Global Organization for EPA and DHA Omega-3s

HAZARD CHARACTERIZATION OF THE LONG-CHAIN POLYUNSATURATED N-3 FATTY ACIDS, DHA, EPA AND DPA

Prepared for and on behalf of the GOED membership by Spherix Consulting, Inc.

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Executive Summary

Background

Interest in long-chain n-3 fatty acids has been growing with new research in the areas of potential beneficial effects on cardiovascular disease, renal disease, inflammatory disorders (arthritis, psoriasis, colitis), asthma, infectious diseases and cancer. In response to consumer interest in consumption of long-chain n-3 fatty acids to derive health benefits, several regulatory and authoritative assessments have been completed to evaluate levels of ingestion determined to provide an adequate intake as well as establish a tolerable upper intake level of these fatty acids.

The most important natural sources of n-3 PUFA are marine organisms (e.g., fish, seafood, algae), which are fed, directly or indirectly, from marine phytoplankton, the primary producer of n-3 in the trophic chain. PUFA in marine oils are usually found as triglycerides and linked to the sn-2 position of the glycerol backbone, which is related to a higher stability against oxidation. Fish oil usually contains higher amounts of n-3 PUFA than seed oils or microalgae and therefore, has become a source of raw material for n-3 concentrate production and an ingredient in n-3 enriched food products (Rubio-Rodriguez et al., 2010).

The vast majority of fish oil concentrates sold globally, including those sold in North America are EPA and DHA ethyl ester concentrates. A small percentage of fish oil concentrates on the market are natural TAGs. Fatty acid ethyl esters are a class of lipids that are derived by reacting free fatty acids with ethanol (alcohol). Called trans-esterification, the process involves a reaction whereby the glycerol backbone of a TAG is removed and substituted with ethanol (Rubio-Rodriguez et al., 2010). The resulting ethyl esters allow for the fractional distillation (concentration) of the long chain fatty acids at lower temperatures. Commonly referred to as molecular distillation in the fish oil industry this step allows for the selective concentration of the EPA and DHA fatty acids to levels greater than found naturally in fish oil. The resulting EPA and DHA concentrate (as ethyl ester form) is typically the end product that is subsequently marketed and sold as "Fish Oil concentrate".[1]

The Steering Committee of the Norwegian Scientific Committee for Food Safety (2011) evaluated positive health effects of n-3 fatty acids in the areas of cardiovascular diseases, inflammation and immune function, CNS and mental health functioning. They put forth the

[1] Because the term fat or oil refers only to TAG, the EPA and DHA ethyl ester concentrate is, by definition, no longer a fat or oil. Because ethyl esters rarely occur in nature, this affects the way they are digested and absorbed in the body.

opinion that 1) in patients given either 0.8 g EPA and DHA or 1.8 g of EPA as ethyl ester daily, the risk of cardiovascular events and mortality was reduced; 2) consumption of 0.25 to 0.50 g of EPA and DHA daily decreases the risk of mortality from coronary heart disease and sudden cardiac death; 3) 1.6 to 7.1 g/day EPA and DHA might lessen symptoms o reduce the use of anti-inflammatory drugs in patients with rheumatoid arthritis; 4) EPA and DHA in doses ranging from 0.5 to 2.8 g/day have reported positive effects in various CNS disorders. FAO/WHO (2010) concluded that the total n-3 fatty acid intake can range between 0.5 to 2E% (energy percent or percent of total energy intake; 0.5 E% is equivalent to 1.3 g n-3 fatty acids per day). The Nordic Nutrition Recommendations (NNR Project Group 2004) have no specific recommendations for EPA, DPA or DHA but recommend up to 1E% (2.0 to 2.6 g) of n-3 fatty acids per day. The Nordic recommendations are adopted in the Norwegian recommendations and by Sweden. Taking into account that available data are insufficient to derive an average requirement, an AI of 0.25 g/day for EPA and DHA for adults was set on the basis of scientific evidence that 0.25 to 0.50 g of EPA and DHA daily decreases the risk of mortality from coronary heart disease and sudden cardiac death.

In 2002, the Institute of Medicine concluded that insufficient data were available to define dietary reference intakes (DRI) for eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), noting only that EPA and DHA could contribute up to 10% toward meeting the Adequate Intake (AI) for α-linolenic acid (ALA) (report available online at http://www.iom.edu/)[2]. The Technical Committee on Dietary Lipids of the International Life Sciences Institute North America sponsored a workshop on 4-5 June 2008 to consider whether the body of evidence specific to the major chronic diseases in the United States - coronary heart disease (CHD), cancer, and cognitive decline-had evolved sufficiently to justify reconsideration of DRI for EPA+DHA (Harris et al 2009). The workshop participants arrived at these conclusions: 1) consistent evidence from multiple research paradigms demonstrates a clear, inverse relation between EPA+DHA intake and risk of fatal (and possibly nonfatal) CHD, providing evidence that supports a nutritionally achievable DRI for EPA+DHA between 250 and 500 mg/d; 2) because of the demonstrated low conversion from dietary ALA, protective tissue levels of EPA+DHA can be achieved only through direct consumption of these fatty acids; 3) evidence of beneficial effects of EPA+DHA on cognitive decline are emerging but are not yet

[2] According to the Continuing Survey of Food Intakes by Individuals (CSFII) 1994–1996 and 1998 (now replaced by NHANES), the Panel concluded that, because roughly 10% of the total (n-3) PUFA intake currently comes from EPA and DHA, 10% of the AI for ALA could be met by these 2 (n-3) LCPUFA.

sufficient to support an intake level different from that needed to achieve CHD risk reduction; 4) EPA+DHA do not appear to reduce risk for cancer; and 5) there is no evidence that intakes of EPA+DHA in these recommended ranges are harmful.

There is consensus among several authoritative and regulatory bodies that intake of EPA and DHA is associated with potential beneficial outcomes; however, there is inconsistent or missing guidance on safe upper intake levels for these fatty acids. With regard to the conclusions from the Technical Committee on Dietary Lipids of the International Life Sciences Institute North America Workshop (Harris et al 2009) , the basis of their conclusion that there is no evidence of harm at intakes of EPA and DHA in the recommended ranges came from data available from three large trials, Japan Eicosapentaenoic Acid Lipid Intervention Study (JELIS) (Yokoyama et al 2007), Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione (GISS 1999), and GISSI-Heart Failure (GISSI 2008), that reported no clinically relevant adverse effects in over 35,000 individuals. In 1997 the FDA determined that intakes of EPA+DHA of up to 3 g/d are safe for the general population (Federal Register Vol. 62, No. 108: Thursday, June 5, 1997. 21CFR §184 Substances Affirmed as Generally Recognized as Safe: Menhaden Oil Final Rule). This determination was based on a consideration of the effects of fish oils on bleeding time, glycemic control, and low-density lipoprotein (LDL) cholesterol.

The IOM (2005) in its report on dietary reference intakes for dietary fats indicated that at the time of their review, there were not sufficient data to support establishing a Tolerable Upper Intake Level (UL) for EPA and DHA. The conclusion reached was that while there was evidence to suggest that high intakes of n-3 polyunsaturated fatty acids, particularly EPA and DHA, may impair immune response and result in excessively prolonged bleeding times, data was insufficient to establish a UL. Studies on immune function were done *in vitro* making it difficult or impossible to extrapolate to an *in vivo* response. Adverse effects in specific populations on bleeding time and incidence of bleeding could not be linked to EPA and DHA as the sole cause of these effects.

The Norwegian Food Safety Authority requested the Norwegian Scientific Committee for Food Safety, VKM, (VKM 2011) to evaluate the positive and negative human health effects from intake of n-3 fatty acids from food supplements and fortified foods. They investigated the following negative health effects: bleeding tendency, lipid peroxidation, impaired inflammation

and other immune functions, impaired lipid and glucose metabolism and gastrointestinal disturbances. Based on the review, they concluded that it was not possible to identify clear adverse effects from EPA and/or DHA, which could be used for setting upper intake levels. The conclusion was based on a determination that no negative health effects were seen regarding bleeding complications, none of the oxidative stress biomarkers noted are defined as risk factors of disease making the clinical relevance of lipid peroxidation unclear, the clinical relevance of an increase of low-grade systemic inflammation is uncertain, the effect on LDL-cholesterol is minor and of uncertain clinical significance and negative effects regarding gastrointestinal function could not be ascribed specifically to EPA and/or DHA.

In 2009, the German Federal Institute for Risk Assessment (BfR) evaluated DHA and EPA, regardless of the source (fish oil, algae oil or novel fatty acid ethyl esters) according to the criteria of the rule of Article 8 of Regulation (EC) No 1925/2006 of the European Parliament and Council of December 20th 2006 on the addition of vitamins, minerals and certain other food additives. BfR[3] recommended that no more than 1.5g unsaturated n-3 fatty acids (long-chain n-3) from all sources should be consumed per day and that food not typically containing fat (e.g., water-based beverages) should not be enriched with n-3 poly-unsaturated fatty acids (PUFAs). Although selected studies evaluating several health based endpoints were summarized in the review, the basis for the limit of 1.5 g was not elucidated.

Objective

At the request of the Global Organization for EPA and DHA Omega-3s (GOED), Spherix Consulting prepared a weight of the evidence hazard characterization of the long-chain polyunsaturated n-3 fatty acids, DHA, EPA and docosapentaenoic acid (DPA). The review consolidates and presents a critical analysis of the relevant pivotal information to address the hazard identification of DHA, EPA and DPA in general and with specific reference to fish- and algal-derived oils containing 20% or more of EPA+DHA+DPA as native triacylglycerides (TAGs), reconstituted TAGs or the ethyl esters.

The hazard characterization was completed utilizing published clinical studies supported by review papers and meta-analyses; methodological deficiencies and confounders were identified in studies reviewed that could impact their scientific interpretation. Evaluation of each

[3] Opinion No. 030/2009 of BfR of May 26th, 2009.

individual study considered objectives, study design, endpoints and outcome, exposures concomitant medications and confounders, and interpretation. When possible, amounts and or ratios of EPA, DHA and DPA, and forms (TAG versus ethyl ester of fatty acids) were noted; however, not all studies provide this compositional information.

Methodology

Spherix retrieved and evaluated studies reported in the authoritative reviews from German Federal Institute for Risk Assessment (BfR 2009) and the Norwegian Scientific Committee for Food Safety (VKM 2011) of hazard identification for these fatty acids. The studies presented in these reports covered the period up to 2008. Therefore studies published from 2008-2012 that included data on health endpoints of interest for both healthy and health-compromised or vulnerable populations were retrieved for analysis from a literature search of publicly available data; studies cited in systematic reviews and meta-analyses that were retrieved were searched for identification of additional relevant studies. The search strategy encompassed the terms: clinical, cardiovascular, omega-3 (fatty acid), eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, DHA, EPA, DPA, fish oil(s), cod liver oil(s), Crypthecodinium cohnii Seligo, DHASCO, alpha- linolenic acid, Lovaza, and AMR101 in combination with the safety end-point search terms listed below.

Safety end-points and adverse effects considered include the following:

- Blood lipids, LDL-cholesterol levels; oxidative stress resulting in lipid peroxidation;
- Bleeding parameters;
- Inflammation and modulation of immune parameters;
- Glucose homeostasis (blood glucose control in diabetes);
- Infant/child growth; and
- Gastrointestinal-and taste-related effects.

A summary of the inclusion/exclusion criteria used to identify evaluable studies for each safety end-point, followed by the background and hazard analysis for each endpoint is presented in the following chapters. A discussion of the findings from the critical weight of the evidence analysis is followed in each chapter by a table summarizing the key elements of each study considered evaluable in this review. Additional corroborative support from authoritative reviews and meta-analyses are presented, where relevant, in the discussion sections of the chapter.

Conclusions

Institute of Medicine (IOM 1998) defines the UL as "the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population." A UL cannot be determined when there are no reports of adverse effects from consumption or the data are insufficient or inadequate for a quantitative risk assessment. The conclusions reached on the basis of the hazard characterization for n-3 fatty acids conducted by Spherix Consulting agree with the conclusions reached by IOM (2005) and (VKM 2011) and are consistent with the assessment approach taken by IOM to derive a UL. A weight of the evidence evaluation of the safety of ingestion of n-3 fatty acids on blood lipids, bleeding parameters, inflammation and immune parameters, glucose homeostasis in diabetes, infant/child growth and gastrointestinal- and taste-related effects does not identify any studies appropriate to define specific intake levels or intake/response relationships that can be used to define a UL for these effects.

In conclusion, the following summarizes key findings regarding the upper range of intakes of n-3 fatty acids studied in both healthy and health-compromised individuals that demonstrate no clinically adverse outcomes or a clear association with results necessary to derive a UL for the evaluated hazards.

Studies evaluated the effects of n-3 fatty acid consumption on the lipid parameters total cholesterol, total triglycerides, LDL-cholesterol, HDL-cholesterol, and oxidative stress and lipid peroxidation. The results of consumption of EPA and/or DHA at intakes as high as 4.8 g EPA or 4.9 g DHA/d or 4.4 g EPA + 2.8 g DHA/d show no consistent dose-related effect on total cholesterol. While studies performed in healthy populations delivering up to 7.2 g EPA+DHA/d did not show any consistent effects on total triglycerides, studies in unhealthy populations at intakes up to 4.2 grams EPA+DHA/d showed consistent decreases in total triglycerides. Weight of the evidence suggests that in healthy populations intakes of up to 4.8 g of EPA/d, 4.9 g DHA/d, or 4.2 g EPA+DHA do not increase LDL cholesterol. In unhealthy populations, some studies showed increases in LDL-cholesterol at intakes up to 4.6 g EPA+DHA/d, however an equal number of studies showed no effect on LDL-cholesterol at intakes up to 4.0 g EPA+DHA/d; therefore no dose relationship between intake and adverse effects on LDL-cholesterol could be established. For HLD-cholesterol, studies in healthy and unhealthy populations at intake levels up to 7.2 g EPA+DHA/d showed no consistent dose-related effects.

Studies in both healthy and unhealthy populations looked at effects on specific lipid oxidation or oxidative stress parameters, which are difficult to interpret. Endpoints such as TBARS, lymphocyte phagocytic activity, in vitro or ex vivo determination of lag time and oxidation rate of LDL, and in vitro rate of formation of conjugated dienes do not appear to have a strong evidence base to support their validated in vivo relevance as biomarkers for a disease or compromised health state. Due to the lack of validation of lipid oxidation or oxidative stress endpoints as biomarkers for disease or health-compromised states, or risk thereof, it is not possible to interpret the results to determine any hazard to health from ingestion of EPA and/or DHA.

Assessment of the effect of consumption of n-3 fatty acids on bleeding parameters were evaluated in studies of both healthy and health-compromised individuals using intakes ranging from 1.08 to 36 g/day of n-3 fatty acids given for durations up to 1 year. Results suggest that findings of adverse effect of n-3 fatty acids on bleeding parameters are rare, inconsistently found, not associated with a dose-response and not linked to bleeding complications or clinically adverse outcomes.

The consumption of oils containing up to 2.1 g of EPA + 1.1 g DHA/day (3.2 g/day total omega-3s) do not appear to perturb immune homeostasis or suppress immune responses. Furthermore, EPA- and DHA-rich oils do not appear to induce inflammation, although the studies reviewed were relatively small in size, evaluated their effects on only a limited number of inflammatory markers, and the amount of EPA and DHA administered varied greatly.

Consumption of EPA- and DHA-rich oils at intakes up to 3 g/day of n-3 fatty acids consistently had no effect on glucose control or insulin production and studies delivering less than 11 g/day of n-3 fatty acids does not appear to reproducibly impair glucose control or insulin production. However, due to the presence of multiple confounding variables across studies, additional work on larger groups of diabetic patients should be performed to evaluate the effect of n-3 fatty acids on glucose control..

No adverse effects from DHA supplementation on infant and child growth have been seen in numerous studies that evaluated nursing mothers given fish oil supplements sufficient to attain DHA levels up to 1% DHA in breast milk, term infants given formula supplemented with up to 0.96% DHA for intervention periods from 4 weeks up to 1 yr or preterm infants fed formula with up to 1.1% DHA (of total fatty acids) to term.

Although gastrointestinal disturbances are frequently associated with intake of an oily substance, these effects can not be attributed to intake of n-3 fatty acids. Studies identified from the literature in both healthy and health-compromised individuals evaluated the effect of doses ranging from 0.2 to 6 g/day of n-3 fatty acids given for durations up to 2 years. Results from a detailed review of these studies suggest that intake of n-3 fatty acids is not associated with adverse gastrointestinal disturbances.

CHAPTER 1: EFFECTS ON BLOOD LIPIDS

Background

Lipids (triglycerides and cholesterol) are obtained either from the diet (exogenous pathway) or manufactured primarily by the liver (endogenous pathway). In the exogenous pathway, dietary lipids are first hydrolyzed during their passage through the digestive tract to monoglycerides, free fatty acids, lysophosphatidylcholine and cholesterol, and then absorbed by enterocytes, the primary absorptive cells that line the luminal wall of the small intestine (Stipanuk 2006). Although the mechanisms that mediate lipid uptake are unclear, once inside the enterocyte fatty acids are re-esterified into triglycerides, phosphatidylcholine, phospholipids, and cholesterol esters, packaged into lipoprotein complexes known as chylomicrons, exported into the intestinal lymph, and delivered into the blood stream via the thoracic duct. Intestinal enterocytes also produce very low-density lipoproteins (VLDLs) plus low levels of high-density lipoproteins (HDLs). The function of the chylomicrons and intestinal VLDLs is to transport the dietary lipids and cholesterol to muscle, adipose, and other tissues where the triglycerides are hydrolyzed by lipoprotein lipase (LPL) into free fatty acids. These free fatty acids are then absorbed and either broken down inside the cell for fuel via β-oxidation, or integrated into the cell membrane. In adipocytes, fatty acids can also be stored in lipid bodies for later use during times of energy deficit. The remaining triglyceride-poor, cholesterol rich- chylomicrons and intestinal VLDLs, also known as chylomicron and VLDL remnants, are then removed from circulation via the liver by binding to the low-density lipoprotein receptor (LDLR) and possibly LDLR-related protein-1 (LRP) that is expressed by hepatocytes (Herz et al., 1995; Rohlmann et al., 1998).

