, double-blind, placebo-controlled trial.	420 healthy term boys and girls were assigned to receive a DHA-rich fish oil supplement (0.25 g DHA + 0.06 g EPA/day) or a placebo (olive oil) from birth to 6 months.	Infants; 0-6 months	4.2:1	The results suggest that improved postnatal n-3 LC-PUFA intake in the first 6 months of life using high dose infant fish oil supplementation was not beneficial to global infant neurodevelopment. However, some indication of benefits to early communicative development was observed.	No effect +	Nu-Mega Ingredients (Clover), Australia
(Barbadoro et al., 2013) Molecular Nutrition & Food Research	To evaluate the effects of 3-week supplementation with EPA and DHA on perceived stress/anxiety and hypothalamic-pituitary-adrenocortical activity.	A randomized, double-blind, placebo-controlled trial.	31 male alcoholics undergoing a residential rehabilitation program were randomized to placebo (1 capsule/day containing microcrystalline	> 18	4.2:1	An elevated n-3 intake may reduce distress symptoms and basal cortisol secretion in abstinent alcoholics.	+	FX s.r.l. by SALIX s.r.l., Italy



<p>(Hurtado et al., 2015) Journal of Pediatric Gastroenterology and Nutrition</p>	<p>To elucidate (1) whether a dairy drink enriched with n-3 LC-PUFA could have an impact on the lipid profile of the mother and the newborn, and (2) whether this intervention could affect the newborns' visual and cognitive development.</p>	<p>A randomized, double-blind, controlled trial.</p>	<p>110 pregnant women were randomized into a control (400 mL/d control dairy drink) or supplemented group (400 mL/d fish-oil enriched dairy drink, providing 0.32 g DHA + 0.07 g EPA/day) for ~7 months.</p>	<p>30 (mean)</p>	<p>4.4: 1 Dietary supplementation with n-3 LC-PUFA during pregnancy did not have effects on visual and cognitive/psychomotor development.</p>	<p>No effect Not specified</p>
<p>(Bradbury et al., 2004) Nutrition Journal</p>	<p>To investigate whether perceived stress is ameliorated by DHA in stressed university staff.</p>	<p>A small intervention study nested within a larger prospective cross-sectional study.</p>	<p>30 men and women that scored ≥ 17 on the Perceived Stress Scale were randomized to olive oil (placebo) or fish oil (6 capsules/day containing 1.51 g DHA + 0.36 g EPA) for 6 weeks.</p>	<p>18-60</p>	<p>4.2: 1 As a pilot study, it was not sufficiently powered to find any significant difference between the fish oil group and the placebo group.</p>	<p>No effect Not specified</p>
<p>(Smithers et al., 2008) The American Journal of Clinical Nutrition</p>	<p>To test visual responses of preterm infants fed human milk and formula with a DHA concentration estimated to match the intruterine accretion rate (high-DHA group), compared with infants fed human milk and formula containing DHA at current concentrations.</p>	<p>A double-blind, randomized, controlled trial.</p>	<p>143 Preterm boys and girls born at <33 weeks gestation were fed either human milk or formula containing 1% DHA (high-DHA group) or ~0.3% DHA (control group) for 4 months until reaching their estimated due date.</p>	<p>Preterm infants</p>	<p>4.5: 1 At 2 months corrected age, acuity of the high-DHA group did not differ from the control group. By 4 months' corrected age, the high-DHA group exhibited an acuity that was 1.4 cycles per degree higher than the control group. Authors concluded that the DHA requirement of preterm infants may be higher than currently provided by preterm formula or human milk of Australian women.</p>	<p>+ Nu-Mega Ingredients (Clover), Australia</p>
<p>(Makrides et al., 2009) The Journal of the American Medical Association</p>	<p>To test the effect of meeting the estimated DHA requirement of preterm infants on neurodevelopment.</p>	<p>A double-blind, randomized, controlled trial.</p>	<p>545 lactating mothers consumed 6×500 g capsules of either high DHA fish oil (0.90 g DHA + 0.20 g EPA) or soy oil (placebo) for ~5 weeks.</p>	<p>Infants; 0-18 months</p>	<p>4.5: 1* Overall, a DHA dose of approximately 1% total fatty acids in early life did not increase Maternal Development Index (MDI) scores of preterm infants born earlier than 33 weeks, but did improve the MDI scores of girls.</p>	<p>No effect Nu-Mega Ingredients (Clover), Australia +</p>
<p>(Clayton et al., 2009) European Journal of clinical nutrition</p>	<p>To test the effectiveness of n-3 LC-PUFA supplementation in the treatment of mania and depression in juvenile BD (JBD) when given as an adjunct to standard pharmacological treatment.</p>	<p>An open-label study.</p>	<p>18 boys and girls with juvenile bipolar disorder received supplements (1.56 g DHA + 0.36 g EPA/day) for 6 weeks.</p>	<p>6 (mean)</p>	<p>4.3: 1 RBC EPA and DHA were significantly higher following supplementation. Clinician ratings of mania and depression were significantly lower and global functioning significantly higher after supplementation. Parent ratings of internalizing and externalizing behaviours were also significantly lower following supplementation.</p>	<p>+ Nu-Mega Ingredients (Clover), Australia</p>

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Table 2. (Continued)

Reference & Journal	Aim of study	Type of study	Number of participants / Test formulation	Age range (years)	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Lee et al., 2013) Psychopharmacology	To investigate the effects of fish oil supplementation on cognitive function in elderly persons with mild cognitive impairment (MCI).	A randomized, double-blind, placebo-controlled trial.	36 low-socioeconomic-status elderly men and women were randomized to placebo corn oil or an isocaloric fish oil (0.43 g DHA + 0.15 g EPA) for 12 months.	>60	2.8:1	Fish oil has a potential role in improving memory function in MCI subjects.	+	EPAX 1050TG; EPAX AS, Norway
(Mozurkewich et al., 2013) American Journal of Obstetrics and Gynecology	To test the relative effects of EPA- and DHA-rich fish oils on the prevention of depressive symptoms among pregnant women at an increased risk of depression.	A randomized, double-blind, placebo-controlled trial.	126 pregnant women at risk for depression were randomized to soy oil placebo, EPA-rich fish oil (0.27 g DHA + 1.06 g EPA), or DHA-rich fish oil (0.90 g DHA 0.18 g EPA) for ~26 weeks.	30 (mean)	5.0:1	Supplementation with fish oil high in EPA or DHA did not prevent depressive symptoms during pregnancy or postpartum.	No effect	ProDHA, Nordic Naturals, USA
(Souied et al., 2013) Ophthalmology	To evaluate the efficacy of docosahexaenoic acid (DHA)-enriched oral supplementation in preventing exudative age-related macular degeneration (AMD).	A randomized, placebo-controlled, double-blind, parallel, comparative study.	263 men and women with early lesions of age-related maculopathy were randomized to olive oil or daily fish oil (0.84 g DHA+ 0.27 g EPA) for 3 years.	55-85	3.1:1	In patients with unilateral exudative AMD, 3 years of oral DHA-enriched supplementation had the same effect on choroidal neovascularization incidence in the second eye as the placebo.	No effect	Reti-Nat, Bausch & Lomb, France
(Stonehouse et al., 2013) American Journal of Clinical Nutrition	To investigate whether a DHA supplement improves cognitive performance in healthy young adults, and whether sex and APOE modulate the response.	A randomized, double-blind, placebo-controlled trial.	176 healthy men and women were randomly assigned to DHA group (three 750 mg capsules/day, providing 1.16 g DHA + 0.17 g EPA) or placebo group (high-oleic acid sunflower oil) for 6 months.	18-45	6.8:1	DHA supplementation improved memory and the reaction time of memory in healthy, young adults whose habitual diets were low in DHA. The response was modulated by sex.	+	Efamol, New Zealand
(Phillips et al., 2015) International Journal of Molecular Science	To determine if an increasing intake of DHA and EPA through supplementation is beneficial to cognition and mood in individuals with cognitive impairment no dementia (CIND) or Alzheimer's disease (AD).	A randomized, double-blind, placebo-controlled trial.	57 men and women with CIND, and nineteen with AD, were randomized to olive oil or fish oil (0.62 g DHA + 0.60 g EPA) for 4 months.	71 (mean)	1.0:1	Even though n-3 fatty acid supplementation elevates plasma concentrations of DHA and EPA in individuals with CIND and AD, this does not benefit cognition and mood in these populations.	No effect	Not specified
(Andrieu et al., 2017) The Lancet Neurology	To test the effect of n-3 polyunsaturated fatty acid supplementations and a multidomain intervention (physical activity, cognitive training, and nutritional advice), alone or in combination, compared with placebo, on cognitive decline.	A randomized, placebo-controlled trial.	1680 men and women with memory complaints were randomly assigned to one of four treatment regimens over 3 years, including multidomain intervention plus n-3 PUFA (0.80 g DHA + 0.22 g EPA), multidomain intervention plus placebo, n-3 PUFA, or placebo alone.	≥70	3.6:1	The multidomain intervention and polyunsaturated fatty acids, either alone or in combination, had no significant effects on cognitive decline over 3 years in elderly people with memory complaints.	No effect	Pierre Fabre Médicament, France

(Rogers et al., 2008) British Journal of Nutrition	To evaluate the effects of EPA plus DHA supplementation (1.5 g/d) on mood and cognitive function in mild to moderately depressed individuals.	A double-blind, randomized, controlled trial.	218 mildly moderate depressed male and female individuals were instructed to consume either three fish oil (0.85 g DHA + 0.63 g EPA) or olive oil capsules per day for 12 weeks.	18-70	1.3:1	Substantially increasing EPA plus DHA intake for 3 months was found to have no beneficial or harmful effects on mood in mild to moderate depression.	No effect Minami Nutrition SA, Belgium
(Lauritzen et al., 2004) Lipids	To evaluate the biochemical and functional effects of fish oil supplements in lactating mothers.	A placebo-controlled, double-blind, randomized trial.	122 Danish mothers with habitual fish intake below the 50 th percentile of the Danish National Birth Cohort were randomized to olive oil (placebo) or 4.5 g/d fish oil (0.79 g DHA + 0.62 g EPA) for 4 months.	Infants: 4 months	1.3:1	Maternal fish oil supplementation during lactation did not enhance visual acuity of the infants who completed the intervention. However, the results showed that infants with higher RBC levels of n-3 LC-PUFA had a better visual acuity at 4 months of age.	No effect BASF Health and Nutrition, Denmark
(Grenyer et al., 2007) Progress in Neuro-Psychopharmacology & Biological Psychiatry	To test whether taking tuna fish oil confers any additional benefit to conventional outpatient treatment for major depression.	A randomized, double-blind, placebo-controlled, four-month trial.	83 men and women with major depression consumed 8 × 1 g capsules per day of placebo (olive oil) or south pacific tuna oil (2.2 g DHA + 0.6 g EPA) for 16 weeks.	18-75	3.6:1	Despite large reductions in depression, there were no significant differences at any assessment time point between patients receiving fish oil compared with placebo.	No effect Nu-Mega Ingredients (Clover), Australia
(Rees et al., 2008) Australian and New Zealand Journal of Psychiatry	To test whether n-3 fatty acid treatment is superior to a placebo in the treatment of perinatal depression.	A double-blind, randomized, placebo-controlled trial.	26 females with major depression during the perinatal period received either fish oil (1.64 g DHA + 0.41 g EPA) or a placebo (Sunola oil) for 6 weeks.	Fish oil: 31 (mean) Placebo: 34 (mean)	3.9:1	There was no benefit for n-3 fatty acid supplementation over the placebo in treating perinatal depression.	No effect Nu-Mega Ingredients (Clover), Australia
(Sinn et al., 2012) British Journal of Nutrition	To investigate the benefits of supplementing a diet with n-3 PUFA, DHA and EPA, for depressive symptoms, quality of life and cognition in elderly people with mild cognitive impairment.	A double-blind, randomized, controlled trial.	50 male and female, elderly people with mild cognitive impairment were randomly allocated to EPA-rich fish oil (0.16 g DHA + 1.67 g EPA), DHA-rich fish oil (1.55 g DHA + 0.40 g EPA), or safflower oil (placebo) for 6 months.	>65	3.9:1	The results indicate that high DHA and high EPA fish oils exert variable effects on cognitive and physical outcomes and may be effective in treating depressive symptoms and health parameters.	+ Not specified
(Silvers et al., 2005) Prostaglandins, Leukotrienes and Essential Fatty acids	To determine the effect of adding fish oil to existing therapies in participants who were being treated for depression in a community setting.	A randomized, double-blind, placebo-controlled trial.	77 male and female participants were randomly assigned to receive 8 g of either fish oil (2.4 g DHA + 0.6 g EPA) or olive oil (placebo) per day, in addition to their existing therapy, for 12 weeks.	18-65	4.0:1	Despite an increase in circulating n-3 polyunsaturated fatty acids, there was no evidence that fish oil improved mood when compared to the placebo oil.	No effect Nu-Mega Ingredients (Clover), Australia
(Jackson et al., 2012c) Biological Psychology	To investigate the effects of supplementation with high DHA fish oil (1 g or 2 g) or placebo on cerebral blood flow in healthy adult non-consumers of oily fish.	A double-blind, placebo-controlled study.	65 healthy men and women were randomly assigned to receive high DHA fish oil at a dose of either 1 g/d (0.45 g DHA + 0.09 g EPA) or 2 g/d (0.90 g DHA + 0.18 g EPA), or placebo (olive oil) for 12 weeks.	18-29	5.0:1	Supplementation with both doses of fish oil, in comparison with placebo, resulted in significantly increased concentrations of oxyhemoglobin and total levels of hemoglobin, indicative of increased cerebral blood flow, during the cognitive tasks.	+ Not specified

*Personal communication; the DHA: EPA ratio was not specified in the published paper, but was provided by authors of the study upon request.

Psychological (perceived stress and depression) measurements were performed in eleven studies (Barbadoro et al. 2013; Bradbury et al. 2004; Clayton et al. 2009; Freund-Levi et al. 2008; Grenyer et al. 2007; Hirayama et al. 2004; Makrides et al. 2010; Mozurkewich et al. 2013; Rees et al. 2008; Rogers et al. 2008; Silvers et al. 2005); cognitive function was measured in eleven studies (Andrieu et al. 2017; Dangour et al. 2010; Hurtado et al. 2015; Jackson et al. 2012a; Jackson et al. 2012c; Lee et al. 2013; Phillips et al. 2015; Rogers et al. 2008; Sinn et al. 2012; Stonehouse et al. 2013; Stough et al. 2012); neurodevelopment was measured in five studies (Makrides et al. 2009; Makrides et al. 2010; Meldrum et al. 2012; van Goor et al. 2010); visual function was measured in five studies (Hurtado et al. 2015; Lauritzen et al. 2004; Smithers et al. 2008; Souied et al. 2013; Stough et al. 2012); behavioural outcome was measured in two studies (Hamazaki et al. 2008; Itomura et al. 2005); and physiological function of brain was measured in one study (Jackson et al. 2012b).