In the endogenous pathway, triglycerides and cholesterol are generated by the liver either by 1) the re-esterification of circulating free fatty acids, which may become available due to reduced uptake of free fatty acids by muscle and adipocytes, 2) the hydrolysis of stored triglycerides in adipocytes that occurs during times of energy deficit, such as fasting, or 3) the conversion of excess glycolytic intermediates. The resulting triglycerides and cholesterol are then either stored in the liver or packaged into VLDLs by the liver. If the latter, these particles enter the circulation and transport the triglycerides back to the peripheral tissues where they are hydrolyzed and absorbed. The remaining triglycerides in the liver-derived VLDLs, also known as intermediate-density lipoproteins (IDLs), are then further liberated of their fatty acid content

via hydrolysis by hepatic triacylglycerol lipase (HTGL) and converted to cholesterol-rich low-density lipoprotein LDLs. LDLs are then removed from circulation by LDLR. Importantly, elevated serum cholesterol and LDL-cholesterol are known risk factors of atherosclerosis (Cleeman, 2002, Executive Summary of the Third Report of the Expert Panel).

High-density lipoproteins (HDLs) are another subset of lipoprotein complexes in the blood. HDLs are much denser than chylomicrons, VLDLs, LDLs, and IDLs, and are secreted by the liver as small lipoprotein apo A-1-rich particles. Importantly, the primary function of HDLs is to accumulate cell-derived cholesterol and deliver it back to the liver for elimination in the bile. HDLs are also critical to the metabolism of VLDLs and chylomicrons because they donate the lipoproteins apo C-II and apo E to newly formed chylomicrons and VLDLs, which are necessary for LPL activation and LDLR-binding, respectively. In humans, HDLs also contain cholesteryl ester transfer protein (CETP), which allows them to exchange their cholesteryl esters for triglycerides in VLDLs and chylomicrons. Increasing HDL levels is considered to be beneficial in reducing the risk of cardiovascular disease (Shah 2010; Asztalos et al. 2011; Navab et al. 2011). In particular, decreased content of the large-sized HDL particles and increased content of small-sized HDL have been associated with dyslipidemic states such as hypertriglyceridemia, hypercholesterolemia and mixed dyslipidemia (Tian 2010).

In the laboratory, typical blood lipid measurements that are carried out include total cholesterol, TAG, and HDL, which are measured directly. Then Friedewald's equation is used to calculate the amount of VLDL and LDL-cholesterol present in serum cholesterol based on knowing the amount of total and HDL-cholesterol. Unfortunately, this equation assumes a linear relationship between total cholesterol, HDL-cholesterol, and LDL-cholesterol, and is only valid when the TG level is less than 400 mg/dL (5 mmol/L). This is the reason why fasting blood samples (overnight fast of 12 hours) are routinely used to determine blood lipid levels (http://www.medscape.com/viewarticle/451762_2). Also, fasting reduces the contribution of the exogenous pathway on lipid metabolism results. This provides a picture of the body's innate ability to metabolize its endogenous stores of fat.

Oxidative stress and resulting lipid peroxidation is involved in various pathological states including inflammation, atherosclerosis, neurodegenerative diseases, and cancer. Evidence for the role of oxidative stress in the pathogenesis of cardiovascular disease (CVD) is primarily based on experimental cell culture studies and observational human studies. A recent review

concluded that the ability of oxidative stress biomarkers to predict cardiovascular diseases in cell culture studies and observational human studies has yet to be established (Strobel *et al.*, 2010). It is also important to note that the methods used to assess lipid peroxidation in human samples including malondialdehyde (MDA), lipid hydroperoxides, conjugated dienes, oxidized LDL (oxLDL) and F_2-isoprostanes are indirect, and the evidence that these various methods actually reflect lipid peroxidation *in vivo* is limited.

The plasma markers thought to be indicative of lipid peroxidation include malondialdehyde (MDA), lipid hydroperoxides, conjugated dienes, oxidized LDL (oxLDL) and F_2-isoprostanes (Jialal and Devaraj 1996; Favier 1997; Nikolaidis, et al. 2011; Pandi and Rizvi 2011). The most common marker utilized in published reports is quantification of MDA using the thiobarbituric acid reactive substances (TBARS) assay as the readout. Increased oxidation of LDL to oxLDL is linked to the initiation and progression of atherosclerosis (Steinberg *et al.*, 1989); however, it is not known whether increases in specific particle subclasses of oxLDL may be important in assessing the risk for developing atherosclerosis and CVD. Similarly, currently utilized techniques for measuring the amount of oxLDL present in plasma or serum may not discriminate between these particle subclasses; thus, the resulting readout is a general one that likely represents total oxLDL, and this may be important to consider in the interpretation of studies that administer fish oils. The susceptibility of LDL to oxidation can be measured *ex vivo* by isolating LDL directly from plasma and by using the patients' own mononuclear cells, plus Cu2+ or 2,2'-azobis-(2-amidinopropane hydrochloride) (AAPH), as the oxidizing agents. Again, there is no evidence that such techniques generate results that are reflective of oxLDL levels *in vivo* (Moore and Roberts 1998).

Studies reporting on lipid-related endpoints and oxidative stress biomarkers that were cited in the Norwegian Scientific Committee for Food Safety, VKM, (VKM 2011) and the German Federal Institute for Risk Assessment (BfR) 2009 report were initially retrieved for analysis. The review reported by VKM (2011) noted only minor increases in LDL-cholesterol in subjects with Type 2 Diabetes based on meta-analyses of EPA and DHA ingestion of up to 4.8 grams/day. It was also noted that no change in LDL-cholesterol was reported in the large coronary heart intervention trials of subjects with and without Type 2 Diabetes. None of the oxidative stress biomarkers are presently defined as risk factors of disease. The clinical relevance of lipid peroxidation is therefore unclear. In contrast, BfR (2009) highlighted meta-analyses of

omega-3 fatty acids showed significant increases in LDL-cholesterol and did not comment on the effects of omega-3's on lipid peroxidation.

Upon retrieval and review of the cited studies, those that did not include baseline data to compare against final data in an "omega-3" only group were considered not evaluable for purposes of a weight of the evidence evaluation. Numerous studies presented results and statistical analyses for effects of fish oil or EPA+DHA treatment groups versus a control or placebo. However, because the effects of the control interventions alone, which included corn oil, olive oil and other various oils and oil blends, on blood lipid parameters, versus baseline levels, was not reported in many studies, it was not possible to account for the background effect of the control oil interventions on blood lipids. Therefore, we summarize and present the effects that are reported for fish oil or EPA+DHA treatment groups versus *baseline* lipid measurements whenever possible. Using this approach, the hazards from ingestion of EPA+DHA or fish oil could more readily be identified.

Results

Total Cholesterol

Of the studies that were considered evaluable for the purpose of a weight of the evidence hazard characterization, 31 studies looked at the effects of EPA and/or DHA on total cholesterol. Seventeen of these studies were carried out in healthy populations (Bogl et al., 2011; Buckley et al., 2004; Conquer et al., 1996; Engstrom et al., 2003; Geppert et al., 2006; Grimsgaard et al., 1997; Hamazaki et al., 1996; Harris et al., 2008; Higgins et al., 2001; Maki et al., 2005; Mann et al., 2010; Neff et al., 2001; Stark and Holub 2004; Turini et al., 2001; Wander et al., 1996; Wu et al., 2006) and 15 were carried out in health-compromised or vulnerable populations (Bonanome et al., 1996; Contacos et al., 1993; Emsley et al., 2008; Eritsland et al., 1996; Kelley et al., 2007; Maki et al., 2011; Mori et al., 1992; Pedersen et al., 2003; Schwellenbach et al., 2006; Shidfar et al., 2003; Shidfar et al., 2008; Stalenhoef et al., 2000; Suzukawa et al., 1995; Tholstrup et al., 2004; Westerveld et al., 1993).

Across the 16 studies done in healthy populations, only four found an effect of the consumption of fish products or EPA- and/or DHA-rich oils on total cholesterol (Maki et al., 2011; Engstrom et al., 2003; Wu et al., 2006, Grimsgaard et al., 1997). Grimsgaard et al. (1997), Engstrom et al. (2003), and Maki et al. (2011) found that the consumption of fish products or oils delivering up to 3.8 g EPA/d (25 g caviar paste + fish oil/d) and 1.52 g DHA + 0.08 g EPA/d,

increased total cholesterol. Wu et al. (2006) found that the consumption of 2.14 g DHA/d by postmenopausal women reduced total cholesterol. The remaining 13 studies found that the consumption of EPA and/or DHA rich oils delivering up to 4.8 g EPA/d and 4.9 g DHA/d independently, and 4.39 g EPA + 2.8 g DHA/d had no effect on total cholesterol (Bogl et al., 2011; Buckley et al, 2004; Conquer et al., 1996; Geppert et al., 2006; Hamazaki et al., 1996; Harris et al., 2008; Higgins et al., 2001; Mann et al., 2010; Neff et al., 2001; Stark and Holub 2004; Turini et al., 2001; Wander et al., 1996; Wander and Du 2000). The Maki study was carried out in a population having below average HDL, but otherwise normal lipid levels (Maki et al., 2005).

Six of the 15 studies carried out in health-compromised or vulnerable populations, found that the consumption of fish products and EPA- and/or DHA-rich oils on total cholesterol (Bonanome et al., 1996; Eritsland et al., 1996, Einsley et al., 2008; Schwellenbach et al., 2006; Stalenhoef et al., 2000; Tholstrup et al., 2004). Bonanome et al. (1996) and Eritsland et al. (1996) found that the consumption of oils delivering of up to 2.04 g EPA + 1.28 g DHA/d increased total cholesterol whereas Einsley et al. (2008), Schwellenbach et al. (2006), Stalenhoef et al. (2000), and Tholstrup et al. (2004) found that the consumption of oils delivering up to 2 g/d of EPA alone and 1.76 g EPA + 1.44 g DHA/d decreased total cholesterol. The remaining nine studies found that intakes as high as 2.8 g EPA+1.8 g DHA/d and 1.76 g EPA+2.4 g DHA/d had no effect on total cholesterol (Contacos et al., 1993; Kelley et al., 2007; Maki et al., 2011; Mori et al., 1992; Pedersen et al., 2003; Shidfar et al., 2003; Shidfar et al., 2008; Suzukawa et al., 1995; Westerveld et al., 1993). Importantly, these studied included subjects with mixed hyperlipidemia (Contacos et al., 1993; Shidfar et al., 2003; Tholstrup et al., 2004), hypertriglyceridemia (Bonanome et al., 1996; Kelley et al., 2007; Schwellenbach et al., 2006; Stalenhoef et al., 2000), hypercholesterolemia (Maki et al., 2011), diabetes (Pedersen et al., 2003; Shidfar et al., 2008), and hypertension (Suzukawa et al., 1995).

Total Triglycerides

A reduction in plasma triglycerides is thought to be beneficial in cardiovascular disease (Di Minno et al. 2010; Barter and Ginsberg 2008; McKenney and Sica 2007), especially in those having diabetes or metabolic syndrome (De Luis et al. 2009; Hartweg et al. 2009). Of the studies that were considered evaluable for the purpose of a weight of the evidence hazard characterization, 33 studies looked at the effects of EPA and/or DHA on triglycerides. Seventeen

studies were carried out in healthy populations (Bogl et al., 2011; Buckley et al., 2004; Conquer et al., 1996; Engstrom et al., 2003; Geppert et al., 2006; Grimsgaard et al., 1997; Hamazaki et al., 1996; Harris et al., 2008; Higgins et al., 2001; Maki et al., 2005; Mann et al., 2010; Neff et al., 2001; Piolot et al., 2003; Stark and Holub 2004; Turini et al., 2001; Wander et al., 1996; Wander and Du 2000; Wu et al., 2006) and 16 were carried out in health-compromised or vulnerable populations (Bonanome et al., 1996; Contacos et al., 1993; Emsley et al., 2008; Eritsland et al., 1996; Hassan et al., 2010; Kelley et al., 2007; Maki et al., 2011; Mori et al., 1992; Pedersen et al., 2003; Schwellenbach et al., 2006; Shidfar et al., 2003; Shidfar et al., 2008; Stalenhoef et al., 2000; Suzukawa et al., 1995; Tholstrup et al., 2004; Westerveld et al., 1993).

Nine of the 18 studies performed in healthy populations found that the consumption of oils delivering up to 4.8 g of EPA or 4.9 g DHA/d independently, and 2.46 g EPA + 1.8 g DHA/d decreased total triglycerides (Buckley et al., 2004; Conquer et al., 1996; Geppert et al., 2006; Grimsgaard et al., 1997; Higgins et al., 2001; Maki et al., 2005; Piolot et al., 2003; Stark and Holub 2004; Wander and Du 2000). The remaining nine studies found that intakes as high as 4.4 g EPA + 2.8 g DHA/day had no effect on total triglycerides (Bogl et al., 2011; Engstrom et al., 2003; Hamazaki et al., 1996; Harris et al., 2008; Mann et al., 2010; Neff et al., 2011; Turini et al., 2001; Wander et al., 1996; Wu et al., 2006).

Thirteen of the 16 studies carried out in health-compromised or vulnerable populations found that intakes as high as 3 g DHA/d, 1.76 g EPA + 2.44 g DHA/d, and 2 g EPA + 2 g DHA/d decreased total triglycerides (Bonanome et al., 1996; Contacos et al., 1993; Eritsland et al., 1996; Hassan et al., 2010; Kelley et al., 2007; Mori et al., 1992; Pedersen et al., 2003; Schwellenbach et al., 2006; Shidfar et al., 2003; Shidfar et al., 2008; Stalenhoef et al., 2000; Suzukawa et al., 1995; Tholstrup et al., 2004). Four studies were carried out in hypertriglyceridemic individuals (Bonanome et al., 1996, Kelley et al., 2007 Schwellenbach et al., 2006; Stalenhoef et al., 2000), three were in mixed hyperlipidemic subjects (Contacos et al., 1993; Shidfar et al., 2003; Tholstrup et al., 2004), two were in diabetics (Pedersen et al., 2003; Shidfar et al., 2008), and one was in hypertensive patients (Suzukawa et al., 1995). The remaining three studies found that the consumption of oils delivering up to 2 g EPA/d and 3.4 g of EPA+DHA/d had no effect on total triglyceride levels (Emsley et al., 2008; Maki et al., 2011; Westerveld et al., 1993).

LDL – Cholesterol

Twenty-nine evaluable studies looked at the effects of EPA and/or DHA on LDL cholesterol. Fifteen studies were carried out in healthy populations (Bogl et al., 2011; Buckley et al., 2004; Conquer et al., 1996; Engstrom et al., 2003; Geppert et al., 2006; Grimsgaard et al., 1997; Hamazaki et al., 1996; Harris et al., 2008; Higgins et al., 2001; Maki et al., 2005; Mann et al., 2010; Neff et al., 2001; Stark and Holub 2004; Wander et al., 1996; Wu et al., 2006) and 14 were carried out in health-compromised or vulnerable populations (Bonanome et al., 1996; Contacos et al., 1993; Emsley et al., 2008; Eritsland et al., 1996; Hassan et al., 2010; Kelley et al., 2007; Maki et al., 2011; Mori et al., 1992; Schwellenbach et al., 2006; Shidfar et al., 2003; Shidfar et al., 2008; Stalenhoef et al., 2000; Suzukawa et al., 1995; Westerveld et al., 1993).

Three of the 15 studies performed in healthy populations indicated that the consumption of fish products or oils delivering up to 0.9 g DHA/d alone and 1.52 g EPA + 0.08 g DHA/d increased LDL-cholesterol (Engstrom et al., 2003; Geppert et al., 2006; Maki et al., 2005). It is noteworthy that Maki et al. (2005) assessed the effects of EPA+DHA consumption in a population having only below average HDL, but otherwise normal lipid levels (Maki et al., 2005). The remaining 12 studies delivering up to 4.8 g EPA/d or 4.9 g DHA/d independently, and 2.46 g EPA + 1.8 g DHA/d and found no effect on LDL-cholesterol (Bogl et al., 2011; Buckley et al, 2004; Conquer et al., 1996; Grimsgaard et al., 1997; Hamazaki et al., 1996; Harris et al., 2008; Higgins et al., 2001; Mann et al., 2010; Neff et al., 2001; Stark and Holub 2004; Wander et al., 1996; Wu et al., 2006).

Seven of the 14 studies carried out in health-compromised or vulnerable populations showed that consumption of EPA- and/or DHA-rich oils delivering up to 1.8 g EPA/d alone, and 2.8 g EPA + 1.8 g DHA/d affected LDL cholesterol (Bonanome et al., 1996; Eritsland et al., 1996; Kelley et al., 2007; Mori et al., 1992; Stalenhoef et al., 2000; Westerveld et al., 1993; Maki et al., 2011). LDL-cholesterol was increased in six of the studies (Bonanome et al., 1996; Eritsland et al., 1996; Kelley et al., 2007; Mori et al., 1992; Stalenhoef et al., 2000; Westerveld et al., 1993) and reduced in one (Maki et al., 2011). In the remaining seven studies, the intakes as high as 1 g DHA/d alone, 2 g of EPA+ 2 g DHA/d, and 1g EPA + 2.4 g DHA/d had no effect on LDL-cholesterol (Contacos et al., 1993; Emsley et al., 2008; Hassan et al., 2010; Schwellenbach et al., 2006; Shidfar et al., 2003; Shidfar et al., 2008; Suzukawa et al., 1995). Patients enrolled in these studies included those with hypertriglyceridemia (Bonanome et al., 1996; Kelley et. al.,

2007; Stalenhoef et al., 2000;Shidfar et al., 2003), hypercholesterolemia (Maki et al., 2011), hyperlipidemia (Contacos et al., 1993); mental disorders (Emsley et al., 2008), peripheral vascular disease (Mori et al., 1992), diabetes (Westerveld et al., 1993; Hassan et al., 2010; Shidfar et al., 2008), coronary artery disease (Schwellenbach et al., 2006), hypertension (Suzukawa et al., 1995), and patients undergoing coronary artery bypass grafting (Eritsland et al., 1996).

Five studies examined the effect of EPA and/or DHA on the size of LDL particles, as larger-sized LDL particles can carry more lipids and may be less likely to penetrate the endothelium and cause plaque-forming deposits (Cottin, et al. 2011; Maki et al., 2005;Contacos et al., 1993; Tholstrup et al., 2004; Neff et al., 2001; Suzukawa et al., 1995). Three of these studies found that the consumption of oils delivering up to 2 g DHA/d alone and 1.76 g EPA + 2.44 g DHA/d increased LDL particle size (Maki et al., 2005; Neff et al., 2001, Suzukawa et al., 1995). The remaining two found no effect (Contacos et al., 1993; Tholstrup et al., 2004). Contacos et al. (1993) delivered 2 g of EPA + 2 g DHA/d and Tholstrup et al. (2004) was unquantifiable. Patients enrolled in these studies were either healthy (Maki et al., 2005), hyperlipidemic (Contacos et al., 1993; Tholstrup et al., 2004), hypertensive (Suzukawa et al., 1995) or obese (Neff et al., 2011).

HDL – cholesterol

Twenty-nine evaluable studies assessed the effects of EPA and/or DHA on HDL cholesterol. Fifteen were carried out in healthy populations (Bogl et al., 2011; Buckley et al., 2004; Conquer et al., 1996; Engstrom et al., 2003; Geppert et al., 2006; Grimsgaard et al., 1997; Hamazaki et al., 1996; Harris et al., 2008; Maki et al., 2005; Mann et al., 2010; Neff et al., 2001; Stark and Holub 2004; Turini et al., 2001; Wander et al., 1996; Wu et al., 2006) and 14 were carried out in health-compromised or vulnerable populations (Bonanome et al., 1996; Contacos et al., 1993; Emsley et al., 2008; Eritsland et al., 1996; Hassan et al., 2010; Kelley et al., 2007; Maki et al., 2011; Mori et al., 1992; Pedersen et al., 2003; Schwellenbach et al., 2006; Shidfar et al., 2003; Shidfar et al., 2008; Stalenhoef et al., 2000; Suzukawa et al., 1995).

Eight of the 15 studies performed in healthy populations found that the consumption of DHA and/or EPA affected HDL-cholesterol. Seven found that intakes as high as 3.6 g DHA/d and 4.3 g EPA + 2.8 g DHA/d increased HDL (Conquer et al., 1996 Engstrom et al., 2003;

Geppert et al., 2006;Grimsgaard et al., 1997; Maki et al., 2005;Mann et al., 2010; Turini et al., 2001). Buckley et al. (2004) found that the consumption of 4.9 g of DHA/day decreased HDL-cholesterol. The remaining seven studies found that the consumption of up to 2.8 g DHA/d and 2.46 g EPA + 1.8 g DHA/d did not affect HDL-cholesterol (Bogl et al., 2011; Hamazaki et al., 1996; Harris et al., 2008; Neff et al., 2001; Stark and Holub 2004; Wander et al., 1996; Wu et al., 2006).

Five of the 14 studies carried out in health-compromised or vulnerable populations found DHA and/or EPA-rich oil-related effects on HDL-cholesterol. Four studies showed that intakes as high as 2.04 g EPA + 1.28 g DHA/d and 1.76 g EPA + 1.44 g DHA/d increased HDL-cholesterol (Eritsland et al., 1996; Pedersen et al., 2003; Schwellenbach et al., 2006; Stalenhoef et al., 2000). Emsley et al (2008) found that an intake of 2 g EPA/d decreased HDL-cholesterol. The remaining 9 studies found intakes as high as 3 g DHA/d, 2.8 g EPA+1.8 g DHA/d, and 1.76 g EPA + 2.4 g DHA/d had no effect on HDL-cholesterol (Bonanome et al., 1996; Contacos et al., 1993; Hassan et al., 2010; Kelley et al., 2007; Maki et al., 2011; Mori et al., 1992; Shidfar et al., 2003; Shidfar et al, 2008; Suzukawa et al., 1995). Patients enrolled in these studies included those with diabetes (Pedersen et al., 2003; Shidfar et al., 2008; Hassan et al., 2010), hypertriglyceridemia (Schwellenbach et al., 2006; Stalenhoef et al., 2000, Bonanome et al., 1996; Kelley et al., 2007), hyperlipidemia (Contacos et al., 1993; Shidfar et al., 2003), hypercholesterolemia (Maki et al., 2011), mental disorders (Emsley et al., 2008), hypertension (Suzukawa et al., 1995), peripheral vascular disease (Mori et al., 1992), and those undergoing coronary artery bypass grafting (Eritsland et al., 1996).

Eight studies examined the effect of EPA and/or DHA on the size of HDL particles (Bogl et al., 2011; Maki et al., 2005; Neff et al., 2001; Kelley et al., 2007; Maki et al., 2011; Mori et al., 1992; Suzukawa et al., 1995; Tholstrup et al., 2004) as larger HDL particles may be more protective against dyslipidemia (Cottin, et al. 2011). Four studies reported that the consumption of up to 2 g DHA/d, 1.52 g DHA+ 0.08 g EPA/d and 3.4 g EPA+DHA/d significantly affected HDL particle size (Bogl et al., 2011; Maki et al., 2005; Neff et al., 2001; Maki et al., 2011). The remaining four studies found no effect at intake levels as high as 3 g EPA/d alone, 3.4 g EPA+DHA/d, and 2.8 g EPA+1.8 g DHA/day (Kelley et al., 2007; Mori et al., 1992; Suzukawa et al., 1995; Tholstrup et al., 2004). Patients enrolled in these studies included those with no abnormalities (Bogle et al., 2011), lower levels of HDL-cholesterol (Maki et al., 2005), hypercholesterolemia (Maki et al., 2011), hyperlipidemia (Tholstrup et al., 2004),

hypertriglyceridemia (Kelly et al 2007), peripheral vascular disease (Mori et al 1992), hypertension (Suzukawa et al., 1995), and obesity (Neff et al., 2001).

Oxidative stress and lipid peroxidation

Of the thirty studies that were retrieved which mentioned lipid oxidation and oxidative stress-related endpoints (Bloomer et al., 2009; Bonanome et al., 1996; Brude et al., 1997; Engstrom et al., 2003; Grundt et al., 2004; Higdon et al., 2000; Higgins et al., 2001; Himmelfarb et al., 2007; Jain et al., 2002; Koletzko et al., 2003; Mesa et al., 2004; Mori et al., 2000 (2 studies); Mori et al., 2003; Pedersen et al., 2003; Piolot et al., 2003; Rhodes et al., 2003; Shidfar et al., 2003; Shidfar et al., 2008; Shoji et al., 2006; Siahanidou et al., 2007; Stalenhoef et al., 2000; Stier et al., 2001; Suzukawa et al., 1995; Tholstrup et al., 2004; Turini et al., 2001; Wander et al., 1996; Wander and Du 2000; Wu et al., 2006; Yaqoob et al., 2000), 27 were regarded to be evaluable based on their inclusion of baseline data and data for an omega-3 only intervention group (Bloomer et al., 2009; Bonanome et al., 1996; Brude et al., 1997; Engstrom et al., 2003; Grundt et al., 2004; Higgins et al., 2001; Himmelfarb et al., 2007; Jain et al., 2002; Koletzko et al., 2003; Mesa et al., 2004; Mori et al., 2000 (2 studies); Mori et al., 2003; Pedersen et al., 2003; Piolot et al., 2003; Rhodes et al., 2003; Shidfar et al., 2003; Shidfar et al., 2008; Shoji et al., 2006; Stalenhoef et al., 2000; Stier et al., 2001; Suzukawa et al., 1995; Turini et al., 2001; Wander et al., 1996; Wander and Du 2000; Wu et al., 2006; Yaqoob et al., 2000).

Thirteen studies were carried out in healthy populations (Bloomer et al., 2009; Engstrom et al., 2003; Higgins et al., 2001; Koletzko et al., 2003; Mesa et al., 2004; Piolot et al., 2003; Rhodes et al., 2003; Shoji et al., 2006; Turini et al., 2001; Wander and Du 2000; Wander et al., 1996; Wu et al., 2006; Yaqoob et al., 2000) and fourteen were carried out in health-compromised or vulnerable populations (Bonanome et al., 1996; Brude et al., 1997; Grundt et al., 2004; Himmelfarb et al., 2007; Jain et al., 2002; Mori et al., 2000 (two studies); Mori et al., 2003; Pedersen et al., 2003; Shidfar et al., 2003; Shidfar et al., 2008; Stalenhoef et al., 2000; Stier et al., 2001; Suzukawa et al., 1995).

Studies in healthy populations reporting an observed effect of n-3 fatty acids on oxidative stress-related endpoints include the following: increased maximum diene concentration (Higgins et al., 2001), decreased urinary malondialdehyde levels (Koletzko et al, 2003), increased formation of conjugated dienes in oxidized LDL particles (Mesa et al., 2004), decreased diene propagation rate for LDL oxidation (Piolot et al., 2003; Turini et al., 2001; Wander et al., 1996),

increased erythemal threshold for ultraviolet radiation (Rhodes et al., 2003), decreased lag time for formation of oxidized LDL (Turini et al., 2001; Wander et al., 1996), increased TBARS levels in the absence of added alpha-tocopheryl acetate (Wander and Du 2000; Yaqoob et al. 2000), and increased LDL-TBARS levels (Wu et al., 2006). The repeat findings for increased diene propagation rate and decreased lag time for formation of oxidized LDL are interesting but the clinical significance is unclear.

Studies in health-compromised populations generated the following results for oxidative stress-related effects of n-3 fatty acids: temporarily increased copper-mediated LDL oxidation (Brude et al., 1997), decreased plasma homocysteine (Grundt et al., 2004), decreased IL-6 levels (Himmelfarb et al., 2007), decreased lipid peroxides and diene conjugates, increased levels of reduced glutathione (Jain et al., 2002), decreased urinary F2-isoprostanes (Mori et al., 2000; Mori et al., 2003), increased excretion of urinary F2-isoprostanes (Stier et al., 2001), decreased lag time to form oxidized LDL (Stalenhoef et al., 2000; Pedersen et al., 2003; Suzukawa et al., 1995;), decreased propagation rate of oxidized LDL (Suzukawa et al., 1995), increased malondialdehyde content in LDL (Pedersen et al., 2003), increased diene content of LDL (Stalenhoef et al., 2000), decreased malondialdehyde levels (Shidfar et al, 2008), increased TBARS levels (Suzukawa et al., 1995), and increased uptake of oxidized LDL by macrophages (Suzukawa et al., 1995). Again, it is notable that multiple studies report that n-3 fatty acids can decrease the lag time to the formation of oxidized LDL.

There were no effects on oxidative stress-related endpoints in the following studies: Bloomer et al., 2009 (trolox equivalent antioxidant capacity, protein carbonyls, oxidized LDL, malondialdehyde levels); Engstrom et al., 2003 (plasma malondialdehyde levels); Shoji et al., 2006 (malondialdehyde and 8-OHdG excretion); Bonanome et al., 1996 (theoretical susceptibility of LDL to oxidation); and Shidfar et al., 2003 (malondialdehyde levels). Refer to Table 1 for doses and durations of EPA and/or DHA in these and the abovementioned studies.

Effects on specific lipid oxidation or oxidative stress parameters are difficult to interpret, as endpoints such as TBARS, lymphocyte phagocytic activity, *in vitro* or *ex vivo* determination of lag time and oxidation rate of LDL, and *in vitro* rate of formation of conjugated dienes do not appear to have a strong evidence base to support their validated *in vivo* relevance as biomarkers for a disease or compromised health state. Due to the lack of validation of lipid oxidation or oxidative stress endpoints as biomarkers for disease or health-compromised states, or risk

thereof, it is not possible to interpret the results of the abovementioned studies as pertains to the existence of a specific hazard to health from ingestion of EPA and/or DHA.

Conclusions

Some studies report that the consumption of EPA- and DHA-rich oils increase LDL-cholesterol levels; however, this must be considered in light of the total effect on blood lipids, which also necessitates consideration of triacylglycerides (TAGs), HDL, and total cholesterol, at a minimum, to provide the proper context for clinical interpretation of changes in cardiovascular disease risk. Additionally, as HDL particles are the main lipid fraction into which excess cholesterol may partition for its ultimate removal from the body, any favorable effects of consumption of fish oil-derived fatty acids on HDL must also be considered. Evidence is also emerging that supports the existence of variously-sized particle subclasses within the major blood lipid fractions; for instance, there exist LDL particles which are small and dense and those which are larger and less dense, each of which may have different reactivity regarding their ability to penetrate into the vascular epithelium and cause atherogenic depositions. Larger LDL and HDL particles can carry more cholesterol; in particular, larger HDL particle size may be more protective against dyslipidemia (Cottin, et al. 2011). The clinical implications resulting from alteration of the balance of these LDL subclasses within the overall lipid profile are currently unknown, but will likely be elucidated with future research. For these reasons, it is considered useful to discuss the hazard related to intake of omega-3 fatty acids in relation to effects which are observed for overall changes in a blood lipid profile, including effects on LDL, HDL, total cholesterol and TAGs. A blood lipid profile change after consumption of fish oil that includes a shift toward increased LDL, increased HDL, and reduced TAGs would still be considered a favorable outcome, as lowered HDL and raised fasting levels of TAGs are considered cardiovascular disease risk factors (Cottin, et al. 2011). Authoritative researchers have suggested that the reduction in TAGs may be due to reduced hepatic *de novo* lipogenesis which, over the long term, might contribute to lower cardiovascular risk (Mozaffarian and Wu 2011).

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
Barden *et al.*, 2004	Randomized, double-blind, placebo-controlled	Pregnant atopic women (n=15)	Placebo: 4 g olive oil/d (67% oleic acid and <3% n-3 PUFA) Fish oil: 4 g fish oil (27.7% EPA and 56% DHA)	From 20 weeks of pregnancy to delivery – approxima tely 16 weeks	Allergic disease confirmed by a history of allergic rhinitis and/or asthma and one or more positive skin prick tests to common allergens. Placebo was olive oil.	Cord plasma F2-isoprostanes Lymphocyte stimulation assays				Approximately 20% of the fish oil supplements was other fatty acids and the fatty acid composition of the placebo differed greatly from the fish oil supplement. Baseline measurements were not taken.
Bays et al. 2010	Assess the long-term efficacy and safety of prescription omega-3-acid ethyl esters coadministered with simvastatin in hypertriglyceridemi c patients. Open-label extension study of subjects completing 8 wk treatment with simvastatin ± Lovaza.	Patients participating in the Combination of Prescription Omega-3 Plus Simvastatin (COMBOS) trial [Davidson et al. 2007]. The COMBOS trial utilized an 8 wk lead-in phase wherein all patients received simvastatin 40 mg/d then were randomized to a further 8 wk of Lovaza (4 g fish oil ester/d; GlaxoSmithKline, NC, USA) or placebo. This reference reports data from the extension study. Patients had triglyceride levels ≥ 200 mg/dL and < 500 mg/dL.	Those patients who received placebo + simvastatin (40 mg/d) in COMBOS switched to open-label Lovaza (four 1 g capsules containing 0.465 g EPA and 0.375 mg DHA) plus simvastatin ("switchers;" *n*=100 enrolled; 97 in safety population; 73 completing extension). Those who received Lovaza + simvastatin continued on the same regimen ("non-switchers;" *n*=88 enrolled; 85 in safety population; 62 completing extension). 1.86 g EPA plus 1.5 g DHA = 3.36 g EPA+DHA/d with concomitant 40 mg/d simvastatin × 24 mo	24 mo	Simvastatin 40 mg/d No control group using Lovaza only.	• Switchers had statistically significant decreases in non-HDL cholesterol, triglycerides, and VLDL cholesterol, versus baseline levels, beginning at 4 mo on combination therapy. These effects persisted through 24 mo. • Switchers also exhibited statistically significant reductions in total cholesterol, versus baseline levels, beginning at 12 mo on combination therapy. The effect persisted through 24 mo.				• No placebo group for comparisons. •No Lovaza-only group. • Non-switchers had essentially already benefited maximally from the addition of Lovaza to their simvastatin therapy. Therefore, during the extension study, non-switchers appear to have little change from "baseline" which is an equilibrated treatment effect from the COMBOS study. • Switchers exhibited favorable changes, versus baseline levels, for non-HDL cholesterol, total cholesterol, triglycerides, and VLDL cholesterol parameters by or before 24 mo therapy on simvastatin plus the newly-added Lovaza. • Lovaza was generally well-tolerated in this population. • Most adverse events were mild and not related to treatment.

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
										• Of the 182 patients considered to be the safety population (not all completed the extension study), 28 had serious adverse events. 20 resulted in study withdrawal, but only one serious adverse event was considered to be possibly related to study treatment (myocardial infarction; *n*=1). No deaths occurred during the study period. • Hypertension (*n*=20) • Arthralgia (*n*=12) • Myalgia (*n*=9) • Back pain (*n*=8) • Anxiety (*n*=8) • Depression (*n*=7) • Increased weight (*n*=6) • Blood glucose and fructosamine levels increased slightly during treatment (data not shown); however, median plasma HbA_{1c} levels did not substantively increase during treatment in patients having diabetes or elevated fasting glucose. • There was minimal effect of Lovaza on glycemia, according to the authors.