The effect of DHA on attention-deficit/hyperactivity disorder (AD/HD) symptoms was studied by Hirayama et al. (2004), and findings of this study suggest that DHA supplementation does not improve AD/HD-related symptoms. Itomura et al. (2005) found that physical aggression in girls increased significantly in the control group but did not change in the high DHA fish oil group; whereas there were no significant changes in physical aggression in boys. Freund-Levi et al. (2008), studying AD patients and psychiatric and behavioral symptoms, reported that, overall, supplementation had no significant effects on neuropsychiatric symptoms or on daily activities. Hamazaki et al. (2008) reported that the concentrations of DHA and EPA in the phospholipid fraction in red blood cells were significantly increased in the DHA group compared to the control. Although there were no differences in behaviour between groups, children in the DHA group had a significantly higher school attendance rate. van Goor et al. (2010) reported that neither DHA alone, nor DHA + arachidonic acid (20:4n-6) during pregnancy and lactation, influenced outcomes in the traditional examination of infant's brain development. General movement quality of infants in the DHA group was lower than that of infants in the other two groups. The authors concluded that general movement quality at 12 weeks postpartum is sensitive to the maternal dietary DHA/ arachidonic acid balance. Makrides et al. (2010) ran a trial (DHA to Optimize Mother Infant Outcome [DOMInO] trial) to determine whether increasing DHA during the last half of pregnancy ameliorates maternal depression and enhances the neurodevelopment of their children. The authors found that use of high DHA fish oil did not affect the levels of postpartum depression in mothers, nor modify the cognitive and language development in their children. The effect of n-3 LC-PUFA on cognitive function in elderly people was tested and no change in cognitive function scores over the 24-month period was observed in the high DHA fish oil group compared to control (Dangour et al. 2010). Jackson et al. (2012a) suggested that supplementation with n-3 LC-PUFA, high DHA and high EPA fish oils, in healthy, normally developing, and impairment-free populations, is unlikely to result in cognitive enhancement. In another study conducted by Jackson et al. (2012b) it was reported that supplementation with high DHA fish oil, in comparison with the placebo and

high EPA fish oil supplement (which did not result in differences compared to the placebo), significantly increased the concentrations of oxygenated-haemoglobin and total levels of haemoglobin, indicating an increase in the cerebral blood flow during the cognitive tasks. No significant effects on cognitive function following DHA supplementation compared to the control were observed (Stough et al. 2012); but for participants with corrected vision, the group receiving DHA were found to have significantly better right eye visual acuity post-supplementation with DHA in comparison to the control. Meldrum et al. (2012) found no significant difference in infant neurodevelopment between the two tested groups (DHA or control), but some indication of benefits to early communicative development was observed. Barbadoro et al. (2013) showed that an elevated DHA intake reduced distress symptoms and basal cortisol secretion in abstinent alcoholics. Hurtado et al. (2015) found that there was no significant difference in the visual and cognitive/psychomotor development in the newborns between treatment groups (high DHA fish oil vs control). Bradbury et al. (2004) reported no significant difference in perceived stress between the high DHA fish oil group and the placebo group. Smithers et al. (2008) assessed the effects of a high DHA dose on the visual acuity of preterm infants. At 4-months corrected age, higher visual acuity was found in the high-DHA group compared to the control group, and authors suggested that the DHA requirement of preterm infants might be higher than currently provided. Makrides et al. (2009) ran the Docosahexaenoic acid for the Improvement of Neurodevelopmental Outcome in pre-term infants (DINO) trial. At 18 months' corrected age, the mental development index (MDI) among girls fed the high DHA diet was higher than girls fed the standard DHA in both unadjusted and adjusted analyses. The MDI among boys did not differ between groups. Clayton et al. (2009) showed that clinician ratings of mania and depression in children and adolescents with juvenile bipolar disorder were significantly lower, and global functioning significantly higher, after high DHA fish oil supplementation. Parent ratings of internalizing and externalizing behaviours were also significantly lower following supplementation. Lee et al. (2013) reported the results of a trial in elderly subjects with mild cognitive impairment. Individuals in the high DHA fish oil group displayed a significant improvement in short-term and working memory, immediate verbal memory and delayed recall capability. Mozurkewich et al. (2013) suggested that neither high DHA nor high EPA fish oil supplementation during pregnancy prevents depressive symptoms during pregnancy or postpartum. Souied et al. (2013) studied the effects of high DHA fish oil on preventing age-related macular degeneration over a period of 3 years. No significant differences were found in the time to occurrence, and the incidence of choroidal neovascularization, between the DHA and placebo groups. The effect of DHA on cognitive performance in healthy young adults was studied (Stonehouse et al. 2013), and findings of this study suggest that DHA supplementation improves memory and memory reaction time in healthy young adults whose habitual diets were low in DHA. Phillips et al. (2015) found that even though omega-3 fatty acid supplementation elevates plasma concentrations of DHA and EPA, this did not result in any measurable benefits on cognition and mood in cognitively impaired

subjects with dementia or AD. The effect of DHA supplementation and multidomain intervention (physical activity, cognitive training, and nutritional advice), alone or in combination, on cognitive decline in elderly adults with memory complaints was studied (Andrieu et al. 2017). No significant difference in 3-year cognitive decline between any of the intervention groups and the placebo group was recorded. Rogers et al. (2008) reported no beneficial, nor harmful, effects of increasing DHA + EPA intake on mood in mild to moderately depressed individuals. The effect of maternal supplementation with high DHA fish oil on the visual acuity of infants was studied by Lauritzen et al. (2004). Infant visual acuity was not significantly different between the high DHA supplementation and control, but was positively associated with infant RBC-DHA at 4 months; suggesting that n-3 LCPUFA may influence visual maturation. Grenyer et al. (2007) showed that, in outpatients with major depression, despite a large numerical reduction in depression following both treatments, no statistically significant differences between patients receiving fish oil compared with placebo could be measured at any assessment time point. No significant difference in depression scores between females with major depression during the perinatal period receiving high DHA fish oil or those receiving the sunola oil was recorded (Rees et al. 2008). The beneficial effects of supplementing a diet with n-3 LC-PUFA on enhancing depressive symptoms, quality of life and cognition in elderly people with a mild cognitive impairment were investigated in a double-blind, randomized, controlled trial (Sinn et al. 2012). Findings of this study indicate that DHA may be equally, if not more, effective than EPA for improving mood. Depressive symptom scores were significantly reduced after 6 months following high EPA and high DHA supplementation compared to the control. As for the cognitive outcomes, significant improvements in initial letter fluency a, test of fluid thinking ability, were only detected in the DHA group. Silvers et al. (2005) investigated the effect of fish oil supplementation in participants being treated for depression. Though circulating n-3 LC-PUFA increased resulting from high DHA fish oil supplementation, there was no evidence that high DHA fish oil improved mood when compared with the placebo. Jackson et al. (2012c) examined the effect of supplementation with different doses of high DHA fish oil on cerebral blood flow in healthy adult non-consumers of oily fish over a 12-week period. Supplementation with fish oil at both dosages, in comparison with the placebo, resulted in a significant increase in the concentrations of oxyhemoglobin and total hemoglobin, which is indicative of increased cerebral blood flow during cognitive tasks. However, these changes in hemodynamic response to tasks were not accompanied by consistent changes in cognitive performance.

3.1.3. Other medical aspects

Details of the 23 studies reporting findings on the effect of high DHA fish oil administration on immunity, allergy, growth (body mass) and other medical aspects that were included in this review are summarised in Table 3.

All of these studies were long-term, intervention trials, with a duration of 14 days to 18 months. The number of participants per group varied from 8 to 1202. Majority (67%) of these trials were performed with male and female participants, while 24%

were performed with males only. In addition, two trials were conducted with females only, and one trial did not specify the gender of participants. A total of 56% of the studies used healthy individuals and 44% were either disabled, overweight, obese, preterm, osteoporotic, or suffering from burns, AD, type 2 diabetes, or multiple sclerosis.

The doses of EPA plus DHA administered to subjects were < 1.0 g/day in two trials (Sabour et al. 2012; Zhou et al. 2012), 1.0–1.9 g/day in nine trials (Atwell et al. 2013; Damsgaard et al. 2012; Helland et al. 2001; Hill et al. 2007b; Manley et al. 2011; Mansoori et al. 2016; Marques et al. 2015; Santos et al. 2013; Toupchian et al. 2016), 2.0–2.9 g/day in seven trials (Freund-Levi et al. 2014; Gorjão et al. 2006; Munro and Garg 2012 2013a, b; Ramirez-Ramirez et al. 2013; Vedin et al. 2008), and 3–6 g/day in two trials (Kew et al. 2004; Tihista and Echavarría 2017). Few studies used more than one dose: Kew et al. (2003) used two dosages (0.77 g and 1.79 g EPA + DHA/day), Wallace et al. (2003) and Gunnarsdóttir et al. (2008) used three dosages (0.44, 0.94 and 1.9 g EPA + DHA/day; and 0.26, 1.06 and 2.14 g EPA + DHA/day, respectively). DHA: EPA ratio in these trials were from approximately 1.48: 1 (Helland et al. 2001) to 8: 1 (Zhou et al. 2012).

Cytokines were measured in eleven trials (Freund-Levi et al. 2014; Gorjão et al. 2006; Hill et al. 2007b; Kew et al. 2003; Kew et al. 2004; Marques et al. 2015; Ramirez-Ramirez et al. 2013; Sabour et al. 2012; Santos et al. 2013; Vedin et al. 2008; Wallace et al. 2003); peripheral blood mononuclear cell functions (phagocytic activity, lymphocyte proliferation) were measured in seven trials (Gorjão et al. 2006; Hill et al. 2007b; Kew et al. 2003; Kew et al. 2004; Marques et al. 2015; Santos et al. 2013; Wallace et al. 2003); the marker of monocyte/macrophage activation factor was measured in one trial (Toupchian et al. 2016); allergy-related events, such as the incidence of bronchopulmonary dysplasia (Manley et al. 2011) and the proportion of children hospitalised for lower respiratory tract conditions (Atwell et al. 2013), were measured in two trials; bone mass and markers of bone formation were measured in one trial (Damsgaard et al. 2012); the incidence of gestational diabetes mellitus and preeclampsia was measured in one trial (Zhou et al. 2012); weight and fat mass were measured in six trials (Gunnarsdóttir et al. 2008; Helland et al. 2001; Mansoori et al. 2016; Munro and Garg 2012 2013a, b); and infectious complications were measured in one trial (Tihista and Echavarría 2017).

Hill et al. (2007b) reported that, while there was no effect on cytokine production by T cells and monocytes following supplementation with high DHA fish oil or exercise, supplementation with high DHA fish oil reduced superoxide production from stimulated neutrophils, while regular moderate exercise training protected bactericidal activity. Additionally, neutrophil chemotaxis and adherence were not significantly affected by exercise, oil, or the combination of the two. Marques et al. (2015) suggested that high DHA fish oil could reduce inflammatory disturbance and neutrophil death induced by acute exercise in wheelchair athletes. Munro and Garg (2013a) found significant correlations between leptin and weight loss, and leptin and plasma EPA and DHA levels, in high DHA fish oil group. The authors reported also that there were no significant effects on weight reduction, fat mass reduction and inflammatory biomarkers between high DHA fish oil treatment and

Table 3. A summary of experimental design and results from human studies testing the effects of high DHA fish oil on other medical aspects.

Reference & Journal	Aim of study	Area of study	Type of study	Number of participants / Test formulation	Age range (years)	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Hill et al., 2007b) British Journal of Nutrition	To test the combined effect of exercise and n-3 PUFA on immune function, particularly in patients with risk factors for CVD.	Immunity	A randomized, placebo-controlled study.	50 volunteers randomly consumed 6 × 1 g capsules of either sunflower oil (placebo) or high DHA fish oil (1.56 g DHA + 0.36 g EPA) per day for 12 weeks.	25-65	4.3:1	There was no effect of high DHA fish oil on cytokine production by T cells and monocytes. Superoxide anion production from stimulated blood neutrophils was decreased by fish oil but not by exercise, and this change was negatively correlated with the incorporation of DHA into erythrocytes.	No effect	Nu-Mega Ingredients (Clover), Australia
(Marques et al., 2015) Applied Physiology Nutrition and Metabolism	To investigate the effects of DHA-rich fish oil supplementation on the lipid profile, levels of plasma inflammatory mediators, markers of muscle damage, and neutrophil function in wheelchair basketball players before and after acute exercise.	Immunity	Before-after study in the same patient.	8 male wheelchair basketball athletes with paraplegia received 3 g/d fish oil (1.50 g DHA + 0.30 g EPA) for 30 days.	33.8 (mean)	5.0:1	High DHA fish oil supplementation reduces the markers of muscle damage, inflammatory disturbances, and neutrophil death induced by acute exercise in wheelchair athletes.	+	DHA OMEGA, Naturalis, Brazil
(Munro and Garg, 2013a) Obesity Research & Clinical Practice	To test whether n-3 LC-PUFA, combined with a reduced energy diet, facilitated weight loss and improvements in blood lipids and inflammatory mediators.	Growth / Body mass	A double-blind, randomized, controlled trial with two parallel groups.	35 obese male and female subjects received 6 × 1 g capsules per day of either sunola oil (placebo) or fish oil (1.62 g DHA + 0.42 g EPA/day) for 12 weeks.	18-60	3.9:1	There was no significant difference within and between the placebo and the fish oil groups for weight reduction, fat mass reduction or changes in inflammatory biomarkers. Blood lipids, apart from TAGs, reduced by 27% in the fish oil group. There were significant correlations between leptin and weight loss, and leptin and EPA and DHA in the fish oil group.	No effect +	Nu-Mega Ingredients (Clover), Australia
(Gunnarsdottir et al., 2008) International journal of obesity	To assess the effects of fish (lean or oily) and fish oil consumption on blood lipid concentration during weight loss.	Weight loss / Body mass	Randomized, controlled 8-week trial.	324 men and women were randomized to 4 treatment regimens for 8 weeks: sunflower oil capsules, cod diet (3 × 150 g/week, including 0.21 g DHA + 0.05 g EPA/day), salmon diet (3 × 150 g/week including 1.37 g DHA + 0.77 g EPA/day), fish oil capsules (0.43 g DHA + 0.63 g EPA/day).	20-40	Cod diet: 3.8:1 Salmon: 1.8:1 Fish oil capsules: 0.6:1	Weight-loss diets, including oily fish with high DHA, resulted in greater TAG reduction than a diet without fish or fish oil. HDL cholesterol tended to decrease less in salmon and fish oil groups.	+	Lipid Nutrition, The Netherlands
(Sabour et al., 2012) Journal of Research in Medical Sciences	To evaluate the possible effect of n-3 PUFAs on proinflammatory cytokine bone change in chronic spinal cord injured patients.	Immunity	A double-blind, placebo-controlled trial.	82 osteoporotic men and women with a spinal cord injury were randomized to two capsules of placebo oil or high DHA fish oil (435 mg of DHA and 65 mg of EPA/day) for 4 months.	38-40 (mean)	6.7:1	High DHA fish oil supplementation for 4 months had no significant effect on inflammatory markers.	No effect	Minami Nutrition Co. Belgium
(Zhou et al., 2012) American Journal of Clinical Nutrition	To determine whether n-3 LC-PUFA supplementation in pregnancy reduces the incidence of gestational diabetes mellitus (GDM) or preeclampsia.	Gestational diabetes	A double-blind, multicenter, randomized control.	2399 pregnant women at < 20 weeks' gestation were randomized to vegetable oil without DHA (placebo) or high DHA fish oil (0.80 g DHA + 0.10 g EPA/day) from trial entry to birth.	29 (mean)	8.0:1	DHA supplementation of 800 mg/day in the second half of pregnancy does not reduce the risk of GDM or preeclampsia.	No effect	Incromega 500 TG; Groda Chemicals, England

(Manley et al., 2011) Pediatrics	To report the effect of DHA supplementation on long-term atopic and respiratory outcomes in preterm infants. (The Infant Fish Oil Supplementation Study (IFOS)).	Asthma / Respiratory and allergy	A multicentre, randomized, controlled trial.	657 preterm infants (< 33 weeks' gestation) consumed expressed breast milk from mothers who took six 500-mg capsules of either high DHA tuna oil or high DHA concentration that was 1% of total fatty acids) or placebo soy oil capsules for 18 months.	Pre-term infants	4.5: 1 *	DHA supplementation for infants of <33 weeks' gestation reduced the incidence of bronchopulmonary dysplasia in boys and in all infants with a birth weight of <1250 g and reduced the incidence of reported hay fever in boys at either 12 or 18 months. However, the hospitalisation rate for lower respiratory tract issues was not reduced compared with the control.	No effect	Nu-Mega Ingredients (Clover), Australia
(Santos et al., 2013) Journal of Sports Science and Medicine	To investigate the effects of DHA-rich fish oil supplementation on lymphocyte function before and after a marathon race.	Immunity	A double-blind, randomized, placebo-controlled trial.	21 male athletes were randomized to vegetable oil capsules or 3 g/d fish oil (1.5 g DHA + 0.3 g EPA) for 60 days.	37±2	5.0: 1	High DHA fish oil supplementation has beneficial effects in preventing some of the changes in lymphocyte function induced by marathon participation.	+	Omega DHA, Naturalis, Brazil
(Toupchian et al., 2016) Journal of Clinical Lipidology	To investigate the effects of DHA-enriched fish oil on the marker of monocyte/macrophage activation factor soluble CD163, asymmetric dimethyl arginine (ADMA), and insulin resistance in type 2 diabetic patients.	Immunity	A double-blind, randomized controlled trial.	72 type 2 diabetic men and women were randomized to placebo oil or high DHA fish oil (1.45 g DHA + 0.40 g EPA/day) for 8 weeks.	56 (average)	3.6: 1	Short-time supplementation with DHA-enriched fish oil decreased sCD163 levels without significant differences in ADMA.	+	Pure encapsulations, USA
(Mansoori et al., 2016) Journal of Nutrigenetics and Nutrigenomics	To investigate (1) the impact of high DHA fish oil supplementation on body composition, plasma adiponectin level, and peroxisome proliferator-activated receptor γ (PPAR γ) gene expression, and (2) whether the effect of high DHA fish oil supplementation on the variables is modulated by PPAR γ Pro12Ala polymorphism.	Body mass	A randomized, double-blind placebo-controlled trial.	72 patients with type 2 diabetes were randomized to 2.4 g/d placebo oil (paraffin oil) or fish oil (1.45 g DHA + 0.4 g EPA) for 8 weeks. Gender was not specified.	55 (mean)	3.6: 1	High DHA fish oil supplementation favorably modulated body composition in patients with T2DM and could be useful to reduce visceral obesity. Weight, body mass index, fat-free mass, adiponectin level, and PPAR γ gene expression changes showed no significant difference between treatments.	+	Pure Encapsulation Co, USA
(Atwell et al., 2013) Journal of Paediatrics and Child Health	To test the effect of supplemental enteral DHA in high dose (1% DHA) versus a standard dose (0.3% DHA) on the longer term respiratory health of prematurely born babies.	Asthma / Respiratory	A multicentre, randomized, controlled trial.	657 mothers received either a high-DHA diet to achieve a breast milk DHA concentration that was 1% of total fatty acids, or a standard-DHA diet (0.3% of total fatty acids) for 18 months.	Pre-term infants	4.5: 1 *	Hospitalisation for lower respiratory tract problems in the first 18 months for preterm infants was not reduced by neonatal supplementation with 1% DHA compared with 0.3% DHA.	No effect	Nu-Mega Ingredients (Clover), Australia
(Damsgaard et al., 2012) Pediatric Research	To investigate whether bone mass, and markers of bone formation and growth, were associated with DHA status and affected by fish oil supplementation in adolescent boys.	Bone	A randomized trial.	78 healthy, slightly overweight boys received breads with high DHA fish oil (0.89 g DHA + 0.19 g EPA/day) or a control for 16 weeks.	13-15	4.7: 1	Fish oil strongly increased DHA status. No associations were found between DHA status and bone mineral content, bone area, bone mineral density or the markers of bone formation and growth at baseline. There was a positive association between changes in DHA status and plasma insulin-like growth factor-1 (IGF-1) during intervention.	No effect	Nu-Mega Ingredients (Clover), Australia

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Table 3. (Continued)

Reference & Journal	Aim of study	Area of study	Type of study	Number of participants / Test formulation	Age range (years)	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Holland et al., 2001) Pediatrics	To investigate whether dietary supplementation of n-3 PUFAs to pregnant and lactating mothers would affect gestational length, birth weight, and the biochemical status of the neonates.	Gestational length, birth weight	A double-blind, randomized trial.	590 pregnant women were randomized to receive 10 mL of cod liver oil (1.18 g DHA + 0.80 g EPA) or corn oil (placebo) from 18 weeks' gestation until 3 months after delivery.	Infants: 3, 6, 9, 12 months	1.5:1	Neither harmful nor beneficial effects of maternal supplementation of n-3 PUFAs was found regarding pregnancy outcome, cognitive development, or growth, as compared with supplementation with n-6 fatty acids.	No effect	Peter Moller, Norway
(Gorjão et al., 2006) Clinical Nutrition	To investigate the effect of high DHA fish oil supplementation on human leukocyte function.	Immunity	Before-after study in the same patient.	10 male subjects received 3 g/d fish oil (1.62 g DHA + 0.78 g EPA) for 2 months.	25-45	2.1:1	High DHA fish oil increased the phagocytic activity in neutrophils and monocytes, neutrophil chemotactic, and the rate of production of reactive oxygen species by neutrophils.	+	Naturalis Alimentos Naturais Ltda (Sao Paulo, Brazil)
(Vedin et al., 2008) American Journal of Clinical Nutrition	To determine the effects of dietary supplementation with an n-3 fatty acid preparation rich in DHA on release of cytokines and growth factors from peripheral blood mononuclear cells (PBMCs).	Immunity	A randomized, double-blind, placebo-controlled trial.	204 men and women with Alzheimer disease (AD) were randomized to placebo or fish oil (1.7 g DHA + 0.6 g EPA/day) for 6 months.	73 (mean)	2.8:1	AD patients treated with high DHA supplementation increased their plasma concentrations of DHA (and EPA), which were associated with reduced release of IL-1 β , IL-6, and granulocyte colony-stimulating factor from PBMCs.	+	EPAX 1050TG; Pronova Biocare A/S, Lysaker, Norway
(Ramirez-Ramirez et al., 2013) Oxidative Medicine and Cellular Longevity	To evaluate the efficacy of fish oil supplementation on serum proinflammatory cytokine levels, oxidative stress markers, and disease progression in multiple sclerosis (MS).	Immunity	A double-blind, randomized, placebo-controlled trial.	50 men and women with relapsing-remitting MS were randomized to placebo or 4 g/d fish oil (1.6 g DHA + 0.8 g EPA) for 12 months.	18-55	2.0:1	Fish oil supplementation is highly effective in reducing the levels of cytokines and nitric oxide catabolites in patients with relapsing-remitting MS.	+	Omega Rx capsules, USA
(Freund-Levi et al., 2014) Journal of Alzheimer's Disease	To evaluate systemic oxidative stress and inflammatory biomarkers following oral supplementation of dietary n-3 fatty acids.	Immunity	A randomized, double-blind placebo-controlled trial.	40 men and women with moderate Alzheimer's disease (AD) were randomized to placebo or an isocaloric oil containing 1.7 g DHA + 0.6 g EPA, for 6 months.	70 (mean)	2.8:1	Supplementation of n-3 fatty acids in patients with AD for 6 months does not have a clear effect on free radical-mediated formation of F ₂ -isoprostane or cyclooxygenase-mediated formation of prostaglandin F ₂ alpha.	No effect	EPAX1050 TG, Pronova Biocare A/S, Norway
(Munro and Garg, 2012) British Journal of Nutrition	To test whether n-3 LC-PUFA, combined with a very-low-energy diet, facilitated weight loss, weight maintenance, and improvements in blood lipids and inflammatory mediators.	Growth / Body mass	A double-blind, randomized, parallel-controlled trial.	32 obese male and female subjects received 6 \times 1 g capsules per day of high DHA fish oil (1.62 g DHA + 0.42 g EPA) or sunola oil (placebo) for 14 weeks. Both groups were on a very low energy diet for 4 weeks, which was then followed by 10 weeks of weight maintenance.	18-60	3.9:1	There was a significant reduction in fat mass for the fish oil group at week 14 but not for the placebo group. However, supplementation with n-3 LC-PUFA had no significant effect on weight loss or weight maintenance over the 14 weeks.	+	Nu-Mega Ingredients (C/lover), Australia



(Munro and Garg, 2013b) Food & Function	To test whether supplementation with n-3 LC-PUFA alone, compared to being consumed concomitantly with a very low energy diet, facilitated weight loss, improvements in blood lipids and positive changes to inflammatory mediators.	A double-blind, randomized, controlled trial, with two parallel groups.	39 obese male and female subjects received 6 × 1 g capsules per day of either sunola oil (placebo) or fish oil (1.62 g DHA + 0.42 g EPA/day) for 14 weeks. Both groups were on a very low energy diet for 4 weeks, which was then followed by 10 weeks of weight maintenance.	18-60	3.9:1	At 4 weeks, levels of EPA and DHA increased two-fold in the fish oil group, with no significant changes to anthropometric measurements for either group. At 8 weeks, a significant 3-way interaction between time, group and gender was observed for percentage reduction in weight and BMI, with a greater percentage decrease for females in fish oil group compared with placebo for weight and BMI. Supplementation with n-3 LC-PUFA had a time dependent effect on weight loss in females.	+ Nu-Mega Ingredients (Clover), Australia
(Kew et al., 2004) American Journal of Clinical Nutrition	To determine the effects of supplementation with an EPA- or DHA-rich oil on a range of immune outcomes representing key functions of human neutrophils, monocytes, and lymphocytes in healthy humans.	A placebo-controlled, double-blind, parallel study.	42 healthy men and women were randomized to olive oil, EPA-rich fish oil (0.73 g DHA + 4.75 g EPA) or DHA-rich fish oil (4.91 g DHA + 0.85 g EPA) for 4 weeks.	23-65	5.8:1	Supplementation with DHA, but not with EPA, suppresses Tlymphocyte activation, as assessed by expression of CD69. EPA alone does not, therefore, influence CD69 expression. No other marker of immune function assessed in this study was significantly affected by either EPA or DHA.	+ Not specified
(Tihista and Echavarría, 2017) Clinical Nutrition	To investigate the effect, particularly on infectious Complications, of enteral nutrition enriched with n-3 PUFAs in burn patient	A prospective, randomized double-blind Trial.	106 male and female burn patients admitted to intensive care randomly received either a standard low-fat diet or a low-fat diet in which 50% of the fat was replaced by fish oil, for 14 days.	40 (approx.)	2.5:1	The inclusion of n-3 PUFAs in a low-fat diet in ICU burned patients was associated with significant clinical benefits compared with a conventional low-fat diet, with lower rates of severe sepsis, septic shock and pyloric dysfunction.	+ VIPEZ of Landasur Laboratory, Uruguay
(Kew et al., 2003) American Journal of Clinical Nutrition	To determine the effects of enriching the diet with ALA or EPA plus DHA on immune outcomes representing key functions of human neutrophils, monocytes, and lymphocytes.	A placebo-controlled, double-blind, parallel study.	150 moderately hyperlipidemic, but otherwise healthy men and women were randomized to control oil, 0.28 g DHA + 0.18 g EPA/day, 0.76 g DHA + 0.49 g EPA/day, 3.7 g ALA/day, or 8.7 g ALA/day for 6 months.	25-72	1.5:1	An intake of ≤ 9.5 g ALA/day or ≤ 1.7 g EPA and DHA/day does not alter the functional activity of neutrophils, monocytes, or lymphocytes, but it changes the fatty acid composition of mononuclear cells.	+ No effect Roche Vitamins Ltd, Basel
(Wallace et al., 2003) British Journal of Nutrition	To determine the effect of enriching the diet with different doses of fish oil or with a modest dose of ALNA on a range of functional responses of human monocytes and lymphocytes.	A randomized, placebo-controlled, double-blind, parallel study.	40 healthy males were randomized to control oil, 3.5 g/d ALNA, 0.44 g/d DHA + EPA, 0.94 g/d DHA + EPA, or 1.9 g/d DHA + EPA, for 12 weeks	18-39	1.9:1 2.5:1 2.8:1	Except for IL-6 production, a modest increase in intake of either ALNA or EPA plus DHA does not influence the functional activity of mononuclear cells. The threshold of EPA and DHA intake that results in decreased IL-6 production is between 0.44 and 0.94 g/d.	+ No effect Not specified

*Personal communication; the DHA: EPA ratio was not specified in the published paper, but was provided by authors of the study upon request.

placebo. Gunnarsdottir et al. (2008) conducted a trial to determine the effects of fish (lean or oily) and fish oil consumption on blood lipid concentration during weight loss, and tested four treatments: sunflower oil capsules, high EPA fish oil capsules, a cod diet (lean fish, high EPA) or a salmon diet (oily fish, high DHA). Overall, the reduction in total cholesterol was greater in the fish groups (cod and salmon) compared to the control group, however when adjusting for weight loss, this result is of borderline significance. The overall decrease in HDL was greater in the control group compared to the fish and fish oil groups. In addition, there were significant correlations between weight-loss diet and the reduction of TG following the consumption of high DHA oily fish, compared to groups receiving either high EPA fish oil or sunflower oil capsules. The effects of n-3 LC-PUFA on inflammatory cytokines were studied by Sabour et al. (2012) in osteoporotic patients, who showed that high DHA fish oil supplementation for 4 months had no significant effect on inflammatory markers, and in a trial aimed to determine the effect of DHA supplementation during pregnancy on the incidence of gestational diabetes mellitus or preeclampsia (Zhou et al. 2012). The overall incidence of gestational diabetes mellitus and preeclampsia did not differ significantly between the high DHA fish oil and the placebo groups. The effect of DHA supplementation on long-term atopic respiratory outcomes in preterm infants was reported (Manley et al. 2011). A significant reduction in bronchopulmonary dysplasia was observed in the high DHA group, compared to the placebo, at either 12 or 18 month's in infants with a birth weight of < 1250 g. However, as reported in a following study, the hospitalisation rate for lower respiratory tract conditions was not reduced compared with the control (Atwell et al. 2013). Santos et al. (2013) investigated the effects of high DHA fish oil supplementation on lymphocyte function in male athletes before and after a marathon race. High DHA fish oil supplementation increased lymphocyte proliferation and prevented the reduction of cytokine production, suggesting it may be beneficial in preventing some of the changes in lymphocyte function following marathon participation. Toupchian et al. (2016) reported that, when compared with the placebo group, serum level of Scd163, the marker of monocyte/macrophage activation factor, decreased significantly in the high DHA fish oil group in type 2 diabetes patients. Mansoori et al. (2016) reported that supplementation with high DHA fish oil, compared to the placebo, decreased waist circumference, body fat mass, body fat percent and visceral fat rating, as well as trunk fat mass, however weight, body mass index, fat-free mass, adiponectin level, and PPAR γ gene expression changes did not differ significantly between treatment groups. Damsgaard et al. (2012) showed that although high DHA fish oil increased DHA status, no associations were found between high DHA fish oil supplementation and bone mineral content, bone area, bone mineral density, and the markers of bone formation. Helland et al. (2001) found no difference in gestational length, birth weight, or cognitive function scores, between the high DHA cod liver oil group and the corn oil group in healthy, pregnant women. Gorjão et al. (2006) found that supplementation with high DHA fish oil increased the phagocytic activity in neutrophils and monocytes, neutrophil chemotactic, and the rate of production of reactive oxygen species by neutrophils.

Vedin et al. (2008) reported a significant decrease of IL-6, IL-1 β , and granulocyte colony-stimulating factor, was observed in AD patients treated with high DHA supplementation. In a trial evaluating the efficacy of high DHA fish oil supplementation on serum proinflammatory cytokine levels (Ramirez-Ramirez et al. 2013), high DHA fish oil treatment decreased the serum levels of TNF- α , IL-1 β , and IL-6 compared to the placebo oil treatment. Freund-Levi et al. (2014) reported that supplementation of DHA in AD patients does not affect inflammatory response. Munro and Garg (2012) reported a significant reduction in fat mass for the high DHA fish oil group after 14 weeks intervention in obese male and female subjects, but not for the control sunola oil group, and while the metabolic profiles of both treatment groups improved, overall there was no significant effect on weight loss or weight maintenance over the 14 weeks. Successively, Munro and Garg (2013b) reported that obese females consuming high DHA fish oil over sunola oil exhibited a greater percentage decrease in weight (-7.21% vs -5.82%) and BMI (-7.43% vs -5.91%), respectively, but not in males. The authors suggested that supplementation with high DHA fish oil had a time dependent effect on weight loss in females. Kew et al. (2004) found that supplementation with DHA, but not EPA, suppressed T lymphocyte activation in healthy subjects. Neither a high DHA or a high EPA fish oil had any significant effect on monocyte or neutrophil phagocytosis, cytokine production, or adhesion molecule expression by peripheral blood mononuclear cells. Tihista and Echavarria (2017) performed a trial to investigate the effect of n-3 LC-PUFA on infectious complications in burn patients. Compared to burns patients receiving the conventional low-fat diet, the inclusion of high DHA fish oil in a low-fat diet was of clinical benefit, where a decrease in the rate of severe sepsis, septic shock and pyloric dysfunction was observed. Kew et al. (2003) showed that intakes of plant (ALA)- or marine- derived n-3 LC-PUFA (high DHA), up to 9.5 or 1.7 g/day, respectively, did not influence circulating inflammatory and immune cell numbers compared to the placebo. Eventually, a study determined the effect of enriching the diet with different doses of high DHA fish oil (Wallace et al. 2003). Compared to the control, interventions did not influence the functional activity of mononuclear cells; but the two higher doses of fish oils resulted in a significant decrease in IL-6 production by stimulated mononuclear cells.