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
Bloomer et al., 2009	Randomized, double-blind, placebo-controlled	Exercise-trained men (n=14)	Placebo: 8 x gel capsules of soybean oil Treatment: 8 gel capsules containing EPA and DHA. Total daily consumption of EPA = 2.224 mg and DHA = 2.208 mg	6 weeks	Placebo capsules contained soybean oil.	The purpose of this study was to determine the effect of EPA and DHA supplementation on resting and exercise-induced inflammation and oxidative stress 1. Blood levels of EPA/DHA. 2. Blood levels of the inflammatory markers C-reactive protein and TNFα. 3. Blood levels of the oxidative stress markers (protein carbonyls, oxidized LDL, IgG autoantibodies to oxidized LDL, malondialdehyde, hydrogen peroxide, nitric oxide, and lactate) 4. Blood levels of creatine kinase activity and muscle soreness .		2.224 mg EPA + 2.208 mg DHA		The exact fatty acid composition of the placebo and EPA/DHA supplements were not noted. Blood was harvested pre and post exercise. 1. There was no statistical difference in the dietary intakes of those subjects consuming the placebo or EPA/DHA supplement 2. EPA/DHA supplementation resulted in increased blood levels of EPA and DHA. 3. Resting blood levels of C-reactive protein and TNFα were significantly reduced and there was no difference in resting oxidative stress markers (protein carbonyls, IgG autoantibodies to oxidized LDL, malondialdehyde, hydrogen peroxide, nitric oxide, and lactate) 4. EPA/DHA supplementation had no effect on resting creatine kinase activity or muscle soreness.

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
Bogl et al. 2011	Monozygotic twin pair study.	Twenty-four healthy monozygotic twin pairs aged 23-33 yr. One male subject was obese and had recently developed type II diabetes and used insulin; all other subjects were healthy.	Not stated. A fat substitution model was carried out to evaluate the effect of substituting 1% of the energy intake with omega-3 fatty acids on HDL particles and apolipoprotein A1 levels.	3 d	No medications	Examine the relationship between macronutrient composition and lipoprotein particle size and HDL subspecies. LDL was calculated based on the Friedewald equation; HDL was isolated and mean particle size distribution was determined by polyacrylamide gradient gel electrophoresis. • Substitution of up to 1% of energy intake with n-3 polyunsaturated fatty acids caused a statistically significant: --increase in HDL particle size --increase in number of HDL_{2b} particles --decrease in number of HDL_{3a} and HDL_{3b} particles			Changes in HDL particle size: 0.82 ± 0.24% energy intake as n-3 fatty acids	• Study implications are limited by lack of reporting of the composition and dose of the test article. • Study results are independent of genetic effects • Statistically significant effects on HDL_{2b}, HDL_{3a} and HDL_{3b} were observed between co-twins consuming 0.58 ± 0.21 vs. 0.82 ± 0.24% of energy intake as omega-3 polyunsaturated fatty acids.
Bonanome et al., 1996	Sequential phase	Hypertriglyceridemic hemodialyzed patients (n=12)	First phase: 2.5 g EPA and DHA ethyl esters/day at a 1.2:1 ratio. Each capsule contained 0.3 mg α-tocopherol. Second phase: n-3 supplementation was discontinued	2 months	Chronic renal failure Eight patients were also taking calcium supplements and a daily dosage of 0.5 µg calcitriol	1. Fatty acid composition of LDLs 2. Blood lipid, lipoprotein complex, and LDL vitamin E levels 3. 2,2'-azobis (2-amidinopropase dihydrochloride (AAPH) induced LDL oxidation, 4. Theoretical susceptibility of LDL to oxidation. <u>EPA/DHA supplementation, versus baseline</u>: 1. Significantly increased the amount of			↑Total cholesterol, LDL; ↓TAG, VLDL: 1.36 g EPA + 1.14 g DHA/d	Blood was measured before and after hemodialyis treatments

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
						EPA and DHA in LDLs, with no effect on other LDL fatty acids. 2. Significantly increased total cholesterol (9%; *P*<0.01) and LDL cholesterol (28%; *P*<0.001), reduced total triglycerides (30%; *P*<0.001) and VLDL cholesterol (35%; *P*<0.001), and had no effect on HDL levels or LDL-vitamin E levels. 3. Had no effect on AAPH-induced LDL oxidation, i.e. lag phase or peroxidation rate. 4. Had no effect on the theoretical susceptibility of LDL to oxidation.				
Bowden et al. 2009	Double-blind, permuted-block, randomized, placebo-controlled.	Eighty-seven patients having endstage renal disease patients.	Subjects consumed 6 capsules of either fish oil concentrate (0.96 g EPA, 0.6 g) (*n*=44) or corn oil (*n*=43) per day. Two capsules were taken with each meal.	6 mo	Patients having a life expectancy of < 6 mo, pregnant, having a history of hemodialysis or medication noncompliance or <18 yr old were excluded. Subjects also consumed supplements containing 15 mg vitamin B_6, 12 mg vitamin B_{12}, and 2.5 mg folic acid. Placebo capsules contained corn oil.	Serum lipids were measured. • HDL and LDL were significantly increased in the fish oil group versus corn oil group (*P* = 0.012 and 0.001, respectively). However, the corn oil group exhibited a trend for decreased HDL and increased LDL over time. • There was also a trend for HDL to increase over time in the fish oil group (nonsignificant), and an increase in LDL particle size, and decrease in TAGs was also noted in the fish oil group by the end of the study, versus baseline levels.				• The corn oil control group exhibited a trend for decreased HDL and increased LDL over time.
Brude et al., 1997	Randomized, double-blind, placebo-controlled	Male smokers with combined hyperlipidemia (40-60 years old; n=42)	Placebo: 8 g oil that represented the fatty acid pattern similar to an ordinary	6 weeks	Inclusion criteria included smoking ≥ 10 cigarettes/d, cholesterol levels between 6-9 mmol/L,	Lipid parameters and measures of oxidized lipids Omega-3				One patient did not complete the trial due to a nonfatal myocardial infarction.

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
			Norwegian diet Treatment 1: n-3 fatty acids (5 g EPA and DHA/day; 39.3 % EPA, and 23.7% EPA) Treatment 2: n-3 fatty acids (5 g EPA and DHA/day; 39.3 % EPA, and 23.7% EPA) + antioxidants, contained a variety of fatty acids but not EPA and DHA. Treatment 3: antioxidants		and TAG 2-5 mmol/L. Patients with heart, kidney, liver or malignant diseases, plus vegetarians, alcoholics or drug abusers were excluded. Subjects had to discontinue intake of fish oil, cod liver oil and vitamins at least 3 mo prior to beginning the study. None took any prescribed medications. Composition of control oil capsules was not provided.	supplementation, versus control, significantly decreased the formation rate of conjugated dienes during 2,2'-azobid-(2-amidinopropane hydrochloride) induced oxidation (P<0.05).				
Buckley et al. 2004	Double-blind, placebo-controlled, parallel.	Forty-two normolipidaemic subjects	Subjects consumed 9 1 g capsules per day containing either EPA-rich oil (4.8 g EPA/d), DHA-rich oil (4.9 g/d), or control oil (olive oil: 72 g oleic acid + 12.1 g palmitic acid + 10.2 g linoleic acid/100 g total fatty acids). Oils also contained 2-4 mg mixed natural tocopherols/g oil. The EPA- and DHA-enriched oils were supplemented with 7 mg mixed natural tocopherols per capsules as antioxidant. The EPA-rich oil contained 8.1 g DHA/100 g total fatty acids and the DHA-rich oil contained 9.4 g EPA/100 g total fatty acids.	4 wk	Exclusion criteria included diagnosed diabetes or fasting glucose > 6.8 mmol/L, liver or endocrine dysfunction, evidence of CVD, hypolipidaemic therapy or other medication known to affect lipid metabolism, consumption of fatty acid supplements or more than one portion of oily fish per week, weight-reducing diet, BMI < 20 or > 32 kg/m^2, blood pressure > 160/95 mmHg, fasting TAG > 4.0 mmol/L and fasting total cholesterol > 8.0 mmol/L. Olive oil was the control.	Serum lipid-related parameters and apolipoproteins were measured. •Both the EPA and DHA groups exhibited significantly lower TAG levels and significantly higher apoE levels ($P \leq$ 0.006 for both) at 4 wk, versus baseline. • The DHA group exhibited a significant decrease in apoA1:HDL versus baseline levels (P=0.037).		4.8 g EPA/d or 4.9 g DHA/d (total cholesterol)	↓TAG, ↑ApoE: 4.8 g EPA/d or 4.9 g DHA/d ↓ApoA1: HDL: 4.9 g DHA/d	

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
Cairns et al., 1996	Double blind, placebo controlled	Patients after PTCA. MaxEPA (n=325), corn oil placebo (n=328)	18 capsules/d of either MaxEPA (providing 3.24g EPA and 2.16g DHA) or corn oil placebo	18 wk	Heparin (during PTCA), low-molecular-weight heparin, aspirin Corn oil was the placebo.	Reduction of restenosis	LDL .			[Cannot interpret Table 4 due to formatting ambiguity as appearing in online version.]
Conquer et al. 1996	Double-blind, placebo-controlled	Twenty-four healthy vegetarians aged 29.6 ± 1.7 yr who reported having no meat, poultry or fish for a period of at least 6 mo. Subjects were assigned to take 9 capsules containing DHASCO or corn oil/d.	DHASCO capsules contained 0.5 g oil, 180 mg of which was DHA.. Total dose of oil per day was 4.5 g, containing 1.62 g DHA. DHASCO was from Martek Biosciences, Columbia, MD	6 wk	Corn oil was the placebo. Authors report that the fat consumed in the capsules represented < 10% of the dietary fat intake, that there were no differences between daily energy intakes, or intakes of specific fat types between the two groups.	Versus baseline levels, the DHASCO oil intervention significantly increased HDL and decreased TAG levels (P< 0.05).			1.62 g DHA as constitue nt in 4.5 g DHASC O oil	
Contacos et al. 1993	Compare the effects of a statin, fish oil and placebo on plasma lipids and lipoproteins in patients having mixed hyperlipidemia. Randomized Placebo-controlled Single-blind No placebo group for second phase of study	Patients aged 32-70 yr having a history of primary mixed (type IIB) hyperlipidemia (22M/10F).	6 wk dietary run-in period after cessation of lipid-modifying drugs, then randomization to one of three groups: placebo (not specified), pravastatin 40 mg/d, or fish oil (himega 6 g/d; contained 3 g omega-3 fatty acids, EPA:DHA, 2:1) × 6 wk. After 6 wk, those not achieving target lipid levels were put on combination therapy of pravastatin 40 mg/d plus himega 6 g/d for 12 wk. EPA+DHA = 3 g/d alone × 6 wk, then in combination with 40 mg/d pravastatin × 12 wk	12 wk	Pravastatin 40 mg/d Placebo composition not mentioned.	• In the group initially taking fish oil alone, plasma triglycerides were reduced by 30% versus baseline (p<0.05). VLDL decreased by 40% (p<0.05). • Patients taking pravastatin + fish oil during the second part of the study exhibited reductions in total cholesterol, triglycerides, LDL and apolipoprotein B, versus baseline levels.			↓TAG, VLDL: 2 g EPA + 2 g DHA/d	• No drug-related adverse events were reported and laboratory safety parameters were acceptable over the course of the study. • Group mean body mass index did not significantly change during the study (data not shown).

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Davidson et al., 1997	Double blinded, placebo controlled	Persons with combined hyperlipidemia. Placebo (n=8), low dose DHA (n=9), high dose DHA (n=9)	Twelve 0.5-g capsules of either 6 g corn/soy bean (1:1) oil, 6g DHASCO® oil (providing 2.5g DHA TAG form) or 3g DHASCO® oil (providing 1.25g DHA TAG form) plus 3 g corn/soy bean (1:1) oil	6 weeks	Control was corn:soybean oil (1:1).	Lipid profile including LDL			↑Non-HDL cholesterol, LDL, ↓TAG: 2.5 g DHA/d ↓TAG: 1.25 g DHA/d	<u>Versus baseline levels</u>: 2.5 g DHA/d increased non-HDL cholesterol (P<0.004), increased LDL (P<0.001), and decreased triglycerides (P<0.01). 1.25 g DHA/d decreased triglycerides (P<0.01).
Davidson et al. 2007	Evaluate the effects of adding Lovaza to preexisting statin therapy in patients with persistent hypertriglyceridemia. Multi-center Randomized Double-blind Placebo-controlled	Adults from 18-79 who had received a stable dose of statin to control LDL cholesterol levels for ≥ 8 wk and had mean fasting triglyceride level ≥ 200 and <500 mg/dL and a mean LDL cholesterol level below or within 10% of the patient's National Cholesterol Education Program Adult Treatment Panel III goal. 256 patients were enrolled and randomized after the 8 wk lead-in period on statin.	8 wk lead-in period: all patients on simvastatin 40 mg/d. Randomized to either simvastatin + placebo ("vegetable oil") (n=133; 1 exclusion from analysis) or simvastatin + 4 g Lovaza (Reliant Pharmaceuticals, Inc., Liberty Corner, NJ)/d (n=123; 1 exclusion from analysis) × 8 wk Lovaza: each 1 g capsule contains 0.465 g EPA and 0.375 g DHA. Four 1 g capsules: 1.86 g EPA, 1.5 g DHA = 3.36 g (EPA+DHA)/d plus 40 mg/d simvastatin × 8 wk.	8 wk	Simvastatin 40 mg/d No control group using Lovaza alone.	• Simvastatin + Lovaza caused a statistically significant decrease in non-HDL cholesterol, triglycerides, and VLDL cholesterol (p<0.001 for all), versus simvastatin alone. • A significant increase in HDL cholesterol (p<0.001) and a decrease in total cholesterol (p=0.001) and apolipoprotein B (p=0.023) was observed for the combination therapy, versus simvastatin alone.				• There is a mild effect of EPA+DHA on glucose in patients being treated with simvastatin. However, the clinical significance of this is unclear. • The adverse event rate was similar across treatment groups.
Dehmer et al., 1988	Placebo controlled, randomized	Male patients scheduled for angioplasty ; with test article (n= 44); without test article (n=39)	MaxEPA containing EPA (3.2g) + DHA (TAG; 2.2g); one dose level at 5.4	6-month study	Aspirin and dipyridamole. No modification or control was made in patients' diets or	Rate of early restenosis	LDL, VLDL LDL levels were similar at baseline in			

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

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						Primary	Secondary	NEL	EL	
			g/d		medications, except that each was encouraged to stop smoking. No placebo.		both groups and increased in both after 3 months, but the 3-month value was higher in the treatment group, vs. control group (no statistics). VLDL levels were also similar at baseline in both groups; they did not change after 3 months in the control group but decreased in the treatment group (no statistics).			
Durrington et al. 2001	Study the efficacy, safety and tolerability of concentrated fish oil omega-3 fatty acids in coronary heart disease patients having hypertriglyceridemia. Randomized Placebo-controlled Follow-up open-label phase (24 wk)	59 patients aged ≤ 75 yr (mean age 55 yr, range 37-75) with established coronary heart disease and serum triglycerides > 2.3 mmol/L. 55 patients completed the study; 46 agreed to take fish oil for the open-label follow-up phase	2 g concentrated fish oil/d (Omacor, *a.k.a., Lovaza*; 44% EPA, 36% DHA) or corn oil control. 0.88 g EPA + 0.72 g DHA = 1.6 g (EPA+DHA)/d × 24 wk Then open-label (24 wk) of 1.6 g EPA+DHA/d.	24 wk	Simvastatin 10-40 mg/d; dose unchanged for at least 3 mo prior to study Use of aspirin, beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, calcium channel antagonists and oral hypoglycemic agents was similar across groups. Corn oil was the control. No fish oil-only group.	• Serum triglycerides and VLDL cholesterol were statistically significantly reduced in the treatment group, versus baseline levels, at 12, 24, and 48 (continuers) wk (p<0.0005 and 0.0005, respectively, per endpoint). • Serum cholesterol was reduced in the treatment group, versus baseline levels, at 12 (p<0.025), 24 (p<0.025) and 48 (continuers; p<0.005) wk. • Patients on placebo who switched to treatment at 24 wk had a significant reduction in serum triglycerides and VLDL cholesterol by 48 wk (p<0.0005 and				• Baseline serum triglyceride levels in the treatment and placebo groups were 4.6 and 3.8 mmol/L, respectively. This may have contributed in part to the observed change in triglyceride levels noted in the treatment group, versus placebo. • There were no untoward effects on LDL or HDL levels. • EPA+DHA decreased triglycerides. • No significant change in fasting blood glucose or HbA_{1c} in either diabetic or non-diabetic patients

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
						0.005, respectively, per endpoint). • No significant changes in apolipoproteins AI or B, Lp(a) between groups.				receiving fish oil capsules for 48 wk. • No significant changes in blood pressure or laboratory values outside the reference range between groups.
Emsley et al., 2008	Double blinded, placebo controlled	Psychiatric patients. Blinded trial (EPA arm n=39; placebo arm, n=33). Open label extension (n=23 from the EPA arm and 22 from the placebo arm)	2g/d encapsulated ethyl ester-EPA (Amarin) or placebo (liquid paraffin)	12 weeks blinded followed by 40 weeks open label extension	Antipsychotic medication; non-steroidal anti-inflammatory agents or aspirin Placebo was liquid paraffin.	Safety factors including blood lipids. No change in LDL in EPA group vs. baseline levels, during blinded portion of study.	LDL		↓Total cholesterol, HDL; ↑ BMI: 2g/d (blinded portion of study)	• Increase in bleeding time and BMI in EPA group, versus baseline levels, during blinded portion of study (P<0.05). • Total cholesterol and HDL levels were significantly decreased (P=0.03) versus baseline levels, during the EPA extension phase. However, BMI was also significantly increased (P=0.001).
Engstrom et al., 2003	Randomized, double-blind,	Healthy (n=16; 8 men and 8 women)	25 g of ordinary caviar paste (65% cod roe paste and 35% rapeseed oil. 25 g caviar paste + fish oil (65% cod roe paste, 15% rapeseed oil, and 20% fish oil (18% EPA and 12% DHA)).	3 weeks	In the comparison diet, 20% of the rapeseed oil was substituted with fish oil.	1. Blood lipids 2. Fatty acid composition of plasma phospholipids 3. Lipid peroxidation Both treatments significantly reduced the amount of linoleic acid and increase the amount of EPA and DHA in plasma phospholipids. Fish oil-supplemented paste significantly increased the amount of total (P=0.010), HDL (P=0.027) and LDL cholesterol (P=0.024), versus baseline levels. There was no effect on plasma glucose, platelet activatory inhibitor-1, lipoprotein(a), and malondialdehyde equivalents.			↑Total cholesterol, HDL, LDL: 25 g caviar paste + fish oil (65% cod roe paste, 15% rapeseed oil, and 20% fish oil (18% EPA and 12% DHA)).	