3.2. Follow-up studies

A number of follow-up studies were initially retrieved, but were later excluded from this review paper as they did not meet the inclusion criteria (Ayer et al. 2009; Skilton et al. 2012; Skilton et al. 2014). Details and characteristics of the 10 high DHA follow-up trials that were considered for analysis are shown in Table 4. All studies were follow-up, long-term intervention trials, where subjects of an initial intervention trial were followed for a period of time ranging from 5 months to 12 years.

In a follow-up study from the DINO trial (Makrides et al. 2009), Smithers et al. (2010) evaluated the long-term effects of feeding preterm infant's with milk containing high levels of DHA on their language, behaviour and temperament at

Table 4. A summary table for follow-up studies testing the effects of high DHA fish oil in humans.

Reference & Journal	Aim of study	Area of study	Type of study	Number of participants / Test formulation	Age range (years)	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Smithers et al., 2010) The American Journal of Clinical Nutrition	To test whether feeding preterm infants milk with a higher DHA content than that used in current practice influences language or behavior in early childhood.	Brain	A follow-up study in a subgroup of infants enrolled in DINO (a double-blind, randomized, controlled trial).	Female participants received either tuna fish oil capsules (0.90 g DHA + 0.20 g EPA) which increased breast-milk DHA concentrations to 1% of total fatty acids, or soy oil capsules (no DHA) which does not alter the DHA content of breast milk; 0.2–0.3% total fatty acids as DHA.	26 months (corrected age), and between 3 and 5 years (corrected age)	4.6: 1*	Feeding preterm infants milk containing 3 times the standard amount of DHA did not result in any clinically meaningful change to language development or behavior when assessed in early childhood.	No effect	Nu-Mega Ingredients (Clover), Australia
(Lauritzen et al., 2005) Reproduction Nutrition Development	To determine if an increased content of DHA in breast-milk via maternal fish oil supplementation affects mental development in term infants.	Brain	A double-blind, randomized trial, with follow up.	122 Danish mothers were randomized to olive oil (placebo) or 4.5 g/day fish oil (0.79 g DHA + 0.62 g EPA).	Children; 9, 12, 24 months	1.3: 1	Maternal fish oil supplementation during the first four months of lactation was found to have no beneficial effect on problem solving, although the data indicates there may be an effect in girls. Maternal fish oil supplementation was found to result in a reduction in vocabulary comprehension at one year of age, especially in boys.	No effect	BASF Health and Nutrition A/S, Denmark
(Cheatham et al., 2011) Lipids	To examine whether fish oil supplementation during lactation affects processing speed, working memory, inhibitory control, and socioemotional development at 7 years.	Brain	A double-blind, randomized trial, with follow up.	122 Danish mothers were randomized to olive oil (placebo) or 4.5 g/d fish oil (0.79 g DHA + 0.62 g EPA).	7 (mean)	1.3: 1	The authors found that boys whose mothers consumed fish oil had lower prosocial scores relative to the control group. Moreover, speed of processing scores and inhibitory control/working memory scores were differentially negatively predicted by maternal n-3 LCPUFA intake and infant RBC-DHA status.	-	BASF Health and Nutrition A/S, Denmark
(Helland et al., 2003) Pediatrics	To examine the effect of supplementing pregnant and lactating women with n-3 PUFAs on mental development of the children, compared with maternal supplementation with n-6 PUFAs.	Brain	A double-blind, randomized trial, with follow up.	590 pregnant women were randomized to receive 10 mL of cod liver oil (1.18 g DHA + 0.80 g EPA) or placebo corn oil from 18 weeks' gestation until 3 months after delivery.	4 (mean)	1.5: 1	Maternal intake of n-3 PUFAs during pregnancy and lactation may be favourable for mental development of children at 4 years of age.	+	Peter Moller, Norway
(Helland et al., 2008) Pediatrics	To examine the effect of supplementing pregnant and lactating mothers with n-3 PUFAs on children's IQ.	Brain	A double-blind, randomized trial, with follow up.	590 pregnant women were randomized to receive 10 mL of cod liver oil (1.18 g DHA + 0.80 g EPA) or placebo corn oil from 18 week' gestation until 3 months after delivery.	7 (mean)	1.5: 1	No significant differences in IQ scores were found between children at 7 years of age whose mothers had taken cod liver oil or corn oil.	No effect	Peter Moller, Norway
(Dunstan et al., 2008) Archives of Disease in Childhood. Fetal and Neonatal Edition	To assess the effects of antenatal n-3 PUFAs on cognitive development in a cohort of children whose mothers received high-dose fish oil in pregnancy.	Brain	A double-blind, randomized trial, with follow up.	98 pregnant women randomly received either fish oil (2.2 g DHA + 1.1 g EPA/day) or olive oil (placebo) from 20 weeks' gestation until delivery.	2.5 (mean)	2.0: 1	Children in the fish oil-supplemented group attained a significantly higher score for eye and hand coordination than those in the placebo group.	+	Ocean Nutrition Canada, Ltd., Canada

(Continued on next page)

Table 4. (Continued)

Reference & Journal	Aim of study	Area of study	Type of study	Number of participants / Test formulation	Age range (years)	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Meldrum et al., 2015) Nutrients	To investigate the long-term effect (12 years) of fish oil supplementation in pregnancy on neurodevelopment.	Brain	A double-blind, randomized trial, with follow up.	98 pregnant women randomly received either fish oil (2.2 g DHA + 1.1 g EPA/day) or olive oil (placebo) from 20 weeks' gestation until delivery.	12 (mean)	2.0: 1	There were no significant differences for any of the neurodevelopmental assessment measures completed.	No effect	Ocean Nutrition Canada, Ltd., Canada
(Collins et al., 2011) British Journal of Nutrition	To test the effect of higher-dose DHA (approximately 1% dietary fatty acids) on the growth of pre-term infants up to 18 months corrected age, compared with standard feeding practice.	Growth in pre-term infants	A multi-centre, randomized, controlled trial (DINO trial).	Lactating mothers consumed 6 × 500 g capsules of either high DHA fish oil (0.90 g DHA + 0.20 g EPA) or placebo soy oil per day.	Infants; 0–18 months	4.5: 1*	There was no effect of higher DHA on weight or head circumference at any age, but infants fed a higher dose of DHA were 0.7 cm longer at 18 months corrected age.	No effect +	Nu-Mega (Clover), Australia
(Muhlhauser et al., 2016) American Journal of Clinical Nutrition	To determine whether maternal DHA supplementation during the second half of pregnancy results in a lower BMI and percentage of body fat in children.	Growth	A follow up study (DOMInO trial).	Women with a singleton pregnancy were provided with high DHA fish oil capsules (0.8 g DHA + 0.1 g EPA/day) or placebo vegetable oil capsules in the second half of pregnancy.	3–5	8.0: 1	Maternal intake of high DHA fish oil during the second half of pregnancy does not affect the growth or body composition of children at 3 or 5 years of age.	No effect	Incromega 500 TG, Croda Chemicals, England
(Best et al., 2016) Pediatrics	To determine whether protective effects for allergy were evident in the 6-year-old offspring of women supplemented with n-3 rich fish oil during pregnancy.	Allergy	A follow up of a double-blind, multi-centre, randomized, controlled trial (DOMInO trial).	Women with a singleton pregnancy were provided with high DHA fish oil capsules (0.8 g DHA + 0.1 g EPA/day) or placebo vegetable oil capsules in the second half of pregnancy.	6	8.0: 1	Prenatal n-3 LC-PUFA supplementation did not reduce IgE-associated allergic disease of children at 6 years of age.	No effect	Incromega 500 TG, Croda Chemicals, England

*Personal communication; the DHA: EPA ratio was not specified in the published paper, but was provided by authors of the study upon request.

26 months and 3–5 years corrected age. Findings of this follow-up trial indicated that feeding preterm infants milk with high DHA did not result in any clinically meaningful changes in language development or behaviour when assessed in early childhood. Lauritzen et al. (2005) conducted a follow-up study of a previous trial (Lauritzen et al. 2004) to see if maternal fish oil supplementation affects mental development in term infants. A total of 122 Danish mothers were randomly assigned to receive high DHA fish oil during the first 4 months of lactation, and a further 53 mothers with a high-fish intake as a reference group. The authors found that maternal fish oil supplementation in the early stage of lactation had no beneficial effect on the problem-solving capacity of the infant, but resulted in improvement in vocabulary comprehension at one year of age. Cheatham et al. (2011) ran a follow-up study of the trial by Lauritzen et al. (2004). The authors found that boys whose mothers consumed fish oil had lower prosocial scores relative to the control group. Moreover, both speed of processing and inhibitory control/working memory scores were differentially negatively predicted by maternal n-3 LCPUFA intake and infant RBC-DHA status. Helland et al. (2001) ran two follow-up trials (Helland et al. 2008; Helland et al. 2003) in which mothers took either high DHA cod liver oil or placebo during gestation until 3 months after delivery. Their children's IQ and BMI were measured at 4 and 7 years of age. The authors found children who were born to mothers that had taken cod liver oil during pregnancy, scored higher on the intelligence measurement at 4 years of age. No significant differences in IQ scores were found between children at 7 years of age. Dunstan et al. (2008) showed that children in the high DHA fish oil-supplemented maternal group attained a significantly higher eye and hand coordination score than those in the placebo group. In contrast, a subsequent follow-up study on the same children at 12 years of age (Meldrum et al. 2015), found no significant differences between treatment groups for any of the neurodevelopmental assessments. In another follow-up study from the DINO trial (Makrides et al. 2009), the effect of high-dose DHA on the growth of preterm infants was tested in comparison to standard feeding practice (Collins et al. 2011). The authors reported no effect on the weight or head circumference of children at any age in the high-dose DHA group, however, infants fed high DHA were 0.7 cm longer at 18 months corrected age compared to the control group. Overall, high-dose DHA increased the length of infants weighing ≥ 1250 g at 4 months corrected age, and both weight and length at 12 and 18 months corrected age. Muhlhauser et al. (2016) ran a follow-up study of children at 3 and 5 years of age who were born to mothers enrolled in the DOM-INO trial (Makrides et al. 2010). Findings of this trial indicate that maternal intake of high DHA fish oil during the second half of pregnancy does not affect the growth or body composition of children at 3 or 5 years of age.

Best et al. (2016) conducted a follow-up study to determine the effect of high DHA fish oil supplementation during pregnancy on allergies. Allergic disease symptoms were assessed, and sensitization to allergens were measured, in their offspring at 6 years of age. Overall, no difference in the percentage of children with any IgE-associated allergic disease was observed between the high DHA fish oil and control groups.

3.3. Animal studies

3.3.1. Heart and exercise

Details and characteristics of the 10 high DHA fish oil trials focusing on heart function and exercise performance in animals that were included in this analysis are presented in Table 5. Although non-mammalian, farmed animals (such as chicken and fish) are excluded in this section, it is interesting to note that a large, and rapidly growing, body of scientific evidence exists on the differences between dietary and physiological roles of EPA and DHA, particularly in fish nutrition studies. Studies observing the effect of omega-3 DHA dietary administration on the meat quality in farmed mammals are also excluded. If the DHA and EPA contents in the animal's diet was not reported by authors, the content of these fatty acids was calculated and estimated by authors of this paper (as previously described).

All of the studies detailed herein were relatively long-term trials (i.e. no short, post-prandial studies) with an intervention duration of 4 weeks to 7 months (the mean trial duration was 7 weeks). In five studies, rats were chosen as an animal model (Abdukeyum et al. 2016; Abdukeyum et al. 2008; Molinar-Toribio et al. 2015; Owen et al. 2004; Slee et al. 2010), while other studies used mice (Arai et al. 2009; Higuchi et al. 2006; Huggins et al. 2009), pigs (Castellano et al. 2010) and rabbits (Ninio et al. 2005) in their research. The number of animals per group ranged from 3 to 9. In all animal studies DHA: EPA ratios were equal or above 2, with the exception of one study where the ratio was 1.2: 1 (Castellano et al. 2010). Five trials were conducted with male animals only (Abdukeyum et al. 2016; Abdukeyum et al. 2008; Castellano et al. 2010; Higuchi et al. 2006; Owen et al. 2004), three trials were conducted with female animals only (Arai et al. 2009; Huggins et al. 2009; Molinar-Toribio et al. 2015), and in two trials the sex of the animals was not specified (Ninio et al. 2005; Slee et al. 2010).

Several different endpoints were assessed in these studies, including: i) lipid and apolipoprotein concentrations, lipoprotein subclass distribution, and markers of oxidative status (Abdukeyum et al. 2016; Higuchi et al. 2006; Molinar-Toribio et al. 2015); ii) myocardial and erythrocyte membrane fatty acid (Abdukeyum et al. 2016; Huggins et al. 2009; Ninio et al. 2005; Owen et al. 2004; Slee et al. 2010); iii) *ex vivo* cardiac performance and reperfusion arrhythmia incidence (Abdukeyum et al. 2008; Huggins et al. 2009), HR and blood pressure (Abdukeyum et al. 2008), and plasma lipid; iv) hepatic cholesterol; v) hepatic mRNA expression of lipogenic and lipolytic genes (Arai et al. 2009), and concentration of plasma glucose (Higuchi et al. 2006); and vii) insulin secretion and sensitivity (Castellano et al. 2010).

Owen et al. (2004) implemented a study to examine the dose and time responses for incorporation of n-3 LC-PUFA into cellular membranes in male Sprague-Dawley rats. The results showed that myocardial DHA levels doubled in 2-days, stabilizing at levels $\sim 200\%$ higher than the control after 28-days of feeding on a meal containing 10% high DHA fish oil. This study showed that low doses of high DHA fish oil produced marked changes in myocardial DHA levels, and the maximal incorporation takes up to 28 days to occur. Abdukeyum et al. (2008), studied the effects of high DHA fish oil on ischemic preconditioning, and its interaction with heart function and injury during myocardial ischemia and reperfusion in male Wistar rats. It was

Table 5. A summary of animal studies testing the effects of high DHA fish oil on heart functioning and exercise performance.

Reference & Journal	Aim of study	Area of study	Number of participants / Test formulation	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Owen et al., 2004) Lipid	To examine the dose and time responses for incorporation of n-3 PUFA into cellular membranes in rats fed diets containing fish oil.	Heart	Male Sprague-Dawley rats were fed a 10% fish oil diet (32 g DHA + 7.9 g EPA per kg of diet) for periods ranging from 0 to 42 days (n = 3). For the dose response study, rats (n = 3) were fed 0, 1.25%, 2.5%, 5% or 10% fish oil for 4 weeks.	4.0: 1	This study shows that low doses of fish oil produce marked changes in myocardial DHA levels; maximal incorporation takes up to 28 days to occur.	+	Nu-Mega Ingredients (Clover), Australia
(Abdukeyum et al., 2008) The Journal of Nutrition	To evaluate the efficacy of dietary fish oil in providing cardio-protection under the same conditioning as ischemic preconditioning (IPC) and to evaluate their potential synergy.	Heart	54 male Wistar rats were fed 10% fat diets containing either 7% tuna oil (20.2 g DHA + 4.9 g EPA/kg diet) + 3% olive oil, 5% sunflower seed oil + 5% olive oil, or 7% beef tallow + 3% olive oil for 6 weeks (n = 6-9).	4.1: 1	Heart function and infarction did not differ among dietary IPC groups. Arrhythmias was significantly reduced by IPC in (n-6) PUFA, and SF hearts were significantly lower in (n-3) PUFA IPC hearts.	+	Nu-Mega Ingredients (Clover), Australia
(Abdukeyum et al., 2016) Clinical Medicine	To test the hypothesis that incorporation of n-3 LC-PUFA into myocardial membranes increases their peroxidation potential and basal fatty acid oxidation.	Heart / Mitochondrial function	54 male Wistar rats were randomly assigned to a diet of either fish oil (7% fish oil + 3% olive oil, providing 20.2 g DHA + 4.9 g EPA/kg diet), sunflower seed oil (5% sunflower seed oil + 5% olive oil) or beef tallow (7% beef tallow + 3% olive oil) for six weeks (n = 6).	4.1: 1	High DHA fish oil raised basal membrane DHA, fatty acid peroxidisability index, concentrations of lipid oxidation products, and superoxide dismutase activity (but not glutathione peroxidase).	+	Nu-Mega Ingredients (Clover), Australia
(Slee et al., 2010) Journal of Lipid Research	To measure the incorporation of DHA into myocardial membranes of rats from low dietary fish oil intake within the human dietary range and quantitatively assess the influence of dietary n-6 PUFA.	Heart	Rats were fed a diet containing one of the following concentrations of fish oil (0.16, 0.31, 0.63, 1.25, 5.0%; n = 4 per diet) or a control diet for 4 weeks. Gender not specified.	4.0: 1	Myocardial membranes are sensitive to absolute dietary intake of n-3 LC-PUFA at low % in the rat, equivalent to a human intake of one meal of fatty fish per week or less. Myocardial DHA concentration increased in a log-linear fashion, with a dietary threshold of 0.019% en as EPA + DHA and half maximal dietary [EPA + DHA] equal to 0.29% en.	+	Nu-Mega Ingredients (Clover), Australia
(Molinar-Toribio et al., 2015) British Journal of Nutrition	To explore the effect of EPA and DHA supplementation in different proportions on spontaneously hypertensive obese rats.	Heart	28 female spontaneously hypertensive, obese rats were randomly divided into four groups, each supplemented with a different oil mixture (control oil or DHA: EPA oil mix at a ratio of 1: 1, 1: 2, 2: 1) for 13 weeks (n = 7).	2.0: 1	Specific mixtures significantly decreased inflammation (CRP levels, DHA: EPA ratio of 1: 1 and 1: 2), increased the activity of antioxidant enzymes (SOD in erythrocytes and abdominal fat, DHA: EPA ratio of 1: 1; SOD, CAT, GR, GPx in kidneys, DHA: EPA ratio of 2: 1). No significant differences were found for HDL-cholesterol in all groups.	+	AFAMPES 121 EPA (AFAMSA), Spain EnerZona Omega 3 RX and Oligen liquid DHA 80% (IFIGEN-Equip 98, S.L.), Spain
(Higuchi et al., 2006) Annals of Nutrition and Metabolism	To examine the weekly changes in plasma glucose and lipid concentrations in mice fed experimental diets that contained fish oil, DHA, and/or EPA to discuss the effects of DHA and/or EPA intake on glucose and lipid metabolism.	Heart (glucose and lipid metabolism)	45 male mice were fed five different experimental diets (n = 9) which contained 6.0% lard, 6.0% fish oil (14.28 g DHA + 4.02 g EPA/kg diet), 1.5% DHA + 4.5% lard, 0.4% EPA + 5.6% lard or 1.5% DHA + 0.4% EPA + 4.1% lard, for 17 weeks.	3.5: 1	The decreases in plasma glucose concentrations in response to intakes of DHA and EPA in mice take place over a longer period of time than similar decreases in the plasma lipid concentrations.	+	Nippon Chemical Feed Co. Ltd, Hakodate, Japan

(Huggins et al., 2009) American Journal of Physiology – Heart and Circulatory Physiology	To investigate whether dietary n-3 PUFA could provide ischemic protection in female mice with an underlying genetic predisposition to cardiac hypertrophy.	Heart	Mature female transgenic mice and littermate wild-type mice were fed a fish oil-derived diet (20.20 g DHA + 4.87 g EPA/kg diet) or control diet, for 4 weeks (n = 8).	4.1: 1* Dietary fish oil did not suppress the incidence of reperfusion arrhythmias in mice.	No effect	Nu-Mega Ingredients (Clover), Australia
(Arai et al., 2009) Journal of Atherosclerosis and Thrombosis	To elucidate the effects of EPA- or DHA-rich fish oil, and of the latter plus fenofibrate, on lipid metabolism in female mice.	Heart (lipid metabolism)	20 female KK mice were divided into four groups and fed one of three diets for 8 weeks: lard/safflower oil (4:6), EPA-rich fish oil (13.6 g DHA + 21.0 g EPA/kg diet), high DHA fish oil (25.2 g DHA + 7.3 g EPA/kg diet), or high DHA fish oil plus 0.2% fenofibrate (n = 5).	3.4: 1 Fish oil inhibited body weight gain and exhibited an anti-obesity effect through the inhibition of lipid synthesis in female KK mice.	+	NOF Corporation (Tokyo, Japan)
(Castellano et al., 2010) British Journal of Nutrition	To examine the effects of a long-term dietary supplement of fish oil in adult male pigs on body condition as well as insulin sensitivity and secretion.	Heart (insulin metabolism)	15 Duroc boars received a daily 2.5 kg basal diet with a supplement of either 62 g animal fat (rich in SFA), 60 g menhaden oil (10.8 g DHA + 9.0 g EPA), or 60 g tuna oil (19.8 g DHA + 3.9 g EPA) for 7 months.	1.2: 1 Long-term supplementation with dietary n-3 PUFA did not affect insulin metabolism in healthy adult male pigs.	No effect	Bluecina Corp (Calgary, AB, Canada)
(Ninio et al., 2005) Journal of Cardiovascular Electrophysiology	To examine the effect of dietary fish oil on the rabbit model of stretch-induced vulnerability to atrial fibrillation.	Heart	Six-week-old rabbits were fed standard rabbit pellets supplemented with 5% tuna fish oil (13 g DHA + 3 g EPA/kg diet, n = 6) or supplemented with 5% sunflower oil (n = 6) for 12 weeks. Gender not specified.	4.3: 1 Red blood cell, atrial, and ventricular n-3 fatty acid levels were significantly higher in the fish oil group and this is associated with protection against stretch-related AF in the rabbit.	+	Nu-Mega Ingredients (Clover), Australia

*Personal communication; the DHA: EPA ratio was not specified in the published paper, but was provided by authors of the study upon request.

shown that high DHA fish oil protected the heart against myocardial infarction and arrhythmias, and improved post-ischemic recovery of heart function, compared with the other diets. A follow-up study on the latter intervention trial was also used to test the effects of n-3 LC-PUFA on myocardial membranes, peroxidation and basal fatty acid oxidation (Abdukeyum et al. 2016). High DHA fish oil increased peroxidability leading to up-regulated mitochondrial superoxide dismutase, but not glutathione peroxidase, antioxidant activity. Slee et al. (2010) investigated the incorporation of DHA into myocardial membranes of Sprague-Dawley rats from low dietary fish oil intake. They found that low dietary intakes of high DHA fish oil in the rat, in the range equivalent to the human intake of 1–2 fish meals per week, increased the myocardial membrane n-3 LC-PUFA concentration. Molinar-Toribio et al. (2015) investigated the effect of EPA and DHA supplementation in different proportions on spontaneously hypertensive obese rats. The authors found that supplementation with DHA + EPA at 2: 1 or 1: 2 ratios in obese rats significantly lowered plasma total cholesterol and LDL cholesterol concentrations, compared with the control group. No significant differences in HDL-cholesterol were found between groups. Supplementation with DHA + EPA at ratios of 1: 1 and 1: 2 significantly decreased inflammation, and increased the activity of antioxidant enzymes (superoxide dismutase in erythrocytes and abdominal fat), while supplementation with high DHA fish oil increased superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase in kidneys, and showed a tendency towards decreasing excretion of urinary isoprostanes. Higuchi et al. (2006) investigated the effects of DHA, EPA and high DHA fish oil on plasma glucose, total cholesterol, TAG and phospholipid concentrations in male mice. They found that plasma phospholipid concentrations were significantly lower in mice fed the high DHA fish oil and DHA + EPA diets than the lard groups. Plasma glucose levels were reduced after the administration of a high DHA fish oil diet, and the DHA and EPA fortified diets. Huggins et al. (2009) studied the effects of dietary n-3 LC-PUFA supplementation on ischemic protection in female mice with an underlying genetic predisposition to cardiac hypertrophy. They reported that a high DHA fish oil diet modestly suppressed hypertrophic growth; however, it did not enhance postischemic recovery, nor did it decrease the incidence of reperfusion arrhythmia in a normal or hypertrophic heart. Arai et al. (2009) performed a study comparing the effects of diets formulated with different fat sources on lipid metabolism in female KK mice. The authors observed a significantly lower final body weight in groups fed EPA and DHA, compared to that of the control and DHA plus fenofibrate groups, suggesting that n-3 LC-PUFA inhibited body gain weight. Compared to the control group, all three treatments containing n-3 LC-PUFA significantly reduced plasma insulin and hepatic lipid levels, and increased plasma adiponectin. The highest increase in the latter parameter was recorded in the DHA plus fenofibrate group. Castellano et al. (2010) investigated the effects of n-3 LC-PUFA supplementation on body condition, insulin sensitivity and secretion in adult-male Duroc boars. Independent from source and EPA/DHA ratios, the authors found that long-term supplementation with dietary n-3 LC-PUFA, did not affect insulin metabolism in healthy adult male pigs. There were no differences in fat gain among the three diets. Ninio et al. (2005) found that, in rabbits

(New Zealand White rabbits), atrial n-3 LC-PUFA levels were dramatically increased with dietary high DHA fish oil supplementation compared to the control group, and this was associated with increased protection against stretch-related atrial fibrillation in the rabbit.

3.3.2. Brain and visual system

Details and characteristics of the 6 retrieved trials studying the effects of high DHA fish oil on brain and visual system in animals are reported in Table 6.

All studies were long-term trials, with a duration ranging from 7 days to 3 months (the median duration was 26 days). Rats were chosen as an animal model in four studies (Avramovic et al. 2012; Engstrm et al. 2009; Fernandes et al. 2008; Trofimiuk and Braszko 2011), one study used mice (Davis et al. 2017), and another used pigs, or more specifically, pregnant sows and their piglets (Clouard et al. 2015). The number of animals per group varied from 6 to 20. DHA: EPA ratios were above 2 in four studies (Clouard et al. 2015; Davis et al. 2017; Engstrm et al. 2009; Fernandes et al. 2008) and between 1–2 in two studies (Avramovic et al. 2012; Trofimiuk and Braszko 2011). All studies were conducted with male animals, with the exception of one study that used females (Clouard et al. 2015) and another that used animals of both sexes (Davis et al. 2017).

The main endpoints assessed in these studies included: i) retention of cognition (Fernandes et al. 2008), ii) brain nitric oxide synthase (Engstrm et al. 2009), iii) behavioural responses (Clouard et al. 2015; Davis et al. 2017; Trofimiuk and Braszko 2011), iv) brain tissue oxidative status (Avramovic et al. 2012), and v) microbiota (Davis et al. 2017).

Fernandes et al. (2008) conducted a study investigating the effects of high DHA fish oil in alleviating both the cognitive and neurodegenerative deficits caused by transient, global cerebral ischemia (TGCI) in male rats. Animals were trained in an 8-arm, radial maze task and then assigned to the following groups: sham operation, ischemia operation plus olive oil, or ischemia operation plus high DHA fish oil. Retention of the previously acquired cognition from the maze task was assessed one week after TGCI and continued weekly for 6 weeks. The results of this study suggest that long-term treatment with high DHA fish oil facilitates functional recovery after ischemic brain damage, but does not prevent hippocampal damage in rats. Engstrm et al. (2009) implemented a study to assess the effects of two fish oils containing different amounts of EPA, DHA, and a commercial natural antioxidant in male rats on brain nitric oxide synthase activity; an enzyme which has been shown to be important for memory and learning ability. The authors found that high DHA fish oil stabilized with antioxidant increased the brain nitric oxide synthase activity while other treatments did not. Trofimiuk and Braszko (2011) showed that cod liver oil prevented the deleterious effects of chronic restraint stress on recall and spatial memory in male rats. Avramovic et al. (2012) showed that a high DHA fish oil supplementation, compared to control, significantly decreased lipid peroxidation, increased SOD activity, and decreased (not significantly) catalase activity in the brain of male rats. The impact of maternal dietary DHA on the behaviour of offspring was assessed in pigs (Clouard et al. 2015). The authors reported that maternal DHA supplementation enhanced the social

Table 6. A summary of animal studies testing the effects of high DHA fish oil on the brain and visual system.

Reference & Journal	Aim of study	Area of study	Number of participants / Test formulation	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Fernandes et al., 2008) Nutrition Research	To investigate whether long-term treatment with commercial, high DHA fish oil could be effective in alleviating both the cognitive and neurodegenerative deficits caused by transient, global cerebral ischemia in rats.	Brain	40 young adult male Wistar rats received cognition training for 10 days. They were then divided into 3 groups: sham operation, ischemia + vehicle, or ischemia + fish oil (0.3 g DHA + 0.06 g EPA/kg body weight).	5.0: 1	Long-term fish oil treatment is able to facilitate functional recovery after ischemic brain damage, an effect that was distinct from hippocampal damage but did not prevent hippocampal damage in rats.	+ No effect	n-3 DHA 250, Biotik do Brasil Industria e Comercio Ltda, Brazil
(Engstrm et al., 2009) Upsala Journal of Medical Science	To investigate the effects of two different types of natural fish oils containing different amounts of the n-3 PUFAs EPA and DHA and antioxidants on plasma and brain fatty acids, blood lipids, vitamin E, and in vivo lipid peroxidation, as well as brain nitric oxide synthase (NOS) activity.	Brain	24 male, pathogen-free Sprague-Dawley rats were divided into four groups and received either butter (control), EPA-rich fish oil plus antioxidant (5.85 g DHA + 8.7 g EPA/kg diet), high DHA fish oil plus antioxidant (14 g DHA + 3.85 g EPA/kg diet), or high DHA fish oil (14 g DHA + 3.85 g EPA/kg diet) not fortified with antioxidant, for 10 days.	3.6: 1	Both the EPA- and DHA-rich diets affected the blood lipids in a similarly positive manner, and they both had a large impact on plasma phospholipid fatty acids. The intake of DHA was more important than that of EPA for brain phospholipid DHA enrichment and brain NOS activity.	+	EPA-rich oil (ESKIMO-3, Cardinova, Sweden High DHA oil (EPAX 0525TG, Pronova, Norway
(Trofimiuk and Braszko, 2011) Lipids	To test the hypothesis that the active constituents of cod liver oil alleviate the negative impact of prolonged restraint stress on cognitive functions of male Wistar rats.	Brain	75 male Wistar rats were divided into four treatment regimens including Cremophor, Cremophor followed by stress procedure, cod liver oil (0.30 g DHA + 0.22 g EPA/kg body weight) and cod liver oil followed by stress procedure.	1.3: 1	Cod liver oil administration significantly prevented the deleterious effects of chronic restraint stress on recall and the spatial memory.	+	Cod Liver Oil, Lysi HF, Island
(Avramovic et al., 2012) Hippokratia	To investigate the effects of dietary n-3 fatty acid supplementation on levels of lipid peroxidation and oxidant/antioxidant status of brain tissue in rats.	Brain	16 male Wistar rats were divided into two groups. One received standard laboratory food and the other received standard laboratory food and 200 μ l fish oil (0.04 g DHA + 0.03 g EPA/day).	1.5: 1	Incorporation of n-3 fatty acids after their supplementation had beneficial effects on brain tissue. Catalase activity decreased but not significant.	+ No effect	Not specified
(Clouard et al., 2015) Journal of Nutrition	To assess the impact of maternal dietary DHA on behavior, brain fatty acid profile, and sickness response of offspring in pigs.	Brain	24 pregnant sows were fed a diet with high DHA fish oil (2.02 g DHA + 0.27 g EPA/kg body weight) or high-oleic acid sunflower oil from day 61 of gestation through to lactation.	7.0: 1	Maternal DHA beneficially affected social behaviour in offspring after weaning, and mildly attenuated sickness behaviour after an inflammatory challenge in pigs.	+	Marinol D-40; Stepan Lipid Nutrition, USA
(Davis et al., 2017) Brain, Behavior, and Immunity	To determine the contribution of DHA on anxiety and depressive-like behaviours through modulation of the gut microbiota in a paradigm of social isolation.	Brain	36 male and female C57BL/6J mice were randomly placed on one of three dietary conditions including a control diet with no performed DHA, a control diet supplemented with 0.1% by weight DHA, or a control diet supplemented with 1.0% by weight DHA.	9.2: 1	DHA altered commensal community composition and produced beneficial effects on anxiety and depressive-like behaviors in a sex-specific manner.	+	Omega Protein Inc., USA

behaviour of offspring after weaning, and suggested that these changes were mediated by increased brain DHA concentrations. Last, Davis et al. (2017) investigated the influence of DHA on suppressing anxiety and depressive-like behaviours through modulation of the gut microbiota in a paradigm social isolation. After 28-days of social isolation, male and female mice were randomized to receive a control diet or a diet supplemented with 0.1% or 1.0% DHA (by weight in diet). The authors found that DHA significantly altered commensal

community composition, and had beneficial effects on anxiety and depressive-like behaviours in a sex-specific manner.