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Eritsland et al., 1996	Placebo controlled;	CABG ; aspirin (n=148), aspirin with supplement (n=143), warfarin (n=145), warfarin with supplement (n=174)	Four 1-g Omacor capsules providing ethyl esters of EPA (2.04g) + DHA (1.28g)	12 months	Aspirin or warfarin. Patients were told to reduce their intake of saturated fatty acids and to refrain from cod-liver oil and other fish oil products during the study period No placebo intervention.	1-year graft potency	LDL Versus baseline, the fish oil intervention: --Increased total cholesterol, HDL, LDL, and decreased TAGs (P<0.001 for all). Versus the control group, the fish oil intervention: --Decreased TAGs (P<0.001). LDL levels also increased significantly in the control group over time (P<0.001).		↑Total cholesterol, HDL, LDL; ↓TAG: 2.04 g EPA + 1.28 g DHA	Diets were isocaloric between groups and over time within groups.
Geppert et al., 2006	Randomized, placebo-controlled, double-blind, parallel design	One hundred fourteen normolipidemic vegetarians (87F/27M) were assigned to take either Nutrinova DHA oil (2.284 g oil, containing 982.12 mg DHA) (n=59) or 2.248 g olive oil (n=55).	Each DHA capsule contained 571 mg oil derived from microalgae *Ulkenia* spp. and contained at least 245.53 mg DHA.	8 wk	Inclusion criteria included vegetarian diet for at least one year, age ≥ 18 yr, and BMI between 18-25 kg/m^2. Exclusion criteria included intake of medication with known effects on lipid metabolism during the last 3 mo, intake of omega-3 fatty acid supplements, presence of metabolic, cardiovascular, renal or neurological diseases or pregnancy and lactation. Olive oil was the control.	DHA treatment significantly reduced TG and increased LDL and HDL, versus baseline levels (P< 0.001 for TG, LDL; P =0.002 for HDL).			2.284 g oil, containing 982.12 mg DHA	Reported side effect rates were similar in both groups. Side effects from DHA-rich oil included flatulence, stomach ache, and belching.

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Grimsgaard et al., 1997	Double blind, placebo controlled	Healthy subjects, DHA arm (n=72), EPA arm (75), corn oil arm (n=77)	4g/d 95% ethyl esters-EPA, 4g/d 90% ethyl esters-DHA or 4g/d corn oil	7 weeks	At the beginning of the run-in period and throughout the study, participants were instructed not to ingest cod liver oil or other fish-oil supplements. Corn oil was the control.	TAG and serum fatty acids	LDL <u>Versus baseline levels</u>: --DHA decreased TAGs (P<0.001), and increased HDL (P<0.001). --EPA decreased TAGs (P<0.01), total cholesterol (P<0.05), apolipoprotein A-I (P<0.001) and apolipoprotein B (P<0.05). --Corn oil increased TAGs (P<0.01).		↓TAG; ↑HDL: 3.6 g DHA ethyl esters/d ↓TAG, total cholesterol, Apolipoproteins A-I and B: 3.8 g EPA ethyl esters/d	Background diet was isocaloric and matched in its fat and polyunsaturated fat intake across groups. Both DHA and EPA decrease serum triacylglycerols, but have differential effects on lipoprotein and fatty acid metabolism in humans.
Grundt et al., 2004	Randomized, double-blind, placebo-controlled	Subjects with an acute myocardial infarction (included between the 4th and 6th day following the acute myocardial infarction; n=300) were enrolled and received treatment. 100 were invited to participate in a withdrawal study	Placebo: corn oil (4000 g/day) Treatment: 2 capsules twice a day, containing 850-882 mg of EPA and DHA as concentrated ethyl esters (total 3.464 g n-3 PUFAs/day) alpha-tocopherol (4 mg) was added to all capsules to prevent oxidation	29 months	Myocardial infarction; simvastatin (20-40 mg) was initiated prior to discharge and continued beyond 12 months follow-up in 95 patients; Simvastatin-receiving patients were compared to individuals free of statins during the evaluation period. Placebo was corn oil.	Serum thiobarbituric acid-malondialdehyde (TBA-MDA). TBA-MDA levels increased during the intervention phase in the EPA/DHA-supplemented group and returned to normal after withdrawal.	1. Serum triglycerides and cholesterol 2. Fatty acid composition of serum phospholipids There was no difference in the levels of serum lipids, plasma total homocysteine, ultrasensitive C-reactive protein, markers of coagulation, and lipid peroxidation between the different groups. Serum HDL-			This study investigates the effect of washing out EPA/DHA- and corn oil-supplements. The exact fatty acid composition of the corn oil and EPA/DHA-enriched capsules was not noted. 1. ACE-inhibitors/AT-II antagonists were prescribed more often in the corn oil group. 2. There were no statistically significant differences in the distribution of cardiac events between the groups.

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

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							cholesterol remained increased in the EPA/DHA-supplemented group whereas the control group experiences a continued increase in HDL-cholesterol after withdrawal of corn oil. Serum triglycerides decreased during the intervention phase and returned back to baseline levels following withdrawal.			
Hamazaki et al., 1996	Placebo controlled	Healthy subjects, DHA arm (n=13), control oil arm (n=11)	3-3.6g/d DHA rich fish oil providing DHA of 1.5-1.8g/d, or 3-3.6g/d control oil	13 weeks	The control oil was a mixture of 97% soybean oil and 3% sardine oil (containing 32% EPA and 16% DHA).		Serum lipids There were no significant changes over time in the DHA group in the following serum lipids: total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, lipoprotein(a), and apolipoproteins Al and B	1.5-1.8 g DHA/d		

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Harris et al., 2008	Double blinded, placebo controlled	Overweight healthy volunteers. N=11 per group	GMO soybean oil (24 ml/d providing ~3.7d stearidonic acid) or regular soybean oil with or without EPA ethyl esters (~1g/d)	16 weeks		Omega-3 Index	Lipids including LDL	1g/d		No significant differences were noted for any of the physiological (heart rate, blood pressure, body weight) or lipid endpoints
Hassan et al. 2010	Evaluate the effect of oral omega-3 fatty acid administration on plasma lipids and inflammatory markers in peritoneal dialysis patients.	Diabetic and nondiabetic adult patients on stable continuous ambulatory or automated peritoneal dialysis for at least 3 mo and who were following a "dialysis diet." (*n*=15)	Oral 2.4 g DHA + 1 g EPA = 3.4 g (DHA+EPA)/d (in 3 divided doses) × 8 wk	8 wk	Simvastatin Excluded (via exclusion criteria): anticoagulants, additional omega-3 supplements, fibrates, sevelamer No control group using fish oil only.	• DHA+EPA caused a statistically significant decrease in triglycerides, versus baseline levels, for all peritoneal dialysis patients (p=0.001). • LDL and HDL levels were unchanged.			↓TAG: 2.4 g DHA + 1 g EPA/d	• No placebo group for comparisons. • DHA+EHA supplementation was well tolerated and was not associated with any significant adverse effects. It is considered safe in the studied patient population by the authors.
Higdon et al., 2000	Cross over	Postmenopausal women (n=15)	15g/d sunflower oil (providing 12.3 g oleate), safflower oil (providing 10.5g linoleate) or fish oil (providing 2.0g EPA and 1.4 g DHA as TAG)	5 weeks followed by 7 weeks wash out interval	All agreed to refrain from taking any nutritional supplements other than calcium or vitamin D and to refrain from eating fish Control groups took sunflower or safflower oil.	Free F2-isoprostanes, MDA, TBARS	Free F2-isoprostanes, MDA, TBARS		↑TBARS: 2.0 g EPA + 1.4 g DHA/d	TBARS was higher after fish-oil supplementation versus after sunflower oil and safflower oil supplementation (P=0.05 for fish oil vs. sunflower oil and safflower oil).
Higgins et al., 2001	Randomized, placebo-controlled	Healthy volunteers (19-63 years old; n=62)	Placebo: 0.9 g olive oil (0.007 g n-3 PUFA/g oil; EPA and DHA not present) Treatment 1: 0.3 g (fish and olive oil blend; 0.118 g n-3 PUFA/g in the fish oil; 0.056 g EPA and 0.036 g DHA/g in the fish oil) Treatment 2: 0.6 g fish oil/day (fish and olive oil blend; 0.227 g n-3 PUFA/g fish oil;	16 weeks	Olive oil was the placebo.	1. Susceptibility of LDLs to oxidation 2. In vitro TBARS production 3. Estimation of carotenoids, tocopherols, and retinol in plasma and LDLs 4. Total fatty acid composition of plasma and LDLs			↓TAG: 0.9 g fish oil/d (0.172 EPA + 0.11 g DHA/d)	Statistical analyses were only performed between the different groups Blood was collected from fasted subjects <u>Fish oil supplementation, versus baseline</u>: 1. Increased the concentrations of EPA and DHA in plasma and LDLs and decreased the amount of 20:3n-6 and 20:4n-6 more dramatically at the highest dose

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			0.114 g EPA and 0.072g DHA/g fish oil) Treatment 3: 0.9 g fish oil/day (fish oil only; 0.337 g n-3 PUFA/g fish oil; 0.172 EPA and 0.11g DHA/g fish oil) Placebo and treatments contained similar amounts of tocopherols							2. Significantly reduced fasting triglycerides at 0.9 g/d (P<0.0001) but not at the lower doses 3. Significantly reduced beta-carotene at 0.6 g/d but not at other doses. The plasma tocopherol, retinol, and carotenoids other than beta-carotene levels were not affected. 4. Had no effect on LDL tocopherols or carotenoids. 5. Had no effect on diene production, the rate of diene production, the lag phase, or TBARS in Cu2+- or AAPH-oxidized LDL.
Himmelfarb et al., 2007	Randomized, double-blind, placebo controlled	Dialysis patients (n=63) Placebo group: n=32, mean age 61 Treatment group: n=31, mean age of 58	Placebo: 6 capsules/day (total of 3 g of high oleic sunflower oil) Treatment: 6 capsules/ day (2 capsules delivering a total of 308 mg of gamma-tocopherol, 13 mg alpha-tocopherol, 11 mg beta- and delta-tocopherol/day and 4 capsules delivering a total of 800 mg of DHA)	8 weeks	Subjects continued all medications and only changes medications and dosages as prescribed by their physician. All subjects received routine IV erythropoietin for the treatment of anemia. Placebo oil is high oleic acid sunflower oil.	1. Numbers of white blood cells, neutrophils in plasma 2. Biomarkers of inflammation and oxidative stress (plasma IL-6, C-reactive protein, F2-isoprostane levels) Tocopherol and DHA-supplementation: 1. DHA treatment significantly reduced, versus baseline levels, plasma IL-6 concentrations by approx. 30% (P<0.05), white blood cell and neutrophil number by			Changes in IL-6, WBCs, neutrophils, erythropoietin/hematocrit: 800 mg DHA/d	The fatty acid composition of the four capsules delivering DHA was not noted Dropouts were attributable to non-compliance and adverse events not related to the study.

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						10% (P<0.05), and erythropoietin/hematocrit by 30% (P<0.05). 2. Had no effect on plasma C-reactive protein concentrations or on F2-isoprostane levels.				
Jain et al., 2002	Randomized, placebo-controlled	Type 2 diabetics (30 years old, n=65)	Patients were prescribed dietary modifications (25% of total caloric intake was due to fats (8-10% saturated fats, 8-10% monosaturated fats, 8-10% polyunsaturated fats n-3 and n-6)) Placebo: 2x ?/day EPA/DHA supplemented group: 2 x 1 capsule of Maxigard (containing 0.6 g n-3 fatty acids, of which 180 mg is EPA and 120 mg is DHA)/day	6 weeks	All patients were instructed to take their oral hypoglycemic agent and/or insulin in the same dose throughout the study Placebo composition was not stated.	1. Lipid peroxides 2. Diene conjugates 3. Glutathione levels	Various lipid parameters and blood pressure Versus baseline, Maxigard alone caused: --Decrease in postprandial blood sugar (P<0.05). --Decrease in systolic and diastolic blood pressure (P<0.001 for both).		↓Postprandial blood sugar, systolic and diastolic blood pressure: (1.2 g n-3 fatty acids) 360 mg EPA + 240 mg DHA/d	Patients with complications exhibited higher levels of lipid peroxides, diene conjugates, and lower levels of reduced glutathione versus patients without complications.
Kelley et al. 2007	Double-blind, randomized, placebo-controlled, parallel study.	Hypertriglyceridemic men aged 39-66 yr received no supplements for 8 d and then were assigned to receive 7.5 g DHA oil (3 g DHA) (n=17) per d or olive oil for 90 d (n=17).	The DHA group received 7.5 g DHA oil from the microalga *Crypthecodinium cohinii* (Martek Biosciences Corp, Columbia, MD), which delivered ~ 3 g DHA/d. The placebo group received 7.5 g olive oil/d. Oils were provided as 15 capsules/d and5 capsules were taken per meal.	8 d wash-out period followed by 90 d study	Exclusion criteria included subjects regularly taking anti-inflammatory medications, antihypertensives, nonsulfonylurea medications or drugs that alter serum lipids. Also excluded were those consuming illegal substances, > 5 alcoholic drinks/wk, > 1 fish meal/wk, and supplements of fish oil, flaxseed oil, or vitamin C or E.	Versus baseline levels, the DHA group exhibited a significant decrease in TG and Apo CIII, and a significant increase in LDL-cholesterol, at the end of the study (P< 0.05 for all). Postprandial TAGs were also reduced in the DHA group, versus baseline levels (P< 0.05). Large LDL was increased and IDL and			7.5 g DHA oil (3 g DHA)	Two subjects in the DHA group had a feeling of gas or bloating. Postprandial blood samples were collected from only 14 subjects in each group.

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						Primary	Secondary	NEL	EL	
					Inclusion criteria included fasting serum concentrations of TAG 150-400 mg/dL, total cholesterol < 300 mg/dL, and LDL < 220 mg/dL and a BMI between 22-35. Three participants were smokers; one in the placebo group and two in the DHA group. Diet was controlled and provided 1 d prior to blood draws.	small LDL were decreased in the DHA group versus baseline levels ($P< 0.05$). There were no effects on the total or HDL cholesterol.				
Kenler et al. 1996	To compare the safety, gastrointestinal tolerance and clinical efficacy of feeding an enteral formula containing fish oil versus a control formula in postsurgical cancer patients. Prospective Blinded Randomized	Patients undergoing major abdominal surgery for upper gastrointestinal malignancies (n=50; 25 per feeding group) Patients were considered evaluable based on ability to reach a tube feeding rate of > 40 mL/h: Experimental formula (n=17) Control isocaloric formula (n=18)	Tube-fed enteric (jejunal) formula containing fish oil (including EPA + DHA) × 7 d. Patients receiving >40 mL/h of the experimental diet received an intake of 3.27 ± 0.22 g EPA and 1.48 ± 0.10 g DHA for a total of 4.75 g EPA+DHA/d × 7d.	7d	Steroid drug intake was excluded indirectly by excluding patients who had chronic diseases requiring daily corticosteroid doses > 15 mg prednisone or equivalent steroid(s).	Plasma lipids • EPA and DHA were significantly increased in plasma triglycerides (p=0.00 for both) • EPA was significantly increased in plasma phospholipids (p=0.00) and red blood cell membrane fatty acids (p=0.00).	Urinary and plasma prostaglandins • No significant changes versus control group.	3.27 ± 0.22 g EPA and 1.48 ± 0.10 g DHA/d		• Patients experienced no untoward side effects from the fish oil treatment. • Diets were isocaloric.