3.3.3. Other medical/physiological aspects

Details and characteristics of the 10 trials retrieved where high DHA fish oil was tested on immunity, muscle function, hepatitis and other medical aspects in animal models are reported in Table 7.

Table 7. A summary of animal studies testing the effects of high DHA fish oil for other medical purposes.

Reference & Journal	Aim of study	Area of study	Number of participants / Test formulation	DHA: EPA	Results / Conclusion	Outcome	Fish oil supplier
(Peoples and McLennan, 2010) British Journal of Nutrition	To investigate the influence of membrane composition on skeletal muscle function.	Muscle / sport	18 male Wistar rats were fed either saturated fat (SF), n-6 PUFA (linoleic acid rich) or n-3 PUFA fish oil; 20.20 g DHA + 4.87 g EPA/kg diet) for 8 weeks.	4.1:1	Hindlimb oxygen consumption during contraction was significantly lower in the n-3 PUFA group compared with the SF group, producing a significantly higher O ₂ efficiency index.	+	Nu-Mega Ingredients (Clover), Australia
(Peoples and McLennan, 2014) British Journal of Nutrition	To test the effects of a fish-oil diet on skeletal muscle fatigue under the stress of contraction using the rat in vivo autologous perfused hindlimb model.	Muscle function	18 male Wistar rats were fed a diet rich in saturated fat (SF), a diet rich in n-6 PUFA or a diet rich in n-3 LC-PUFA DHA (20.20 g DHA + 4.87 g EPA/kg diet) derived from fish oil for 8 weeks.	4.1:1	Rats fed the n-3 PUFA diet developed higher maximum twitch tension than those fed the SF and n-6 PUFA diets. The n-3 PUFA group used less oxygen for tension developed and produced higher venous lactate concentrations, with no difference in glycogen utilisation compared with the saturated fat and n-6 PUFA groups.	+	Nu-Mega Ingredients (Clover), Australia
(Henry et al., 2015) British Journal of Nutrition	To test the hypothesis that nutritionally relevant fish oil doses can modulate membrane fatty acid composition and muscle fatigue.	Sport	18 male Sprague-Dawley rats were fed 10% fat diets of either a control fat, or a low (0.9 g DHA + 0.2 g EPA/kg diet) or moderate (3.6 g DHA + 0.9 g EPA/kg diet) fish oil for 15 weeks.	4.1:1	There were no dietary differences in maximum developed muscle force. Force within bursts was better sustained with fish oil, where maximum rates of force development and relaxation declined more slowly. Incorporation of DHA was greater in the fast-twitch gastrocnemius than in the slow-twitch soleus muscle.	+	Nu-Mega Ingredients (Clover), Australia
(Kohno et al., 2000) Oncology Reports	To investigate the modify effect of dietary tuna oil on the development of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF).	Colonic diseases	20 male rats were randomized into four groups receiving 5% corn oil, 23.5% corn oil, 5% fish oil, or 23.5% fish oil.	3.4:1	Fish oil derived from tuna, which contains high amounts of DHA and VD3, suppresses the formation and growth of ACF and may have a preventive effect on colon carcinogenesis.	+	Maruha Co., Ltd., Japan
(Du et al., 2004) Journal of Nutritional Biochemistry	To examine the effects of dietary fatty acids on hepatitis, hepatic gene expression and survival.	Liver	144 rats were fed a conventional, low-fat diet (CE2), a CE2 diet supplemented with lard, soybean oil, or a mixture of high DHA fish oil (3.2 g DHA + 1.4 g EPA/kg diet) and soybean oil.	2.3:1	Polyunsaturated fatty acids suppress the development of acute hepatitis and prolong survival in females, regardless of whether they are of the n-6 or n-3 type, which are associated with altered gene expressions.	+	Japan Oil Chemicals Inc., Tokyo
(Philip et al., 2015) PLOS ONE	To compare effects of different diets on the metabolic profile and lipid metabolism gene and protein content in red (soleus) and white (extensor digitorum longus) muscles of mice.	Muscle function	Male and female C57BL/6 mice were randomly assigned to one of three diets (n = 9–12/group), fed either a standard chow, high saturated fat (HF-S) or HF-S fish oil diet (19.5 g DHA + 4.5 g EPA/kg diet).	4.3:1	It is likely that HF-fish oil prevents HF-S-induced intramyocellular lipid accumulation by a concurrent increase in mediators of fatty acid uptake and utilisation, and suppression of mediators promoting fatty acid storage and lipogenesis.	+	Nu-Mega Ingredients (Clover), Australia
(Kook et al., 2002) Asian Australasian Journal of Animal Sciences	To investigate the effect of fish oil on growth performance, ruminal metabolism and fatty acid composition, and physical characteristics of longissimus muscle.	Growth/ heart (serum cholesterol)	10 steers and 10 bulls of Korean cattle were divided into two groups receiving corn grain or 5% fish oil (11.9 g DHA + 1.67 g EPA/kg diet).	7.1:1	Average daily weight gain and feed efficiency were not influenced by high DHA fish oil. There was a treatment effect for serum cholesterol concentrations. High DHA fish oil increased concentrations of n-3 fatty acids, including linolenic, EPA and DHA in longissimus muscle.	+	Enerlac®, Chile
(Castellano et al., 2011) Theriogenology	To determine the effect of long-term dietary supplementation of two types of fish oil on lipid composition and steroidogenesis in adult pig testis.	Sex hormone production	24 boars were randomized into three dietary groups and fed animal fat rich in SFA, menhaden oil (10.80 g DHA + 9.6 g EPA/day), or tuna oil (19.80 g DHA + 4.20 g EPA/day), for 7 months.	MO: 1.2:1 TO: 5.0:1	Long-term dietary supplementation of fish oil, regardless of the EPA/DHA ratio, modified the fatty acid compositions in testis and affected steroid production of healthy adult boars.	+	Virginia prime gold Menhaden fish oil: Omega Protein Inc., USA Tuna oil: Bluecina Inc., Canada

(LeBlanc et al., 2008) American Journal of Veterinary Research	To evaluate the effect of diets enriched with EPA and DHA on in vivo production of inflammatory mediators.	Immunity	15 healthy, crossbred-hound dogs were randomly allocated to receive an isocaloric ration supplemented with either sunflower oil, fish oil, or fish oil plus Vitamin E for 12 weeks.	1.3: 1	A fish oil-enriched diet consisting of EPA and DHA was associated with significant reduction in serum PGE ₂ concentrations and IL-1 and IL-6 activities.	+ Omega Protein, USA
(Patten et al., 2002) Nutrition Research	To investigate, in relation to newborn guinea pig growth, caecal digesta pH and SCFA profile, ileal total phospholipid fatty acid content and particularly for in vitro ileal contractility.	Gut	Newly formulated commercial rice porridge was fed to 7 new born guinea pigs and their mothers for 2 months, which consisted of essential nutrients and 3% total fat as safflower oil or 3% tuna fish oil.	4.2: 1	The fish oil supplemented group had a significantly higher caecal digesta pH with a significantly lower propionate concentration. The supplementation of fish oil decreased the threshold for electrically induced contraction while decreasing the sensitivity to 8-iso-PGE ₂ .	+ No effect Nu-Mega Ingredients (Clover), Australia

Studies in this section were long-term, intervention trials with a duration of 5 weeks to 7 months (the median duration was 12 weeks). Rats were chosen as an animal model in 5 studies (Du et al. 2004; Henry et al. 2015; Kohno et al. 2000; Peoples and McLennan 2010 2014) and mice were chosen in another (Philp et al. 2015). Other studies used cattle (Kook et al. 2002), boars (Castellano et al. 2011), dogs (LeBlanc et al. 2008) and guinea pigs (Patten et al. 2002). The number of animals per group ranged from 5 to 15. DHA: EPA ratios were above 2 in eight studies (Du et al. 2004; Henry et al. 2015; Kohno et al. 2000; Kook et al. 2002; Patten et al. 2002; Peoples and McLennan 2010 2014; Philp et al. 2015) and between 1–2 in one study (LeBlanc et al. 2008). In one study, two types of fish oil were used with different ratios (Castellano et al. 2011). Six studies were conducted with male animals (Castellano et al. 2011; Henry et al. 2015; Kohno et al. 2000; Kook et al. 2002; Peoples and McLennan 2010 2014), one with female animals (Du et al. 2004), and three with both male and female animals (LeBlanc et al. 2008; Patten et al. 2002; Philp et al. 2015).

The main endpoints measured in these studies included: i) growth performance (Kook et al. 2002), ii) tension development (Peoples and McLennan 2014), iii) muscle histology (Philp et al. 2015), iv) oxygen consumption (Peoples and McLennan 2010 2014), v) muscle function (Henry et al. 2015), vi) serum metabolites (Kook et al. 2002), vii) plasma biochemistry (Philp et al. 2015), viii) cytokine (LeBlanc et al. 2008), ix) testicular hormones (Castellano et al. 2011), x) serum biochemistry (Du et al. 2004), xi) liver histology (Du et al. 2004), xii) colonic aberrant crypt foci (Kohno et al. 2000), and xiii) ileal contractility (Patten et al. 2002).

Peoples and McLennan (2010) reported that membrane incorporation of DHA following high DHA fish oil feeding increased efficiency of muscle O₂ consumption, and promoted resistance to muscle fatigue in male Wistar rats. In another study by Peoples and McLennan (2014) applying the same experimental design, the authors reported that rats fed a high DHA fish oil diet developed higher maximum twitch tension, and sustained twitch tension through more repetitions before the tension declined to 50% of the maximum twitch tension, compared with those fed the SFA and n-6 PUFA diets. In addition, the high DHA fish oil group used less oxygen for tension, and developed and produced higher venous lactate concentrations with no difference in glycogen utilisation, compared with the saturated fat and n-6 PUFA groups. The effect of high DHA tuna fish oil on membrane fatty acid composition and muscle fatigue was tested in male Sprague–Dawley rats (Henry et al. 2015). The authors reported that rats fed high DHA fish oil fed incorporated higher muscle phospholipid DHA-relative percentages than those in the control group. Incorporation of DHA was greater in the fast-twitch gastrocnemius than in the slow-twitch soleus muscle, which was comparable with the myocardium and in line with muscle fibre characteristics. These findings suggest that DHA may be an essential component of striated muscle for optimal healthy function, and that the failure to include regular fish or fish oil in the diet may lead to a deficiency that is reflected in susceptibility to muscle fatigue. Kohno et al. (2000) reported that tuna oil rich in DHA inhibited the development of colonic aberrant crypt foci in rats. Du et al. (2004) showed that polyunsaturated fatty acids could

suppress the development of acute hepatitis and prolong survival in female rats, which were associated with altered gene expression, regardless of whether they were supplemented with n-6 (soybean oil) or n-3 (high DHA fish oil). Philp et al. (2015) reported that a diet enriched with high DHA fish oil prevented high fat-induced metabolic dysfunction in the skeletal muscle of mice. Kook et al. (2002) showed that fatty acid composition and physical properties of the muscle fat in fattening Korean cattle were altered by feeding on diets containing 5% high DHA fish oil. However, no differences in growth performance and ruminal metabolism were found between treatment groups. Castellano et al. (2011) reported that, in healthy adult pigs, body and reproductive organ weights were not significantly affected by dietary treatments with different DHA: EPA ratios, however, long-term dietary supplementation of fish oil, regardless of the DHA/EPA ratio, affected steroid production. LeBlanc et al. (2008) showed that a high DHA fish-oil enriched diet was associated with significant reductions in PGE₂ concentrations and IL-1 and IL-6 activities in young, healthy dogs.

Eventually, the effect of high DHA fish oil on bowel health, mainly caecal digesta pH and short chain fatty acid profile, and ileal contractility was investigated in guinea pig in a study by Patten et al. (2002). The authors found that the group supplemented with high DHA fish oil had a significantly higher caecal digesta pH coupled with a significantly lower propionate concentration. There was no significant difference in the sensitivity (EC₅₀), and maximal contraction, induced by acetylcholine, histamine, serotonin, and prostaglandins PGE₂ or PGF_{2α}, however, sensitivity to isoprostane, 8-iso-PGE₂ was significantly reduced in the fish oil supplemented group.

4. Discussion

As the vast majority of studies to date either focus on physiological and health related effects of n-3 LC-PUFA or have used a mixture of EPA and DHA, while the specific and distinct roles and effects of the two individual fatty acids on the most prevalent health problems, including CVD and depressive disorders, remain fundamentally untested. Consequently, it is not surprising that dose recommendations from several national and international organizations are based on the combined consumption of EPA and DHA (Mozaffarian and Wu 2011). Irrespective of the lack of available evidence to justify any substantiated reasoning surrounding the relative importance of DHA versus EPA, or any ratio of the two, some specific recommendations have been made regarding daily intake of the two fatty acids. For example, the European Food Safety Authority (2010) suggests that infants (>6 months) and young children (<24 months) should consume 0.10 g of DHA per day, while for older children (2–18 years), the recommended daily intake of DHA is considerably higher (0.25 g of DHA in one or more servings). Similarly, recommendations made by the FAO/WHO (2008) for pregnant and lactating women specified that the minimum daily intake of EPA plus DHA is 0.30 g, with at least 0.20 g being DHA. In the last few years, the specific effects of DHA on medical aspects has drawn a great deal of scientific attention. As such, the aim of this paper was to review the currently available literature in order to substantiate whether DHA has a beneficial role in influencing risk factors associated

with heart disease, brain and visual disorders, immune disorders and body weight, and/or to highlight knowledge gaps.

Of the 113 studies analysed herein, 46.0% reported statistically significant positive results following the use of high DHA fish oils (22 in heart, 14 in brain and 16 in other medical aspects); 31.0% reported results that were not statistically significant (7 in heart, 18 in brain, 10 in other medical aspects); 21.2% reported a significant positive result for some outcomes, however other measured outcomes were not statistically significant (5 in heart, 9 in brain, 10 in other medical aspects); and 1.8% reported a statistically significant negative effect (2 in brain).

The variations in responses to DHA supplementation might be accounted by the variances in the basal DHA levels. It is reported that the larger the difference of a subject's basal PUFA status from the "change threshold" the higher the change that will be observed with supplementation (Osterman and Schebb 2017). Also, cases where the effects of high DHA fish oil administration were not statistically significant, may be due to an absence of biological effects from increased dietary DHA levels. However, the risk of a type II error should not be underestimated in such intervention trials. In fact, a series of factors can contribute to this occurrence, such as an inadequate power of the statistical test, decreased experimental duration, analytical methodology issues, inappropriate dosage of treatments, improper consideration of body weight, age or gender bias within the subject groups, and other confounding effects specific to studies concerned with n-3 LC-PUFA, that can impact on the robustness of the experimental design (Ghasemifard et al. 2014). In addition, many studies did not measure omega-3 status before entry into the trials or at completion (James et al. 2014). Although the topic of specific research methodologies is beyond the scope of this review, the authors believe it is important to consider this possibility before formulating any conclusions regarding the lack of benefits or risks associated with such dietary interventions. Over the following sections, an attempt is made to summarise and interpret the key findings achieved by the studies retrieved and presented herein, and, where suitable, information from other studies that did not meet the selection criteria are integrated.

4.1. DHA in the heart and cardiovascular system

DHA is known to be the main n-3 LC-PUFA in myocardial membranes in human and rats (Brochot et al. 2009; Harris et al. 2004; Sexton et al. 1995), and its concentration has been reported to rapidly increase following an additional intake of fish oil, even at low quantities (Harris et al. 2004; Slee et al. 2010). The heart is the organ most affected by dietary DHA, whereas brain fatty acid levels appear to be somewhat resistant to dietary DHA (Saito et al. 1998). It has been reported that an increase in myocardial DHA concentrations is negatively associated with chronic stress, reducing both HR and the risk of sudden death (McLennan and Abeywardena 2005). Commonly accepted risk factors involved in heart disease are: plasma TAG, LDL and total cholesterol, blood pressure, HR and vascular reactivity, arrhythmias, and inflammation.

Based on evidence from human and animal studies, it can be inferred that EPA and DHA have differential effects on blood

pressure, heart rate, lipid and vascular reactivity (Mori and Woodman 2006). In general, it has been reported that DHA is more potent than EPA in modifying cardiometabolic risk (Li et al. 2014; Wei and Jacobson 2011). Certainly in animal models, where the underlying mechanisms can be explored more easily, DHA reduced the incidence and severity of ventricular arrhythmias, whereas EPA alone had no effect (McLennan et al. 1996). In human subjects it has been demonstrated that at least 500 mg/day of DHA is sufficient to reduce cardiovascular mortality (Meyer and de Groot 2017). The physiological mechanisms by which DHA exerts its cardioprotection are explained in a review by McLennan (2014), who described the pleiotropic physiological effects of fish oil on heart function as either direct, via membrane incorporation (intrinsic), or indirect, via triacylglycerols, blood pressure and atherosclerosis (extrinsic). McLennan (2014) concluded that DHA is the active component of fish oil which aids in preventing cardiac arrhythmia, lowering the heart rate, and preconditioning the heart to resist a variety of insults, all of which directly affect the fatty acid composition of the heart. The lowering of triacylglycerols and blood pressure to influence plaque formation and ischaemic heart disease are extrinsic to the fatty acid composition of the myocardium (McLennan 2014). Other studies exploring the effects of DHA and EPA on heart function have demonstrated that DHA lowers fasting blood level of C-reactive protein (CRP), interleukin 6 (IL-6) and tumor necrosis factor α (TNF- α) (Li et al. 2014), decreases triglycerides and raises high-density lipoprotein cholesterol (HDL) (Wei and Jacobson 2011), regulates the activity of several transcription factors and gene expression, influences membrane fluidity and downstream cell signalling pathways, and has anti-thrombotic and anti-arrhythmic effects (Adkins and Kelley 2010; Yagi et al. 2017).

The Omega-3 Index is considered to be inversely associated with a high risk of CHD mortality. As reported by Harris and Von Schacky (2004), Omega-3 Index values of $\geq 8\%$ are associated with the greatest cardioprotection, whereas index values of $\leq 4\%$ are associated with the least. As the Omega-3 Index does not differentiate between EPA and DHA, there is evidence that the two fatty acids have a different effect on the Omega-3 Index itself. For example, Allaire et al. (2017) recently demonstrated that the Omega-3 Index was greater after supplementation with 2.7 g/day of DHA, than after 2.7 g/day of EPA, over a 10-week period. As shown by Metcalf et al. (2007), this phenomenon can be explained by the different retention and deposition efficiency of EPA and DHA into erythrocyte and other membranes, particularly myocardial membranes. In this study, the authors reported that DHA accumulated in atrial phospholipids more rapidly than EPA, even though DHA and EPA were present in approximately equal proportions, during treatment with fish oil. Flock et al. (2013) investigated the dose-response of the Omega-3 Index through supplementation with increasing doses of omega-3, reporting a higher Omega-3 Index as the dose of omega-3 increased. This could be due to less β -oxidation and greater deposition following consumption of a single, large dose of dietary omega-3 compared with smaller, daily doses, as described by Ghasemifard et al. (2015b) in a study involving rats.

It has been reported that consumption of high DHA fish oil is effective in reducing TAG levels, which is an independent

risk factor for CVD. A dose response trial with high DHA fish oil supplementation showed a 6%, 20% and 25% TAG level reduction following supplementation with approximately 0.7, 1.3 and 2 g of high DHA fish oil per day, over a period of 6 weeks (Milte et al. 2008). This was supported by another study that showed a 7% reduction in plasma TAG levels following supplementation with 0.8 g of DHA per day (Meyer et al. 2009). There is a growing number of observational studies reporting that fish oils containing a greater amount of DHA relative to EPA, are more effective in reducing TAG levels than fish oils with a higher amount of EPA (Buckley et al. 2004; Caslake et al. 2008; Hill et al. 2007a; Krebs et al. 2006). This is in line with a review paper by Mozaffarian and Wu (2012) reporting that both EPA and DHA lower TAG, though larger effects were seen with DHA. Results from studies using purified EPA and DHA are varied, with some suggesting that DHA has a slightly greater hypotriglyceridemic effect coupled with an ability to raise HDL cholesterol levels (Mori et al. 2000), while others report greater reductions in TAG following EPA intervention (Rambjør et al. 1996). It is also important to highlight that the TAG lowering effect of high DHA fish oil was not observed in some studies (Mann et al. 2010; Ottestad et al. 2012; Pedersen et al. 2010). It is likely that the low dosage of DHA (< 1 g DHA/day) used in these studies resulted in the decreased efficacy. As such, it appears that in order to observe a hypotriglyceridemic effect, there is a dose threshold of at least 1 g of DHA per day. Only one study (Caslake et al. 2008) reported a significant effect on the plasma lipid profile following a dose of DHA as low as 0.6 g per day, however, a greater number of subjects was used over an increased duration (8 weeks) compared to other studies, greatly increasing the statistical power of the study.

Total cholesterol and LDL cholesterol are important determinants of atherosclerotic plaque formation, contributing to determining associated cardiovascular risks. An 8% increase in LDL cholesterol after supplementation with DHA, but not EPA, has been reported in a study testing the effects of an equal dose of EPA or DHA for a period of 6 weeks (Mori et al. 2000). Despite the reported increase in LDL cholesterol after DHA supplementation, in this study it was suggested that the increased LDL particle size may have represented a shift to less atherogenic particles, in which case, the parallel increase in HDL2 cholesterol and decrease in TAG may have represented a more favorable lipid profile than that seen after EPA supplementation (Mori et al. 2000). An increased LDL cholesterol concentration after high DHA fish oil supplementation has been reported by others (Caslake et al. 2008; Milte et al. 2008), while systematic reviews have demonstrated that DHA is associated with significant increases in LDL cholesterol in patients with and without hypertriglyceridemia (Jacobson et al. 2012; Wei and Jacobson 2011). Further, products with EPA that lack DHA did not significantly raise LDL levels when compared with placebo or control products (Ballantyne et al. 2012; Bays et al. 2011; Yokoyama et al. 2007). In contrast, other studies have found no significant differences in total and LDL cholesterol between high DHA fish oil and placebo oil groups (Buckley et al. 2004; Krebs et al. 2006; Ottestad et al. 2012). Within these studies participants typically had normal baseline TAG levels and, as such, no significant changes in LDL

cholesterol were observed, although an increase in LDL was significantly related to baseline TAG measures in the DHA treated group (Jacobson et al. 2012).

Two review papers concluded that fish oil supplementation affects HDL cholesterol (Balk et al. 2006; Harris 1997), however, evidence on the specific effects of high DHA fish oil on HDL cholesterol is inconsistent. In some studies, no change has been found in HDL cholesterol after consumption of high DHA fish oil (Buckley et al. 2004; Mann et al. 2010; Molinar-Toribio et al. 2015; Ottestad et al. 2012), while other trials showed an increase in HDL cholesterol concentration (Caslake et al. 2008; Hill et al. 2007a; Pedersen et al. 2010). The latter findings are further supported by a study demonstrating the increased efficiency of DHA over EPA where a decrease in HDL cholesterol was observed in groups consuming a higher EPA diet (salmon diet, DHA: EPA = 1.8: 1; or fish oil capsules, DHA: EPA = 0.7: 1) compared with a higher DHA diet (cod diet, DHA: EPA = 3.8: 1) (Gunnarsdottir et al. 2008).

Although some trials using high DHA fish oil have reported that blood pressure tended to decrease over the course of the study (Finnegan et al. 2003; Krebs et al. 2006; Sjöberg et al. 2010), no statistically significant differences from the baseline were actually recorded. Nevertheless, a 16-week, randomized trial by Pedersen et al. (2010), reported a significant decrease in both systolic and diastolic blood pressure in teenage boys following the consumption of high DHA fish oil, compared with the control group.

In a number of studies, DHA has been associated with improvements in HR (Dallongeville et al. 2003; Mozaffarian et al. 2006). During submaximal exercise, HR was reduced following high DHA fish oil supplementation, with no observed effects on peak exercise HR. Although this latter observation is consistent across several studies (Buckley et al. 2009; Macartney et al. 2014; O'Keefe Jr et al. 2006), one study reported a positive effect of high DHA fish oil supplementation in lowering peak HR during incremental workloads to exhaustion (Peoples et al. 2008). As for resting HR, high DHA fish oil consumption has been shown to lower resting HR in subjects with a history of myocardial infarction (Mozaffarian et al. 2005; Ninio et al. 2008; O'Keefe Jr et al. 2006), while the resting HR remained unchanged in well-trained subjects (Macartney et al. 2014). There is also a solid body of evidence that supports the positive impact of supplementation with n-3 LC-PUFA on HRV (Brouwer et al. 2002; Holguin et al. 2005; Macartney et al. 2014; Ninio et al. 2008; O'Keefe Jr et al. 2006; Sjöberg et al. 2010), and in one case (Brouwer et al. 2002) a strong association was displayed between DHA and HRV, but not EPA and HRV.

The selective antiarrhythmic effects for DHA over EPA are demonstrable when provided as purified fatty acids in the diet of rats (McLennan et al. 1996), and the heart rate in healthy humans is selectively lowered by DHA (Grimsgaard et al. 1998). Evidence suggests that antiarrhythmic protection, heart rate slowing, and optimised cardiac contractile function are best achieved through regular consumption of fish or fish oil which promotes incorporation of DHA into membrane lipids; however, many of the effects observed *in vitro* await validation in dietary-modulated cells known to have omega-3-derived arrhythmia protection *in vivo*. Data from *in vitro* (Phang et al. 2009), *ex vivo* (Phang et al. 2012a; Phang et al. 2012b) and *in*

vivo human studies (Park and Harris 2002; Phang et al. 2013) suggest that there are sex-dependent differences in platelet aggregation in response to EPA or DHA. In study by Din et al. (2008), EPA specifically reduced platelet aggregation in males, while Phang et al. (2013) reported that DHA intake was shown to decrease platelet aggregability in females. This may explain the lack of effect on platelet aggregation following DHA supplementation in previously published studies conducted predominantly or exclusively in males (Conquer and Holub 1996; Nelson et al. 1997).

Although the molecular mode of action of DHA is still not fully understood, it has been reported that a significant portion of the (patho)-physiological effects of DHA is mediated by its oxidative metabolites, i.e. eicosanoids and other oxylipins (Ostermann and Schebb 2017). It should be noted that none of the studies reported in the heart section (4.1) of this review used a quantitative targeted DHA derived oxylipin method to allow the comprehensive monitoring of DHA supplementation-induced changes in the pattern of oxylipins, to understand its biology in comparison with EPA. Westphal et al (2011) describe the pathways and some effects of the cytochrome P450 epoxy and hydroxy docosanoids derived from DHA, which may explain the many parallels that the physiological effects of dietary fish oil and myocardial membrane DHA share with attenuation of Ca²⁺ overload.

Based on the above evidence, it can be concluded that dietary DHA has been shown to play an important role in modulating the Omega-3 Index, reducing TAG, improving total cholesterol and LDL cholesterol, and decreasing HR and HRV. Further, these effects are not shared, or are shared to a lesser extent, by dietary EPA. Due to inconsistencies and, therefore, inconclusive results, the possible effects of DHA on HDL cholesterol and blood pressure have not been reported. The disparate intakes and physiological mechanisms underpinning different clinical endpoints and selectivity for DHA, plus the common employment of high EPA fish oil for clinical trials, can perhaps explain the sometimes contradictory outcomes of fish oil clinical trials, and provide insight to nutritional optimisation of cardiac function and selection criteria for clinical trials based on an improved hypothesis regarding mechanism of action. The striking difference of sex-specific responses to EPA or DHA administration on platelet aggregation are not only intriguing, but provide clear evidence that much greater attention should be focused on selecting the population to be tested in future studies. This will likely result in more conclusive and informative studies, which will allow the filling of important knowledge gaps that still exist.

4.2. DHA in the brain and visual function

The brain has a unique fatty acid composition with high levels of palmitate (16:0), the long chain omega-6 PUFA arachidonic acid, and DHA (Brenna and Diau 2007; Crawford et al. 1976; Dyll 2015), but low levels of the other n-3 PUFA, especially EPA, which is reported to be at a concentration typically 250–300 times lower than DHA (Chen et al. 2009). Compared with other tissues, such as liver or plasma, the brain has a very high concentration of DHA (Pifferi et al. 2012). It has been suggested that the brain maintains DHA levels primarily via the uptake of DHA from lipids in circulating blood through the

blood-brain barrier, rather than possessing particularly active endogenous biosynthesis, which is reportedly lower in the brain compared to other tissues (DeMar et al. 2006). There is evidence that a major transporter of DHA to the brain is in the form of lyso-phosphatidylcholine (sn 2-DHA-PC), which is taken up by a specific transporter known as MFSD2A, located in the blood brain barrier (Lagarde et al. 2016). Consequently, a mounting number of studies are focusing on the role for DHA in brain development and the prevention of neurodegenerative and neuropsychiatric disorders.

It is widely accepted that DHA is necessary for the growth and maturation of infants' brain (Koletzko et al. 2008; Simmer et al. 2011). Though infants have been reported to be rather efficient at biosynthesis of DHA from shorter and less unsaturated omega-3 fatty acids (Neu and Polin 2012), this endogenous production can be insufficient, especially during the rapid phase of brain growth and development when DHA is needed in large amounts. Accordingly, it has been argued that DHA needs to be provided to preformed infants via external sources, such as in placental transfer from maternal DHA stores during pregnancy and/or maternal milk after birth (Innis 2005).

Despite the existence of robust biochemical evidence for the fundamental role of DHA in the brain, results of *in vivo* studies are varied and, thus, inconclusive. Although many human studies, including follow-up studies, were unable to record statistically significant differences between treatment and control groups (Hirayama et al. 2004; Makrides et al. 2009; Smithers et al. 2010), the beneficial effects of high DHA fish oil supplementation was observed in the communicative development of infants (Meldrum et al. 2012) and in neurobehavioral outcomes, in children, such as school attendance rates (Hamazaki et al. 2008). An animal study conducted by Clouard et al. (2015) also reported that maternal high DHA fish oil supplementation beneficially affected the neurobehavior of offspring after weaning. On the contrary, a follow-up study of a double-blind, randomized, trial reported a negative effect of high DHA fish oil supplementation during the first 4 months of lactation on the cognitive abilities of offspring (Cheatham et al. 2011). The authors suggested that the first 4 months of life might not be an appropriate time to receive high n-3 LC-PUFA supplementation, while another possible explanation for these negative effects is the recruitment of Danish mothers, who naturally have a higher intake of n-3 LC-PUFA, compared to participants from other studies. According to these somewhat inconclusive and, at times, contradictory findings, two separate meta-analysis studies of randomized, controlled trials were implemented. Both studies were unable to conclusively support or refute that n-3 LC-PUFA supplementation in women during pregnancy, or during lactation, positively or negatively affects the neurodevelopment of their offspring (Delgado-Noguera et al. 2015; Gould et al. 2013). It has been suggested that the apparent effects of DHA on brain development may be affected by the gender of the child, which is a possible explanation for the lack of conclusive findings in other studies. Makrides et al. (2009) found that a high-DHA (1% of total fatty acids) in early life did not increase the Mental Development Index of overall preterm infants, however it did show improvement in girls. The apparent increased physiological importance of DHA in developing girls may also be substantiated by the increased rate of

endogenous synthesis of DHA from the precursor fatty acid α -linolenic acid that has been observed in girls, and not boys (Burdge and Calder 2005). Such gender specific effect was also found in other fatty acids. It was reported that anxiety and hostility scores positively correlate with serum levels of long-chain saturated fatty acids in women, but not in men (Yanagisawa et al 2002).