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
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Kim et al. 2010	Study the effects of omega-3 fatty acid supplements and simvastatin on lipoproteins and heart rate variability in patients with mixed dyslipidemia. Prospective Randomized Open-label	Korean patients having mixed dyslipidemia, defined as triglycerides of 200-499 mg/100 mL and a total cholesterol level of > 200 mg per 100 mL. 171 patients were screened, 62 met inclusion criteria after 6 wk on diet therapy and were randomized.	After 6 wk run-in period, patients were randomized to receive four 1 g Omacor (*a.k.a., Lovaza;* 465 mg EPA, 375 mg DHA, 60 mg other omega-3 fatty acids, 100 mg other substances) plus 20 mg simvastatin/d (*n*=30) or simvastatin (20 mg/d) alone (*n*=32) × 6 wk. 1.86 g EPA, 1.5 g DHA = 3.36 g (EPA+DHA)/d × 6 wk	6 wk	Simvastatin 20 mg/d No control group using Lovaza only.	• Treatment with simvastatin plus Omacor caused a statistically significant reduction in fasting triglycerides (TG), versus the reduction in TG achieved with simvastatin-alone (p<0.01).				• There is a mild effect of EPA+DHA on glucose in patients being treated with simvastatin. However, the clinical significance of this is unclear. • Authors do not mention adverse events occurring with the combination therapy.
Koletzko et al., 2003	Randomized, double-blind	Infants (n=29)	Formula +/- PUFAs (0.5 g linoleic acid metabolites/100 g fat, 0.8 g a-linolenic acid metabolites). Formula with PUFAs contained 0.13% EPA and 0.57% DHA. Milk intake was not statistically significant between the groups and ranged from 145 to 155 ml/kg day	4 weeks		Plasma fatty acid profile, antioxidant status and infant growth were evaluated. No increase over time in urinary malondialdehyde was observed in the PUFA group. There was no significant change over time in growth rate in the PUFA group.		Formula containing 0.13% EPA + 0.57% DHA		

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Leaf et al., 1994	Placebo controlled, single-blind (patient)	Patients undertaking voluntary percutaneous intraluminal coronary angioplasty; treatment (n= 226), placebo (n=221)	Ten 1-g gelatin capsules providing ethyl esters of EPA (4.1g) + DHA (2.8) or ethyl ester of corn oil (control)	6-month	Aspirin. Patients were instructed to follow a Step-One American Heart Association diet, although compliance varied among patients. Corn oil was the control.	Rate of restenosis	Blood lipids At 3 mo, versus baseline in the fish oil group, triglycerides were reduced significantly (by 41%; no statistics). No significant difference was seen between groups.			
Maki et al., 2005	Double blinded, placebo controlled	Healthy people with below-average levels of HDL cholesterol. DHA group (n=27), olive oil (n=30)	Four 1-g capsules of Martek oil (providing 1.52g/d DHA and 0.08g/d EPA) or olive oil	6 weeks	No foods and supplements with significant omega-3 fatty acid content. No use of hypolipidemic medication, dietary fiber supplements, and products containing phytosterols/stanols Olive oil was the control.	Lipid response • 1.52 g DHA + 0.08 g EPA/d reduced, versus baseline: --Triglycerides (*P*=0.015) --small LDL_3+LDL_4 (*P*=0.074) --%LDL_3+LDL_4 (*P*=0.025) --Triglycerides/HDL ratio (*P*=0.010) • 1.52 g DHA + 0.08 g EPA/d increased, versus baseline: --Total cholesterol (*P*=0.021) --HDL (*P*=0.080) --LDL (*P*=0.001) --Large LDL_1+LDL_2 (*P*=0.006)			↓TAG, LDL particle size; ↑total cholesterol, HDL, LDL, LDL particle size 1.52g DHA + 0.08 g EPA/d	
Maki et al. 2011	Double-blind, randomized, cross-over study	Thirty-one men and women with primary, isolated hypercholesterolemia (LDL-C 130-220 mg/dL; TAGs < 150 mg/dL while free of lipid-altering therapies). 15 or 16 subjects per treatment per phase.	4 g POM3/d (Lovaza; GlaxoSmithKline, Research Triangle Park, NC; 3.5 g EPA+DHA/day) or soy oil placebo capsules	6 wk per phase	Lipid-altering drugs were discontinued 3 wk prior to screening and lipid-altering dietary supplements were excluded within 2 wk of screening. Up to two servings of fish per week were allowed. Other substances prohibited include weight loss	Total cholesterol, HDL, VLDL, TAGs, and LDL (calculated), plus apolipoprotein A1 and B particle sizes and concentrations were measured at weeks -1, 0, 6 and 12. Serum chemistry, hematology and urinalysis were carried out at weeks -1, 6 and 12.			↓LDL, changes in VLDL, LDL, HDL particle sizes: 4 g ethyl esters of omega-3 fatty acids/d as	• No wash-out period between treatments after cross-over. • There was a significantly larger (*P* = 0.012) increase in fasting glucose concentration in the treatment group vs. placebo. • Gamma glutamyl transferase was

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					drugs, antiepileptic, corticosteroid, antibiotic, anticoagulant, protease inhibitor, estrogen, progestin, and hypoglycemic medications, plus unstable use of antihypertensive medications or thyroid hormone replacement. LDL-c was calculated using the Friedewald equation. The placebo was soy oil.	• LDL was significantly increased (P = 0.010), while VLDL and TAGs were significantly decreased (P< 0.0001 for both) in the treatment group vs. placebo. • VLDL particle size was significantly decreased (P = 0.002), while LDL and HDL particle size was significantly increased (P = 0.017 and 0.0002, respectively) in the treatment group, vs. control.			Lovaza	significantly increased (P = 0.039) and alkaline phosphatase was significantly decreased (P< 0.0001) in the treatment group vs. control. • Diastolic blood pressure was significantly reduced during treatment (P =0.026) vs. placebo, but systolic blood pressure was unaffected. • There were no serious adverse events. • Adverse events that were judged to be possibly treatment-related included mildly elevated alanine aminotransferase (n=1), mild insomnia (n=1), and albuminuria (n=1).
Mann et al. 2010	Randomized, parallel, double-blind, placebo-controlled	Thirty healthy volunteers.	7 d weighed food record before dietary fatty acid supplementation. Subjects consumed either a placebo oil (n=7), fish oil and placebo oil (n=10) or seal oil (n=10). 10 soft-gel capsules were consumed per day: 1) placebo (10 500 mg capsules of sunola oil, high oleic acid sunflower oil: 85% 18:1 n-9, 5% 18:2 n-6,5% 16:0)	14 d washout period with low omega-3 fatty acid diet, then 14 d	Inclusion criteria included no known metabolic, endocrine or hematological diseases, not smokers, no concomitant medications and moderate physical activity level. Confirmed no consumption of antioxidant or other nutrient supplements after enrollment.	Platelet-related parameters, p-selectin, plasma TAG, and HDL levels were measured. • In the fish oil group, platelet ATP release was significantly elevated versus baseline (P< 0.05). • In the seal oil group, HDL was significantly increased, while platelet activation (measured as P selectin expression) and TAGs were significantly decreased versus baseline (P< 0.05).	•In the fish oil group, the total saturated fatty acid content of platelets was significantly reduced at d 14 vs. d 0 (P< 0.05). The DHA content was significantly increased versus baseline (P>0.005). • In the seal oil group, 16:0, 16:1 n-7, 18:1 n-9, total monounsaturated fatty acids,		Seal oil ↑HDL: 340 mg EPA + 230 mg DPA + 450 mg DHA + 1420 mg oleic acid	• This study may be the first to investigate the effect of a DPA-rich seal oil on platelet- and lipid-related parameters. • DPA is found in low levels in fish but in higher amounts in red meats.

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			(Total consumed: 4250 mg 18:1 n-9), 2) 4 placebo capsules plus 6 capsules tuna oil (Total consumed: 210 mg EPA, 30 mg DPA, 810 mg DHA, 1944 mg 18:1 n-9) or 3) 10 capsules seal oil (Total consumed: 340 mg EPA, 230 mg DPA, 450 mg DHA, 1420 mg 18:1 n-9).				total n-9, and total n-7 fatty acids were reduced at d 14 vs. d 0 (P<0.05). DPA, EPA, and DHA were significantly increased versus baseline (P<0.01).			
Mesa et al., 2004,	Randomized, double-blind, placebo-controlled	Healthy subjects (23-65 years old; n=42)	Three groups: Placebo – 9 g olive oil/day EPA – 9 g EPA-rich oil DHA – 9 g DHA-rich oil EPA- and DHA-rich oils also contained small amounts of other n-3 and n-6 fatty acids. The DHA-rich oil also contained 10.7% DPA. All capsules contained 10IU of mixed natural tocopherols to prevent oxidation.	4 weeks	Placebo was olive oil.	1. LDL oxidation *in vitro* 2. LDL-supported thrombin generation 3. Fatty acid composition of LDLs Placebo significantly reduced the rate at which conjugated dienes were formed *in vitro*; EPA-rich oil significantly increased the rate; the DHA-rich oil had no effect. There were no significant changes in the lag time or oxidation rate of LDLs pre- and post-treatment. EPA- and DHA-rich oils significantly increased the amount of EPA and DHA in LDL phospholipids, respectively. EPA- and DHA-rich oils significantly increased the amount of EPA and DHA in LDL cholesterol esters, respectively, and the			↑Rate of formation of conjugate dienes: 4 g EPA/d	Blood samples were taken from fasted subjects.

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						increase was much more dramatic in the EPA-rich oil supplemented group. Mild oxidation *in vitro* did not significantly alter the composition of LDL- phospholipids or cholesterol esters. There were no statistically significant changes in thrombin generation following supplementation with EPA- and DHA-rich diets.				
Meyer et al. 2007	Assess the dose-dependent effects of fish oil supplementation on blood lipids, blood pressure and arterial compliance in hyperlipidaemic patients. Placebo-controlled Randomized	45 hyperlipidaemic patients on stable stain therapy with persistent elevated plasma triglycerides. 40 subjects completed the trial	Olive oil placebo or 4 or 8 g tuna oil/d × 6 mo Fish oil = HiDHA DHA-rich tuna oil; Clover Corporation: 7% EPA, 27% DHA 0.28 g EPA + 1.08 g DHA = 1.36 g (EPA+DHA)/d or 0.56 g EPA + 2.16 g DHA = 2.72 g (EPA+DHA)/d × 6 mo	6 mo	Simvastatin, atorvastatin, pravastatin, cerivastatin, fluvastatin Placebo was olive oil. No control group using tuna oil alone.	• Subjects in the low-dose DHA-rich fish oil group (4 g/d) had a marginal reduction in total cholesterol at 3 mo (p=0.05). • The high-dose group (8 g/d) had a statistically significant reduction in triglycerides at both 3 and 6 mo (p<0.005), versus the control group.				• The reduction of plasma total cholesterol correlated with initial cholesterol levels. • There were no changes in blood pressure or in proximal or distal arterial compliance (data not shown) during treatment with DHA-rich fish oil.
Mori et al 1992	Double blinded, placebo controlled	Patients with peripheral vascular disease. MaxEPA (n=15), olive placebo (n=14)	Fifteen 1-g capsules/d of either MaxEPA (fish oil concentrate providing 5.2 g ω-3 fatty acids including 2.8 g EPA and 1.8 g DHA) or olive oil placebo (providing 11.2 g oleic acid)	4 weeks	Olive oil was the placebo.	Lipid, platelet and neutrophil function Dietary fish oil reduces platelet and neutrophil activity.	Lipid parameters <u>Versus the olive oil placebo, MaxEPA</u>: --Increases LDL at 4 wk (P<0.05). --Decreases TAGs at 2 and 4 wk (P<0.05).		↑LDL; ↓TAG: 2.8 g EPA + 1.8 g DHA/d (5.2g total omega-3/d)	

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Mori et al., 2000	Randomized	1st Trial: Untrained and sedentary NIDDM patients (n=49)	Low fat diet +/- one daily fish meal (3.6 n-3 fatty acids/ day) +/- moderate or light exercise	8 weeks	Control diet was low fat diet without one daily fish meal. Fish was given in a meal, rather than supplementing DHA/EPA in purified form.	Urinary F2-isoprostanes			↓Urinary F2-isoprostanes: 3.6 g n-3 fatty acids (as one fish meal/d)	The fish meal significantly reduced F2-isoprostanes, versus the control group (P=0.01). Moderate exercise had no effect on the diet.
Mori et al., 2000	Randomized	2nd Trial: Overweight, mildly hyperlipidemia men	Placebo: 4 g olive oil Treatment 1: 4 g purified EPA Treatment 2: 4 g purified DHA	6 weeks	Inclusion/exclusion criteria was not noted Placebo was olive oil.	Urinary F2-isoprostanes			↓Urinary F2-isoprostanes: 4 g EPA or 4 g DHA/d	Because the fatty acid content of the olive oil and EPA+ DHA oil was not noted, no conclusions can be made about the specific roles of EPA and DHA in modulating the observed effects. Baseline values were adjusted to accommodate differences between the groups at the beginning of the trial. EPA significantly reduced F2-isprostanes by 27% (P<0.001). DHA significantly reduced F2-isoprostanes by 26% (P<0.001).
Mori et al., 2003	Randomized double-blind, placebo-controlled	Type 2 diabetics (n=59)	Placebo: 4 g olive oil (n= 16) Treatment 1: 4 g purified EPA ethyl ester (n=17) Treatment 2: 4 g purified DHA ethyl ester (n=18) alpha- and gamma-tocopherol levels were similar in the different treatments.	6 weeks	Subjects took hormone replacement therapy (women only), lipid-lowering drugs, aspirin, or antioxidant vitamins and doses were maintained throughout the study. Olive oil was the placebo.	Urinary F2-isoprostanes and markers of inflammation There was a significant reduction in urinary F2-isoprostanes in the EPA (P=0.017) and DHA (P=0.014) groups, versus baseline levels.			↓Urinary F2-isoprostanes: 1.4 g EPA ethyl esters or 2.4 g DHA ethyl esters/d	Withdrawals were due to personal reasons, not treatment related effects. Baseline values were adjusted to accommodate differences between the groups at the beginning of the trials.

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Mostad et al. 2008	Two-armed, parallel, placebo-controlled, randomized.	Twenty-six normotriglyceridemic subjects with type II diabetes who were not on insulin treatment.	Subjects consumed 20 mL of either corn oil (*n*=14) or fish oil (*n*=12). 60 mg of vitamin C was also ingested daily with the oil. Corn oil contained 8.5 g of 18:2 n-6 and fish oil contained 5.9 g of total n-3 fatty acids, of which 1.8 g was 20:5 n-3 and 3.0 g was 22:6 n-3.	9 wk	Type II diabetes was defined by clinical criteria and by the absence of antibodies to glutamic acid decarboxylase. Subjects either never used supplements containing marine n-3 fatty acids or discontinued such supplements for at least 6 mo before baseline. Exclusion criteria included insulin treatment, hypertriglyceridemia, proliferative retinopathy, pregnancy/lactation, allergy to fish or citrus, smoking, alcoholism and congestive heart failure or other serious diseases. Control was corn oil.	Serum lipid parameters were measured and median values per group were compared statistically. • Total HDL and small HDL were both increased in the fish oil group vs. the corn oil group at 9 wk (*P* = 0.051 and 0.012, respectively) and small HDL • VLDL size and large VLDL were decreased in the fish oil group at 9 wk versus the corn oil group (*P*=0.001 and 0.041, respectively).				• Baseline comparisons were not carried out.
Mostad et al., 2009	Double-blind crossover	Type 2 diabetics (30 – 70 years old; n=12)	All subjects underwent 2 Intralipid (soybean) infusions as isoglycemic hyperinsulinemic clamps with or without Omegaven. Intralipid contained 200g/L soybean oil. Omegaven contained 100 g/L fish oil. In addition, 4 subjects underwent an infusion of insulin and glucose to act as controls.	2 weeks	Eight subjects used standard anti-diabetic treatments (metaformin, metformin and glibenclamide, metformin and glimepiride) Three subjects received statins Four subjects received antihypertensive treatment Control contained soybean oil.					In this study, lipids were infused rather than consumed and pathways of metabolizing fats are different.

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Neff et al. 2011	Randomized, controlled, double-blind	Thirty-six overweight or obese adults.	Algal DHA oil (5 mL/d, delivering 2g DHA/d) or placebo (corn-soybean (1:1) oil mixture). *Intervention was designed to decrease the (n-6):(n-3) fatty acid ratio from ~10:1 to 4:1.	4.5 mo	Individuals were excluded who had high blood pressure, high fasting blood sugar, glucosuria, liver/renal/thyroid disease, HIV infection, or if they took hypertension, dyslipidemia, diabetes or weight control drugs. Fish oil supplementation, hormonal therapy or vitamin supplementation in doses exceeding the RDA were also excluded.	Study was designed to examine the effects of DHA alone on plasma lipid and lipoprotein concentrations in the absence of weight loss. • DHA supplementation decreased: --mean VLDL particle size ($P \leq 0.001$) --concentrations of small LDL particles ($P = 0.009$) --concentrations of medium HDL particles ($P = 0.001$) --VLDL triglyceride and total triglyceride concentrations (NMR measurement) ($P = 0.009$ and 0.006, respectively) versus control. • DHA supplementation increased: --mean LDL and HDL particle sizes ($P \leq 0.001$ for both) --concentrations of large LDL and HDL particles ($P \leq 0.001$ for both) versus control. • Placebo oil was corn oil:soy oil (1:1).	Other biomarkers of cardiovascular risk were evaluated.		Changes in VLDL, LDL, HDL particle sizes: 2 g DHA/d (5 mL algal DHA oil/d)	• Plasma IL-10 levels were increased with DHA supplementation vs. placebo ($P = 0.021$). • No changes were observed in glucose metabolism, insulin sensitivity, blood pressure or inflammatory markers with DHA.

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Nenseter et al., 1992	Randomized, placebo-controlled	Normolipidemic men and women (23-70 years old. Control group (n=11) Treatment group (n=12)	6 x 1 g capsules of either corn oil or highly concentrated ethyl esters of n-3 fatty acids (47.1% EPA, 2.7% DPA, and 29.6 % DHA ethyl esters (Norsk Hydro).	4 months	Corn oil was the placebo.	Fatty acid composition of plasma phospholipids Fish oil: 1. Significantly reduced the concentration of n-6 fatty acids and increased the concentration of n-3 fatty acids in plasma phospholipids. 2. Significantly reduced the cholesteryl ester content of LDL particles versus corn oil (P<0.05).	Fish oil displaces saturated and monounsaturated fatty acids from cholesteryl esters of LDL, displaces the monounsaturated fatty acid content of triglyceride content of LDL, and increases the n-3 content of both triglycerides and phospholipids in LDL.			Comparisons to baseline are only relevant because corn oil is not an appropriate control. Importantly all comparisons except for the analysis of fatty acids in plasma phospholipids were compared to the corn oil group.
Nestel et al., 2002	Double blinded, placebo controlled	Dyslipidemic subjects, EPA arm (n=12), DHA arm (n=12), placebo arm (n=14)	Four 1-g capsules/d containing either ethyl esters-EPA (3.04g), ethyl esters of DHA (2.84g) plus DPA (0.52g) or 2.8 g oleic acid (in olive oil)	7 weeks	Control was 2.8 g oleic acid in olive oil.	Systemic arterial compliance	VLDL, LDL Plasma total and VLDL triacylglycerol were significantly lower in the n-3 fatty acid groups than in the placebo group. Plasma total cholesterol and LDL cholesterol did not change significantly over time with either treatment or placebo.			