It is known that cognitive function reaches its peak during middle adulthood, and declines in the later aging process. Brain aging is accompanied by many widespread changes, such as a reduction in brain volume and weight (Anderton 2002), increased oxidative stress (Perluigi et al. 2014), altered membrane lipid content (Svennerholm et al. 1997) and damage to DNA (Canugovi et al. 2013). The aging brain is more susceptible to neurodegenerative diseases, including Alzheimer's disease, Parkinson's diseases, and impaired cognition. Clinical trials aimed at assessing the possible effect of DHA and n-3 LC-PUFA supplementation on neurodegenerative conditions within adults, have reported mixed results. Though several studies using high DHA fish oils found it had no effect on cognitive function (Andrieu et al. 2017; Dangour et al. 2010; Jackson et al. 2012a; Phillips et al. 2015; Stough et al. 2012), other trials reported a significant improvement in memory function in subjects provided with high DHA fish oil (Lee et al. 2013; Stonehouse et al. 2013). Sinn et al. (2012) directly compared the effects of DHA vs EPA in participants with mild cognitive impairment. Only in the high DHA group, were changes in memory exhibited, and verbal fluency significantly improved. In a study focusing on the effects of DHA in memory improvement (Yurko-Mauro et al. 2010), elderly individuals receiving 0.90 g/day of pure algal DHA exhibited improved learning and memory functions, compared to those receiving the placebo oil (corn and soy oil). Furthermore, a recent meta-analysis of 15 human interventional trials indicated that DHA, alone or combined with EPA, contributed to improved memory function in older adults with mild memory complaints (Yurko-Mauro et al. 2015).

The beneficial effect of DHA in the brain might be related to its unique membrane properties. It was reported that DHA had a much higher tendency to accumulate into sphingomyelin/cholesterol-rich raftlike domains on the membrane than EPA, making it having a much greater potential to affect cell signalling by modifying the structure and composition of these lipid rafts (Williams et al 2012). Crawford et al (2013) proposed a theory for the irreplaceable role of DHA in neural cell. They suggested that the unique molecular structure of DHA allows for quantum transfer and communication of π -electrons, explaining the precise depolarization of retinal membranes and the cohesive, organized neural signalling which characterizes higher intelligence. DHA-derived lipid mediators could also play a key role in its beneficial effect. For example, Neuroprotectin D1 (NPD1, 10R-17S-dihydroxy-docosahexaenoic acid), a DHA-derived mediator, is biosynthesized in response to injury and may have therapeutic potential in a wide range of neurodegenerative conditions (Bazan 2013).

Though a species-specific difference in DHA metabolism might exist, caution should be exercised when directly translating findings across species. Animal studies have the advantage over human studies to allow a much greater environmental

control and, thus, often result in more robust experimental designs. Accordingly, unlike clinical trials, animal studies investigating the effect of high DHA fish oil consistently report statistically significant results. For example, Fernandes et al. (2008) demonstrated that long-term, high DHA fish oil treatment facilitated functional recovery after ischemic brain damage rats; Trofimiuk and Braszko (2011) found that long-term administration of high DHA cod liver oil could prevent the deleterious effects of chronic restraint stress on recall and spatial memory in Wistar rats; and Clouard et al. (2015) found that maternal high DHA fish oil supplementation resulted in positive offspring neurobehavior in pigs.

A growing number of observational and epidemiological studies have suggested that there may be some relationship between mental illness, in particular depression, and a reduced dietary intake of n-3 LC-PUFA (Ross et al. 2007). However, controversy exists as to whether EPA, DHA, or both, are responsible for the reported benefits (Song et al. 2016). Mental disorders are generally characterized by a combination of abnormal thoughts, emotions, behaviour and relationships with others; depression is an increasingly common mental disorder, with a global estimate of over 300 million people in the world being affected by it (WHO 2017). With vast positive ramifications, a greater understanding of the possible roles of n-3 LC-PUFA in the treatment of mental disorders is warranted. In a study conducted by Clayton et al. (2009), clinician ratings of depression were significantly lower after high DHA fish oil supplementation, while in an animal study conducted by Davis et al. (2017), DHA was also found to produce beneficial effects on depressive-like behaviour. In contrast, many other human studies investigating the effect of high DHA fish oil on depression found no positive impact of high DHA fish oil over the placebo in treating depression (Grenyer et al. 2007; Itomura et al. 2005; Makrides et al. 2010; Mozurkewich et al. 2013; Rees et al. 2008; Rogers et al. 2008; Silvers et al. 2005). These findings are consistent with the meta-analyses of randomized, controlled trials performed by Martins (2009), and Sublette et al. (2011), in which EPA, rather than DHA, is identified as the effective n-3 LC-PUFA in the treatment of depression, in conjunction with the appropriate medication. In the latter study, the authors suggest the underlying mechanism might be that EPA has some non-brain effects that cause secondary brain changes. This is supported studies reporting that EPA supplementation increased brain N-acetyl-aspartate, a marker for neuronal health in bipolar disorder (Frangou et al. 2007), and the cerebral phosphomonoesters: phosphodiester ratio, which is an indicator of phospholipid turnover and reversed brain atrophy in a subjects with major depressive disorders (Puri et al. 2001). Furthermore, Zhang et al. (2017) reported that EPA was significantly more effective than DHA in mitigating oxidative stress, while Song et al. (2016) concluded that combining a higher dose of EPA with a lower dose of DHA (e.g. at 2–4: 1 or higher) is effective in treating depression. In the latter study, DHA-rich supplementation appeared to be ineffective.

Despite the inconsistent results in relation to the effects of DHA on mood and cognitive factors, it seems plausible that DHA might possess an unclarified role in physiological brain function given that it is heavily concentrated in the mammalian

brain. This has been studied in many clinical trials and *in vitro* studies. Two randomized, controlled trials conducted by Jackson et al. (2012c) and (2012b), found that high DHA fish oil could increase concentrations of oxyhemoglobin and total levels of haemoglobin, an indication of increased cerebral blood flow during a cognitive task. It is also notable that DHA has been shown to modulate nitric oxide synthesis (Engström et al. 2009), and improve brain tissue oxidative status, such as increasing SOD activity and decreasing lipid peroxidation (Avramovic et al. 2012). These physiological effects of DHA could direct us towards understanding the potential mechanisms by which DHA or n-3 LC-PUFA exerts a positive effect on cognitive function, however, additional studies are necessary, and warranted, to provide a greater understanding of such phenomena.

Aside from the brain, the highest level of DHA in the human body is found in the eye, more specifically, in the retina (Neuringer et al. 1986). DHA is needed for the process of transforming light into an electrophysiological signal (Niu et al. 2001) and for the regeneration of the light sensitive pigment in the retina – rhodopsin (Bush et al. 1994). For this reason, several randomized, controlled trials have investigated the effects of DHA supplementation on visual function in both preterm and term infants. Studies on preterm infants administered formulas containing high DHA and have reported improved retinal function (Birch et al. 1992) or visual acuity (Carlson et al. 1993; Carlson et al. 1996; Faldella et al. 1996) following supplementation with DHA, compared to those fed formula without DHA or with low-DHA content (Smithers et al. 2008). In contrast, studies on infants born at term that were supplemented with DHA-containing formulas obtained mixed results, with some reporting an improvement in visual function (Birch et al. 2005; Birch et al. 1998; Hoffman et al. 2003) and others reporting no effects (Bakker et al. 1999; Hurtado et al. 2015; Lauritzen et al. 2004; Makrides et al. 2000). No studies reporting negative effects on visual function following DHA supplementation in infants could be found. Contrasting infants, only a small number of studies assessed the effects of high DHA fish oil supplementation on visual function in adults and, when performed, produced varied results. Stough et al. (2012) found that participants receiving high DHA tuna oil had better right eye visual acuity post-treatment compared to individuals in the control group, while Souied et al. (2013) found no significant difference in visual acuity between high DHA fish oil and placebo groups.

Based on the evidence detailed above, it can be concluded that DHA is likely to play important roles in brain development and function, which are different from EPA, especially in terms of neurodegenerative conditions; EPA is also important, specifically in terms of depression. Nevertheless, there is currently not enough robust information available to draw conclusions on the actual roles and effects of DHA on brain development and physiological function, or on visual function.

4.3. DHA in other medical/physiological aspects

A total of 36 studies focused on the effects of high DHA fish oils on other medical aspects in humans and animals. Of these studies, 27 trials analysed the effects of high DHA fish oil on immune function and inflammation, and growth/Body Mass

Index. Therefore, these two major medical aspects will be discussed in this section. The reminder of studies focused on the effects of high DHA fish oil on gestational diabetes (Zhou et al. 2012), asthma (Atwell et al. 2013; Manley et al. 2011), bone (Damsgaard et al. 2012), skin (Tihista and Echavarria 2017), colonic disease (Kohn et al. 2000), liver (Du et al. 2004), sex hormone production (Castellano et al. 2011) and the gut (Patten et al. 2002); collectively there are not enough studies to discuss and draw any conclusion for the possible effects of high DHA fish oil on these medical aspects.

4.3.1. DHA in inflammation and immune function

EPA has traditionally been considered a more potent anti-inflammatory fatty acid than DHA (Molinar-Toribio et al. 2015), with some studies finding high DHA fish oil had no direct effect on human immune cell function (Kew et al. 2003; Wallace et al. 2003) and the production of inflammatory markers (Freund-Levi et al. 2014; Hill et al. 2007b; Sabour et al. 2012). In contrast, other studies suggest that DHA has a potent anti-inflammatory effect, possibly more potent than that of EPA (Halade et al. 2010; Oliver et al. 2012; Weldon et al. 2007). As such, it has been proposed that DHA, and not EPA, is the most potent n-3 fatty acid for suppressing glomerulonephritis, and extending the life span of systemic lupus erythematosus-prone short-lived B × W mice, possibly via inhibition of IL-18 induction and IL-18-dependent signalling (Halade et al. 2010). Kew et al. (2004) found that supplementation with DHA, but not EPA, suppressed T lymphocyte activation, while Gorjão et al. (2006) found that high DHA fish oil increased the phagocytic activity of neutrophils and monocytes. Vedin et al. (2008) and Ramirez-Ramirez et al. (2013) reported that high DHA fish oil was effective in reducing the levels of proinflammatory cytokines, which was supported by a similar study in animals (LeBlanc et al. 2008). There are also studies that demonstrate the beneficial effects of high DHA fish oil on human lymphocyte function and neutrophil function (Marques et al. 2015; Santos et al. 2013; Toupchian et al. 2016; Zhang et al. 2017). It has also been reported that both DHA and EPA attenuate apoptosis and improve cell viability, however DHA was found to be more effective than EPA (Zhang et al. 2017). Cabello et al. (2017) reported a more effective inhibitory effect with a mixture of EPA plus DHA, than either DHA or EPA alone, although independently, DHA was more potent than EPA. Dawson et al. (2012) studied the effects of DHA on the pattern of global gene expression in blood cells from hypertriglyceridemic males using microarray gene chip analysis. Their data showed that DHA supplementation significantly suppressed the expression of the LDL receptor and cathepsin L1, which provided supporting evidence for the anti-inflammatory effects of DHA supplementation.

In summary, both EPA and DHA have been shown to possess some important anti-inflammatory and immune functions, but profound differences in their biological actions are manifest. Current knowledge is insufficient to clearly understand the exact roles, mechanisms, and potential of the two fatty acids, both in isolation and in combination. Nevertheless, current evidence clearly suggests that rather than looking at which one of the two fatty acids has the most anti-inflammatory properties, understanding that they both play important roles, and that

any knowledge gained on their specific mechanisms of action will certainly improve the efficiency of their utilisation.

4.3.2. DHA and body mass index

The metabolic and CVD risk in humans significantly increases in overweight/obese individuals (Du et al. 2015). Several studies have focused on the possible anti-obesity effects of fish oil high in EPA, using the traditional 18/12, EPA/DHA ratio. Results from a meta-analysis by Du et al. (2015) indicated that supplementation with fish oil was not associated with a reduction in body weight and BMI when compared to control groups. However, in the few studies available where high DHA preparations were used, it was shown that high DHA fish oil could be effective in reducing body weight and BMI (Munro and Garg 2013b), body fat (Hill et al. 2007a), and waist circumference and waist: hip ratio (Cussons et al. 2009; Munro and Garg 2013a).

In a study by Kunesova et al. (2006), 20 obese females (BMI = 40–45 kg/m²) were given a very low-calorie diet with 2.8 g/day of high EPA fish oil (DHA: EPA = 1: 2) or a placebo. The authors reported an increase in phospholipid DHA that was significantly correlated with a decrease in BMI, suggesting that DHA may be the active component for weight loss in obese females. It is becoming increasingly evident that there is a marked sex difference in the metabolism of n-3 LC-PUFA, with studies showing significantly higher concentrations of DHA in females, as opposed to males (Crowe et al. 2008). Hence, it is likely that there are gender differences in weight change in response to supplementation with n-3 LC-PUFA over time (Munro and Garg 2013b). Other studies on males and females (BMI = 27.5–32.5 kg/m²) that were given one of four supplements/diets including sunflower oil capsules, cod diet (DHA: EPA = 3.8: 1), salmon diet (DHA: EPA = 1.8: 1) or fish oil capsules (DHA: EPA = 0.7: 1), demonstrated greater weight loss in males than in females (Gunnarsdottir et al. 2008; Thorsdottir et al. 2007). In these studies, the average weight loss was 6.5 kg for males and 4.2 kg for females. Interestingly, no significant differences were observed when overweight insulin-resistant females were given omega-3 supplements (4.2 g/d EPA + DHA, DHA: EPA = 2.2: 1) in combination with a very low energy diet (Krebs et al. 2006), or when 138 overweight to obese male and female participants were given omega-3 supplements (3 g/day EPA + DHA, DHA: EPA = 0.2: 1) coupled with an energy-controlled diet and exercise (DeFina et al. 2010). The conflicting results reported above could be due to differences in the amount of omega-3 consumed and the different DHA: EPA ratios used in the studies, as previously described for several studies focusing on omega-3 metabolism (Ghasemifard et al. 2014).

Given the evidence above, it can be concluded that DHA appears to have a body weight lowering effect in obese females, yet current knowledge is insufficient to clearly quantify the extent of its action, and understand the exact roles, mechanisms and potentials of EPA and DHA, in isolation and/or in combination.

5. Conclusions

Although there is sufficient evidence to strongly suggest that DHA plays a series of important and fundamentally beneficial and health promoting roles, the currently available scientific literature describing the roles and potential metabolic effects of

DHA, as an individual nutrient and/or in comparison to EPA, is far from conclusive. The exact mechanisms and the extent of these actions are not yet fully elucidated. DHA appears to play important roles, different to those of EPA, in heart, cardiovascular, brain and visual functions. Important differences have been observed in the possible physiological responses to increased dietary DHA administration relative to age. Vastly different roles and effects were noticed in infants, adults, and elderly individuals. This was also the case in relation to gender, with EPA and DHA playing different roles in male and females. A major limiting factor in our current understanding of such physiological phenomena is directly linked to the intrinsic difficulties in developing and implementing statistically powerful and biologically meaningful intervention trials, as there are several sources of variability which are now increasingly becoming apparent. There is a growing, globally significant market and industry for DHA, and n-3 LC-PUFA in general. New, and more efficient, omega-3 concentration techniques are being developed simultaneously with major agribusiness innovations, such as the production and extraction of novel DHA rich oils from microalgae fermentation and genetically modified oilseed crops. Although omega-3 fatty acids have been extensively studied in the last few decades, there are still many unknowns driving the ongoing need for specific, targeted, tailored, well designed and conceived experimental trials.

Conflict of interest

The Authors, SGF and GE are employees of Nu-Mega Ingredients Pty Ltd, a manufacturer and supplier of high DHA fish oil products. Nu-Mega Ingredients Pty Ltd. provided support in the form of salaries for authors SGF and GE, but did not have any additional role in the study design, data collection, analysis and interpretation, decision to publish, or preparation of the manuscript.

The authors' responsibilities

SGF, AJS and GE conceived the study. SGF, AJS and GMT developed the selection criteria. SGF and FW performed the literature search and review. SGF, FW, AJS, GE and GMT wrote the paper.

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