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Nordøy et al. 2000	Evaluate the effect of omega-3 fatty acids and simvastatin on the hemostatic risk profile and postprandial hyperlipidemia in patients with combined hyperlipidemia. Randomized Double-blind Placebo-controlled	41 patients (12F/29M) aged 25-60 yr having combined hyperlipidemia with a mean fasting serum triacylglycerol (TAG) of 2.0-15.0 mmol/L and serum total cholesterol > 5.3 mmol/L after a 16 wk dietary run-in period.	16 wk dietary run-in period followed by treatment with 20 mg/d simvastatin for 5 to 10 wk. Then patients were randomized into two groups: simvastatin + placebo (corn oil) (*n*=20) or simvastatin + omega-3 fish oil capsules (*n*=21) for 5 wk. Omega-3 as 4 g capsules/d containing **1.8 g EPA + 1.56 g DHA = 3.36 g (EPA+DHA)/d plus 20 mg/d simvastatin × 5 wk.**	5 wk	Simvastatin 20 mg/d No fish oil-only group. Placebo oil was corn oil.	Lipid parameters • Combination treatment significantly reduced postprandial triacylglyceride incremental area under the curve and the triglyceridemic response, versus placebo (p=0.003 and 0.001, respectively).				• Combination treatment significantly increased the postprandial insulin/glucose ratio versus placebo (p=0.045). The physiological effect of this is unknown.
Pedersen et al., 2003	Randomized, double-blinded Placebo-controlled	Type 2 diabetic patients (38-85 years old; n=42)	Four 1 g capsules of corn oil or fish oil (Futura, Dansk Droge) daily. Fish oil capsules contained 40.2% EPA and 25.4% DHA. The remaining 35% consisted of a mixture of n-3, n-6, n-7, and n-9 fatty acids of varying length.	Four week run-in with corn oil followed by an eight week treatment period when subjects consumed either corn oil or fish oil.	Nine subjects were smokers Corn oil was the control.	1. Fatty acid composition of LDLs 2. LDL oxidation 3. Blood glucose 4. HbA1c 5. Body weight 6. LDL concentrations of malondialdehyde Fish oil supplementation at end of study, versus baseline levels: 1. Significantly decreased the concentration of 18:2n-6 and 20:3n-6 fatty acids and increased the amount of EPA and DHA in LDLs the Fatty acid composition of LDLs 2. Significantly decreased the lag time and propagation rate of LDL oxidation *in vitro*			↓Lag time, propagation rate of LDL oxidation *in vitro*; ↑blood glucose; ↓TAG; ↑HDL: 1.608 g EPA + 1.016 g DHA/d	Because of the corn oil run-in period, conclusions between the different groups can be made because the run-in period establish the baseline values that were maintained throughout the study.

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						(*P*<0.001 for both). 3. Significantly increased blood glucose levels (*P*=0.047) but had no effect on HbA1c levels. 4. Significantly reduced total triglycerides (*P*<0.001) , had no effect on total cholesterol, and increased HDL-cholesterol (*P*=0.019) levels. 5. Had no effect on body weight. 6. Increased LDL-malondialdehyde concentrations, although the increase was not statistically significant at p<0.05.				
Piolot et al., 2003,		Healthy normolipidemic subjects (n=28); control group consisted of healthy normolipidemic subjects (n=16) that were not treated	6 g/day of fish oil (Super EPA; each 1 g capsule contained 0.671 mg of a-tocopherol, 300 mg EPA and 200 mg DHA = 4 mg a tocopherol, 1.8 g EPA, and 1.2 g DHA/d)	4 weeks (16 subjects extended treatment for 4 weeks)		1. Phospholipid n-6 and n-3 fatty acid concentrations. 2. Plasma and very low-density lipoprotein, and triglyceride levels 3. LDL- and HDL-cholesterol 4. Lipid oxidation lag time and diene propagation rate. 5. Plasma homocyteine, glutathione, cysteine, and cysteinylglycine levels <u>Fish oil supplementation, versus baseline</u>: 1. Reduced phospholipid n-6 fatty acids and increase phospholipid n-3 fatty acids, specifically EPA and DHA. 2. Significantly decreased plasma and very low-density lipoprotein triglycerides			↓VLDL-TAG, plasma TAG, diene propagation rate; ↑homocysteine, glutathione: (6 g fish oil/d) 1.8 g EPA + 1.2 g DHA/d	Blood was sampled from fasting subjects

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						(P<0.05). 3. Had no effect on LDL- and HDL-cholesterol 4. Significantly reduced diene propagation rate at 4 weeks despite no effect in TBARS and lipid oxidation lag time. 5. Had no effect on plasma TBARS or the lipid oxidation lag time or diene-propagation rate at 8 weeks. 6. Significantly increased plasma homocyteine (P<0.01) and glutathione (P<0.05) concentrations. 7. Had no effect on cysteine and cysteinylglycine levels.				
Pooya et al. 2010	Randomized, double-blind, placebo-controlled	Eighty-one patients with type 2 diabetes.	Three capsules per day containing a total of 2714 mg omega-3 fatty acids (1548 mg EPA; 828 mg DHA; 338 mg other omega-3 fatty acids) or placebo capsules (2100 mg sunflower oil: 12% saturated fatty acids, 71 linoleic acid, 16% monounsaturated fatty acids)	2 mo	Inclusion criteria included no consumption of omega-3 fatty acids, multi vitamin-mineral supplements, or drugs which interact with the lipid profile. Placebo capsules contained sunflower oil.	• LDL and total cholesterol were unaffected by the DHA intervention vs. placebo.				• HbA_1c and homocysteine were significantly reduced in the treatment group, vs. control (P< 0.001 for both). • No effect was observed on malondialdehyde, C-reactive protein, and fasting blood sugar.
Rhodes et al., 2003	Double-blind, randomized	Healthy Caucasians (n=42)	1. EPA ethyl ester (95% EPA, 4% other omega-3s, <1% omega-6s) = 3.8 g/day Control was Oleic acid ethyl ester (95 % OA, <1% omega-3s) = 3.8 g/day	3 months	Control was oleic acid ethyl ester	1. EPA content in the skin 2. Levels and degree of Vitamin E, Vitamin C, and glutathione, and lipid peroxidation. 3. Oxidation of Vitamin C and glutathione			↓Threshold to sunburn: 3.8 g EPA ethyl esters/d	No side effects were reported other than increased flatulence. <u>Versus baseline</u>: 1. EPA supplementation increased the EPA content in the skin by approx. 8-fold and the % of oxidized

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			*Both supplements contained 0.0015% (wt/wt) butylhydroxyanisole.			4. Erythema threshold to UVR 5. p53 expression in the skin 6. UVR induced DNA damage in PBLs.				vitamin C by 2-fold, but did not significantly increase the amount of skin lipid peroxidation or glutathione levels. 2. Vitamin E levels in the skin were lower following EPA supplementation but the reduction was not statistically significant and UVR treatment had no effect. 3. EPA supplementation also increased the threshold to sunburn (P<0.01) and decreased p53 protein expression in the skin 4. EPA supplementation reduced UVR-induced DNA damage in PBLs *in vitro* but did not affect basal oxidative DNA damage *in vivo*.

Table 1. Effects of omega-3 fatty acids on blood lipids and lipid oxidation

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Roth et al. 2009	Study the safety and efficacy of 4 g prescription fatty acids per day plus concomitant fenofibrate in subjects with triglyceridemia. 6 wk dietary lead-in period Randomized Double-blind Placebo-controlled *Note: No control group receiving fish oil only.	Subjects with very high triglyceride levels (≥ 500 mg/dL and < 1300 mg/dL)	4 g/d prescription omega-3 fatty acids (Lovaza, Reliant Pharmaceuticals, Inc., NC), with (*n*=81) 130 mg/d fenofibrate, versus fenofibrate plus placebo (corn) oil capsules (*n*=82). [Manufacturer insert states Lovaza contains 465 mg EPA and 375 mg DHA per 1 g capsule] 4 g capsules: 1.86 g EPA, 1.5 g DHA = 3.36 EPA+DHA/d × 8 wk study plus open-label 8wk+ extension studies (+ fish oil) for patients who completed 8 wk of either therapy.	8 wk	Fenofibrate 130 mg/d Excluded, via exclusion criteria: warfarin, cyclic sex hormone therapy, cyclosporine, systemic corticosteroids, high-dose topical corticosteroids, androgens, phenytoin, isotretinoin, and thyroid hormones. Placebo contained corn oil.	• Combination therapy resulted in a statistically significant decrease in VLDL-cholesterol (*p*=0.016), increase in LDL-cholesterol (*p*=0.030), and decrease in remnant-like particle cholesterol (RLP-C) (*p*=0.029), versus fenofibrate + placebo during the main study. A similar effect was observed for patients on monotherapy who were switched to combination therapy during the first extension (plus a ↓ in triglycerides, *p*=0.003).				• Pharmaceutical-grade fish oil capsules containing EPA+DHA were generally well tolerated at 4 g capsules/d when used in combination with 130 mg fenofibrate per day. • Placebo contained corn oil.
Sanders et al. 2006	Double-blind, randomized, placebo-controlled, parallel design study.	Men and women were recruited within a university setting and randomized to receive either 4 g DHA-rich oil/d (1.5 DHA + 0.6 g EPA) (*n*=40; 20M/20F) or 4 g olive oil/d (*n*=30; 19M/20F).	4 g oil/d containing 1.5 g DHA + 0.6 g EPA (DHA-rich TAG derived from *Schizochytrium* spp.) or 4 g/d olive oil	4 wk	Exclusion criteria included: current tobacco use, history of myocardial infarction or diabetes, current pregnancy, use of lipid-lowering or blood pressure-lowering medication or immunosuppressive drugs or hormone replacement therapy or systemic corticosteroids, ndrogens, phenytoin, erythromycin, thyroid hormone, drugs for regulating haemostasis but excluding aspirin, alcohol intake above	Statistics for endpoints measured in the DHA group versus baseline levels were not reported. A minimal increase in LDL and HDL was noted over the course of the study. However, the amount of change did not exceed the standard deviations provided. There appeared to be no effect on TAG.				

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					a set limit, BMI <18 or >35 kg/m^2, serum cholesterol > 7.8 mmol/L or fasting serum TAG > 3.0 mmol/L, systolic b.p. > 140 mm Hg or diastolic blood pressure > 90 mm Hg, abnormal haematology or liver function test. Subjects were also excluded if they were unwilling to abstain from eating oily fish, fish oil supplements, algal oil supplements or flaxseed oil supplements. Placebo contained olive oil.					
Schwellenbach et al., 2006	Prospetive, randomized, double-blind study.	Patients with coronary artery disease and triglycerides > 200 mg/dL were recruited and randomized to receive 1000 mg DHA/d (*n*=57) or 1252 mg of DHA + EPA (containing 1000 mg DHA)/d (*n*=59).	1000 mg DHA/d (*n*=57) or 1252 mg of DHA + EPA (containing 1000 mg DHA)/d	8 wk	Adult patients enrolled in the Clinical Pharmacy Cardiac Risk Service with a history of coronary artery disease were eligible to participate. Subjects having two consecutive TAG between 200-750 mg/dL, LDL < 100 mg/dL and TSH within normal limits (0.32-5.50 IU/mL) within the past year were considered. Concomitant use of statins, diuretics, beta-blockers, estrogen, levothyroxind and/or oral corticosteroids was permitted if doses did not change 2 mo prior to enrollment. Patients were excluded if	Subjects in both groups experienced a significant decrease in TAG, total cholesterol, and non-HDL cholesterol, versus baseline levels ($p <$ 0.001 for TAG, $p< 0.05$ for cholesterol and non-HDL cholesterol). HDL cholesterol was increased, versus baseline, only in the DHA group ($p< 0.05$).			↓TAG, total cholesterol, non-HDL cholesterol: 1000 mg DHA/d or 1252 EPA + 1000 mg DHA/d ↑HDL: 1000 mg DHA/d	Two subjects, one in each group, stopped treatment due to gastrointestinal disturbance. More subjects in the DHA+EPA group reported fishy taste.

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					pregnant or breast feeding, had history of alcoholism, had HbA1c > 8.5% within the past 6 mo, were taking omega-3 suppleents >400 mg DHA/d, receiving > 5 mg prednisone/d or hospitalized within the previous 2 mo.					
Shidfar et al., 2003	Double-blind, randomize, placebo-controlled	71 hyperlipidemic patients (33-75 years old; total cholesterol and triglycerides greater than 200 mg/dl)	Group 1: 500 mg placebo (300 mg saturated fatty acids, 100 mg mono-unsaturated acids, and 600 mg linoleic acid) (n=19). Group 2: Placebo and 1 g n-3 fatty acids (n=16). Group 3: 500 mg vitamin C and 1 g n-3 fatty acids (n=16). Group 4: Placebo and fatty acid placebo (n=16) *n-3 fatty acids were provided by Advanced Nutritional Technology Co., Super EPA 2000	10 weeks	Placebo contained 300 mg saturated fatty acids, 100 mg mono-unsaturated acids, and 600 mg linoleic acid.	1. Serum lipids and lipoprotein apoB and apo A-1 levels 2. Vitamin C 3. Malondialdehyde			↓TAG: 1 g n-3 fatty acids (uncharacterized)	Blood was taken from fasted subjects 3 subjects withdrew, two due to failure to maintain visit schedule and one due to gastrointestinal problems <u>Versus baseline:</u> 1. n-3 fatty acid supplementation had no effect on total cholesterol, LDL-cholesterol, and HDL-cholesterol, or on serum levels of apo A-1 and apoB. 2. n-3 fatty acid supplementation significantly reduced serum triglyceride (*P*=0.05) 3. n-3 fatty acid supplementation had no effect on serum levels of MDA and vitamin C.

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Shidfar et al., 2008	Double-blind, placebo-controlled	Type 2 diabetes patients (33-75 years old, n=50)	Placebo – 2 g/day (300 mg saturated fatty acids, 100 mg monosaturated fatty acids, and 600 mg linoleic acid/capsule) Omega 3 – 2 g/day (520 mg EPA and 480 DHA/capsule = 1040 mg EPA and 960 mg DHA/day	10 weeks	Control oil had differing fatty acid composition from omega-3 group.	Blood levels of: 1. Glucose 2. Insulin 3. HbA1c 4. ApoA-1 5. Total cholesterol 6. HDL-cholesterol 7. LDL-cholesterol 8. ApoB-100 9.Malondialdehyde 10. Triglycerides • Omega-3 fatty acids significantly reduced, versus baseline, the plasma levels of apoB-100 (*P*=0.02), malondialdehyde (*P*=0.02), an indicator for lipid peroxidation, and triglycerides (*P*=0.02).			↓ApoB100, malondialdehyde (plasma), TAG: 2 g omega-3 (1040 mg EPA + 960 mg DHA)/d	The fatty acid composition of the placebo capsules differed from the omega-3 group, making it difficult to compare between the different groups. Omega3-supplementation had no effect on blood glucose, insulin, HbA1c, apoA-1, total cholesterol, HDL-cholesterol, or LDL-cholesterol levels
Shoji et al., 2006	Double-blind, randomized, placebo-controlled	Pregnant women at 20 weeks of gestation (n=46)	Blemil plus (15 mg) with or without DHA (500 mg/d) and EPA (150 mg/d). Both supplements contained 3.0 mg alpha-tocopherol. Intake was not restricted but it was recommended that the women follow the recommended dosing for pregnant women.	From week 20 to delivery	Eligibility criteria included not using fish oil supplements, nonsmoker status and healthy singleton pregnancy.	1. Urinary malondialdehyde (obtained with thiobarbituric acid and normalized to creatine) 2. Urinary 8-hydroxy-20-deoxyguanosine (8-OHdG) 3. Plasma alpha-tocopherol 4. Plasma phospholipid fatty acids DHA and EPA supplementation had no effect on urinary MDA levels or 8-OHdG DHA and EPA had no effect on plasma alpha-tocopherol levels		Blemil (15 mg) + 500 mg DHA + 150 mg EPA/d		There were no differences in the clinical characteristics of the pregnant women or their offspring between the control and treatment group. After supplementation DHA was significantly higher in the DHA supplemented group than in the control group. EPA levels were higher in the supplemented group at 30 weeks but not at delivery.

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Siahanidou et al., 2007	Randomized	Preterm infants admitted after birth (> or equal to 28 weeks; > 1000 g; n=140)	Commercial infant formula with or without long chain fatty acids. The formula containing long chain fatty acids contains 12 mg arachidonic and 7.1 mg DHA/100 ml formula.	?		1. Serum malondialdehyde (MDA) 2. Serum total peroxide 3. Serum total antioxidant capacity	1. Serum lipids	7.1 mg DHA/100 mL formula		The formula supplemented with LCPUFA: 1. Had no effect on serum MDA levels. 2. Had no effect on total antioxidant levels * Total peroxide concentrations were below the limit of detection, even after correction for serum lipids.
Stalenhoef et al., 2000,	Double-blind, placebo-controlled, randomized trial	Hypertriglyceridemic patients (n=28)	1. Placebo – corn oil (56% linoleic, 26.8% oleic, 2.3 % stearic acid, and 2.4 mg vitamin E /capsule) 2. Gemfibrozil (1200mg/day) vs 3. Omacor (4 g/day; 44% EPA, 36% DHA, 27% other fatty acids, and 3.3 mg/g α-tocopherol = 1.76 g EPA, 1.44g DHA, and 1.4 g other fatty acids/day; n=15)	12 weeks	Patients were consuming American Heart Association Step I diet (<30% of total calories/day from fat and <300 mg/day cholesterol). Placebo was corn oil.	1. Lipid and lipoprotein levels 2. LDL subfraction profile 3. LDL oxidation <u>Versus baseline:</u> 1. Omacor significantly reduced plasma levels of total cholesterol (P<0.05) and triglycerides (P<0.001), and VLDL-triglycerides (P<0.001) and VLDL-cholesterol (P<0.001). 2. Omacor significantly increased HDL-cholesterol (P<0.05) and LDL-cholesterol (P=0.005). 3. Omacor also significantly increased the cholesterol in LDL1, 2, and 3 fractions (P<0.05) but not the LDL 4 and 5 fractions. Importantly LDL1, 2, and 3 are less dense than LDL 4 and 5. 4. Omacor reduced the LDL concentrations of stearic and oleic acids			↓Total cholesterol, TAG, VLDL-TAG, VLDL; ↑HDL, LDL; ↑less dense LDL particles; ↓lag time, formation of dienes *in vitro*: 1.76 g EPA + 1.44 g DHA + 1.4 g other fatty acids/d (as 4 g Omacor/d)	One subject developed excessive hypertriglyceridemia after he stopped his regular medication. Eight patients in the Omacor group had an increase in body weight. The body weights of the remaining seven either remained the same or decreased. No significant side effects were observed in the Omacor group.

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						Primary	Secondary	NEL	EL	
						and increased LDL concentrations of EPA, DHA and vitamin E. 5. Omacor reduced lag time (P<0.001) and the formation of dienes *in vitro* (P=0.01), but not the oxidation rate of LDLs. 6. Omacor had no effect on plasma TBAR levels.				
Stark and Holub 2004	Randomized, double-blind, placebo-controlled cross-over trial.	Thirty-eight postmenopausal women aged 45-70 yr who had their last menses ≥ 1 yr prior to the start of the study were recruited. Subjects refrained from consuming fish 2 wk prior to the study, then were randomized to receive either DHA concentrate (2.8 g DHA/d) or control oil (corn and soy oil) for 28 d, then washed out and crossed over to the opposite therapy for another 28 d. Subjects consumed 12 capsules/d.	Each capsule of DHA concentrate contained 230 mg DHA and no EPA, plus 180 mg fatty acids, 94 mg monounsaturated fatty acids, 234 mg PUFAs, and 0.13 mg alpha-tocopherol equivalents of *R,R,R*-alpha-tocopheryl acetate plus 0.13 mg ascorbyl palmitate. Each placebo oil capsule contained a mixture of corn + soy oil that provided ~ 81 mg saturated fatty acids, 132 mg monounsaturated fatty acids, 300 mg PUFAs (mainly linoleic acid (18:2N-6), and the same amount of antioxidants as in the treatment oil.	2 wk initial wash-out 28 d intervention ≥ 6 wk intermediate wash-out before crossover, then 28 d intervention	Exclusion criteria included those diagnosed with diabetes or cardiovascular disease, who consumed fish more than once/wk, and women consuming supplements containing either n-3 PUFAs or phytoestrogens.	TAG levels were significantly reduced in the treatment group, versus baseline levels (P < 0.05).			↓TAG: 2.8 g DHA/d	Four subjects withdrew from the study; two complained of fatigue and nausea, one was unable to swallow the required number of capsules per day, and one was unable to meet the scheduled sample collection dates due to unforeseen travel complications. Heart rate was also significantly reduced in the treatment group, versus baseline levels (P , 0.05).

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						Primary	Secondary	NEL	EL	
Stier et al., 2001	Prospective, randomized, double-blind study	Preterm infants (8 with long-chain fatty acids (LCP), 7 without LCP, 8 expressed breast milk (EBM)	Infant formula supplement with either linoleic and α-linolenic acid (No LCP) or EPA and DHA (with LCP) Importantly, the formula without LCP had less oleic acid and more linoleic acid than the with LCP formula	3 weeks	Control formula contained less oleic acid and more linoleic acid vs. the test formula.	Urinary excretion of prostanoids, markers of oxidative stress, and creatinine				There was no effect of the LCP-supplemented formula on the excretion of 8-epi $PGF_{2\alpha}$, and PGF_2 compared to baseline levels. F2-isoprostanes increased compared to baseline in all groups but were the increases were not statistically significant.
Suzukawa et al., 1995	Randomized, double-blind, placebo-controlled	Stable hypertensive subjects (n=20); 14 women and 6 men; mean age of 60	1. Placebo – corn oil (58% linoleic, 28% oleic, 2.0 % stearic, 10% palmitic acid and 2.2 mg α-tocopherol/ml) 2. Omacor (4 g/day; 48% EPA, 36% DHA, 3% DPA, 27% other fatty acids, and 2.0 mg/ml α-tocopherol = 1.76 g EPA, 1.44g DHA, and 1.4 g other fatty acids/day; n=15)	6 weeks	Subjects were being treated with either beta-blocker (10F/5M) or diuretics (4F/1M). Placebo was corn oil.	1. Plasma lipids and lipoproteins 2. LDL oxidation <u>Versus baseline,</u> <u>Omacor</u>: 1. Significantly reduced plasma triglycerides ($P<0.01$) but had no effect on plasma cholesterol or LDL-, HDL-, HDL2-, and HDL3-cholesterol or plasma apoB or plasma lipid peroxide. 2. Significantly increased the radius (size) of LDL ($P<0.01$). 3. Significantly increased the concentrations of EPA and DHA, decreased the concentrations of oleic, linoleic, dihomogammalinolenic, and arachidonic acid in LDLs, but had no effect on LDL α-tocopherol concentrations. 4. Significantly decreased LDL oxidation lag time (25%; $P<0.001$) and propagation rate (10%; $P<0.05$). 5. Significantly			↓TAG; ↑LDL radius; ↓LDL oxidation lag time, propagation rate; ↑TBARS formation *in vitro*; ↑uptake of *in vitro* oxidized LDL by macrophages: 1.76 g EPA + 2.44 g DHA + 1.4 g other fatty acids/d	

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						increased TBARS formation *in vitro* at 90 and 180 min (P<0.001) using pooled LDL samples. 6. Significantly increased the uptake of *in vitro* oxidized LDL obtained from the fish oil group. 7. Significantly increased the uptake of *in vitro* oxidized LDL by macrophages (P<0.001) and the generation of TBARS *in vitro* during the culture with macrophages (no statistics).				
Theobald et al., 2004	Double blinded, placebo controlled, crossover	Healthy subjects (n=38)	3 capsules/d (each contains 500 mg DHASO or olive oil). DHASO supplement provided 0.68 g/d DHA (TAG) and <0.005 g EPA/d	3 months followed by a washout phase of ≥ 4 months	Olive oil was the control.	LDL Serum total cholesterol, LDL cholesterol, and plasma apolipoprotein B were 4.2% (0.22 mmol/L; P=0.04), 7.1% (0.23 mmol/L; P=0.004), and 3.4% (P=0.03) higher, respectively, with DHA treatment than with placebo.				Baseline comparisons were not made within groups.
Tholstrup et al., 2004	Randomized, double-blind, crossover	Sixteen men aged 35-75 yr who tended to have an atherogenic lipid profile.	80 g of the daily dietary fat intake was substituted with "O" fat (containing a mixture of mango fat, rapeseed, and safflower oils) or "F" fat (similar to "O" fat, except that 5% of the oleic acid was substituted with EPA and DHA derived from fish oil).	Two study periods of 3 wk each, separated by a washout period of 3 wk.	Selection criteria included males aged 30-75 having plasma TAG > 1.3 mmol/L, HDL ≤ 1.2 mmol/L, total cholesterol < 9 mmol/L, total cholesterol:HDL ratio > 4.5. Four subjects were smokers. One subject took antihypertensive medication. Individual amounts of DHA and EPA added to "F" fat was not specified.	Plasma lipids and selected markers of oxidative stress were measured. Versus baseline diet, the "F" diet group exhibited significantly lower (P<0.05) levels of total cholesterol, VLDL, IDL, total TAG, VLDL-TAG, and LDL-6 apoB at 3 wk. No significant effects of the diets on oxidative stress markers were observed.				Diets were isocaloric and also contained the same amount of fat across groups. All groups consumed equivalent amounts of saturated fat; test groups consumed more monounsaturated and polyunsaturated fat versus the habitual diet. The "O" diet contained more monounsaturated fat vs. the "F" diet.

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Turini et al., 2001	Randomized, "placebo"controlled	Healthy men (50 years old; n=10)	1. Trisun - sunflower seed oil (25 g/day) 2. Fish oil (25 g/day; 4.3 g EPA and 2.8 g DHA/day) *α-tocopherol concentrations were adjusted for equivalency	4 weeks	Sunflower seed oil was the control.	1. Percentage of n-6 and increased n-3 PUFAs in total phospholipid fatty acid composition in monocytes and granulocytes. 2. Monocyte and granulocyte phagocytic activity. 3. Plasma HDL-cholesterol, triglyceride, and LDL concentrations. 4. Plasma vitamin C, either plasma or LDL concentrations of tocopherol, carotene, lycopene, or red blood cell super oxide dismutase levels. 5. Lag time and oxidative rate of LDLs ex vivo.			↑Monocyte and granulocyte phagocytic activity; ↑HDL; ↓lag time and oxidative rate of LDLs *ex vivo*: 25 g fish oil/d (4.3 g EPA + 2.8 g DHA/d)	Fish oil supplementation at 4 wk, versus baseline: 1. Reduced the percentage of n-6 and increased n-3 PUFAs in total phospholipid fatty acid composition in monocytes and granulocytes. 2. Significantly increased monocyte and granulocyte phagocytic activity by 12 and 3%, respectively ($P<0.05$ for both). 3. Increased plasma HDL-cholesterol concentrations by 5% ($P<0.05$) but had no effect on triglyceride and LDL concentrations. 4. Did not affect plasma vitamin C, either plasma or LDL concentrations of tocopherol, carotene, lycopene, or red blood cell super oxide dismutase levels. 5. Significantly reduced lag time and oxidative rate of LDLs *ex vivo* ($P<0.05$).

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von Schacky et al., 1999	Double blind, placebo controlled	Patients with angiographically proven coronary artery disease. Fish oil arm (n=111), placebo arm (n=112)	Over the first 3 months, six 1-g capsules/d; over the next 21 months, three 1-g capsules/d. Each capsule contains an oil mixture with 35.4% EPA, 21.5% DHA and 9.7% DPA (fish oil arm) or an oil mixture with no marine ω-3 fatty acid.	3 mo for loading dose, then 21mo for maintenance dose	Control oil composition was not provided.	Coronary atherosclerosis	LDL			Regarding between-group comparisons, the below were reported but it should be noted that the composition of the control article is not known: Levels of LDL cholesterol (mmol/l) were significantly greater in the fish oil group at 6 mo (3.85±1.09 in placebo vs. 4.3±1.21 in fish oil), 18 mo (3.75±1.06 in placebo vs. 4.1±1.0 in fish oil), and 24 mo (3.5±1.04 in placebo vs. 3.85±0.85 in fish oil). No LDL differences were seen at 1 mo and 12 mo.
Wander et al., 1996	Double-blind, crossover	Postmenopausal women (45-70 years old; n=48) using and not using hormone- replacement therapy	Fifteen grams of fish oil (National Institutes of Health's Fish Oil Test Material Program; 2.46 g of EPA and 1.8 g DHA/d) with or without 100, 200, or 400 mg α-tocopherol/day	5 wk followed by a 4-wk washout	Some subjects were receiving hormone-replacement therapy No placebo control.	LDL resistance to oxidative modifications in vitro (i.e., lag time, propagation rate, production of conjugated dienes) Versus baseline, fish oil supplementation: 1. Shortened *in vitro* lag time ($P \leq 0.05$) and slowed production of conjugated dienes ($P \leq 0.05$) in women using or not using hormone replacement therapy.	1. Plasma and LDL α- and γ-tocopherol concentrations 2. Plasma lipid concentrations		↓*In vitro* lag time, production of conjugate dienes: 2.46 g EPA + 1.8 g DHA/d	Two subjects withdrew for medical reasons but were replaced with comparable subjects Lipid oxidation was assessed *in vitro*. Effect of fish oil on LDL in women taking HRT was the same whether calculated or measured values were used for LDL.

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Wander and Du, 2000	Double-blind, cross-over	Postmenopausal white and Asian women (45-75 years old; n=48).	Fifteen grams of fish oil (National Institutes of Health's Fish Oil Test Material Program; 2.46 g of EPA, 0.5 g DPA) and 1.8 g DHA/d). Total daily dose also included 5.1 g saturated fatty acids, 3.5 g monounsaturated fatty acids, and 0.25 g linoleic acid.	5 wk followed by a 4-wk washout	Not all of the fish oil test articles contained alpha-tocopheryl acetate.	1. Plasma concentrations of triglycerides, EPA, DHA, and α-tocopherol 2. Plasma concentrations of thiobarbituric acid-reactive substances(TBARS) 3. Plasma concentrations of oxidatively modified protein.			↓TAG, ↑TBARS (fish oil without added alpha-tocopheryl acetate only): 2.46 g EPA + 0.5 g DPA + 1.8 g DHA/d (also included 5.1 g saturated FAs, 3.5 g MUFAs, 0.25 g linoleic acid)	Two subjects withdrew because of medical reasons. Versus baseline levels: 1. Fish oil significantly reduced plasma triglycerides and linoleic acid, and increased EPA, DHA (pooled data because there was no effect of α-tocopherol supplementation on any of these parameters. 2. Fish oil without added alpha-tocopheryl acetate significantly increased plasma TBARS by 16% (P<0.05) but had no effect on plasma protein oxidation.
Westerveld et al. 1993	Double blinded, placebo controlled	DIDDM. Two EPA arms (n=8 in each) and one placebo arm (n=8).	6 capsules providing 1.8g/d MND21, or 0.9g/d MND21 plus 0.828g olive oil or 1.656g/d olive oil (placebo). MND21 contains 93.6% ethyl ester-EPA	8 weeks	Insulin, diabetic drugs or diet control. Olive oil was the placebo.	Safety factors including glycemic control, lipid, lipoprotein, platelet aggregation.	LDL At 4 and 8 wk, LDL was higher in the 1.8 g/d group, versus baseline (P=0.04 and 0.014).	0.9 g/d	↑LDL: 1.8 g EPA ethyl esters/d	

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Wu et al., 2006	Single-blind, randomized, placebo-controlled	Postmenopausal vegetarian women (Average age of 52; n=25)	Placebo: 6 g corn oil (n=11) Treatment: 6 g DHA-rich algae oil (n= 14; 2.14 g of DHA/day)	6 wk	Placebo was corn oil.	1. Plasma lipids 2. Breast cancer markers - urinary 2-OHE_1 and 16α-OHE 3. Urinary F_2-isoprostanes 4. Plasma α-tocopherol DHA supplementation, versus corn oil: 1. Significantly increased EPA and DHA concentration in plasma LDLs 2. Decreased plasma cholesterol (*P*=0.040) and increased LDL-TBARS (*P*=0.039). 3. Had no effect on plasma triglycerides, LDL-cholesterol, HDL-cholesterol, α-tocopherol, urinary F2-isoprostanes.			2.14 g DHA ↓ total cholesterol	
Yaqoob et al., 2000	Randomized , double-blind, placebo-controlled	Healthy Caucasians (n=8/group)	This study evaluated the effects of a variety of oils. The effects of only fish oil will be evaluated. Placebo: 3:1 mixture of coconut and soybean oil (contained a mixture of saturated, monounsaturated, and polyunsaturated fatty acid and proportions linoleic and α-linolenic acid and n-6 and n-3 PUFAs similar to those of the current average UK diet Fish oil: Subjects	4 weeks followed by an 8 week washout	Placebo contained a 3:1 mixture of coconut:soybean oil.	1. Fatty acid composition of plasma phospholipids 2. Cell surface expression of CD7, CD21, CD4, CD8, CD64, CD16, CD2, CD54, and CD11b 3. NK cell killing activity 4. ConA-induced proliferation of PBMC 5. ConA- and LPS-induced cytokine production Versus baseline, fish oil supplementation: 1. Significantly reduced dihomo-γ-linolenic acid and increased the		2.1 g EPA + 1.1 g DHA/d		

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			consumed an extra 2.1 g EPA and 1.1 g DHA/day			amount of EPA and DHA in plasma phospholipids. Arachidonic acid was reduced following fish oil supplementation but the reduction was not statistically significant. 2. Significantly increased plasma α-tocopherol concentrations but had no effect on TBARS and total plasma antioxidant activity. 3. Significantly reduced arachidonic acid and increase EPA and DHA levels in PBMCs. 4. Had no effect on the cell surface expression of any of the markers tested, NK cell lytic activity, ConA-induced whole blood and PBMC proliferation, or ConA- or LPS-induced cytokine production.				
PCI=Percutaneous coronary intervention, commonly known as coronary angioplasty; SAC= systemic arterial compliance; MDA=Malondialdehyde; TBARS=Thiobarbituric acid reactive substances;										

CHAPTER 2: EFFECTS ON BLEEDING PARAMETERS

Background

Omega-3 fatty acids (or n-3 fatty acids) have multiple cardiovascular benefits but it also has been suggested that they may inhibit platelet aggregation, reduce blood viscosity and increase bleeding risk. Prolonged bleeding times and increased incidence of bleeding have been observed in Inuits, whose diets are rich in EPA and DHA (mean 6.5 g/day) (Dyerberg and Bang, 1979), however, information is lacking to conclude that EPA and DHA were the sole basis for these observations. Subsequently, a number of studies have been completed to evaluate the impact of n-3 fatty acids on bleeding parameters. Studies reporting on bleeding complication-related endpoints cited in the VKM (2011) and BfR (2009) reports, obtained from searching public databases for studies more recent than 2008 and cited in the review articles Knapp 1997, Hooper et al. 2004, and Harris 2007 were included in this review.

Results

Twenty-three studies identified from the literature in both healthy and health-compromised individuals evaluated the effect of intakes ranging from 1.08 to 36 g/day of n-3 fatty acids given for durations up to 1 year (Table 2).Two of them were conducted with healthy subjects (Nelson et al., 1997; Olsen et al., 1992) and the remaining studies were conducted with health-compromised individuals.

Seven studies with intakes of n-3 fatty acids ranging from 1.8 to 21.3g/d found no statistically significant prolongation of bleeding time compared to baseline measurements and no significant difference in this parameter between the treatment and placebo groups (Dehmer et al., 1988; Donnelly et al., 1992; Eritsland et al., 1996; Keck Jr. et al., 2006; Lempert et al., 1988; Rapp et al., 1991; Westerveld., 1993).

Two studies with intakes of n-3 fatty acids ranging from 2 to 6.9 g/d showed either significant prolongation of bleeding time compared to the baseline measurement or a significant difference in this parameter between the n-3 fatty acid and placebo groups (Emsley et al., 2008; Leaf et al., 1994). However, the prolongation of bleeding time reported in these studies did not exceed normal limits and did not produce clinically significant bleeding episodes.

Two studies with intakes of n-3 fatty acids at 1.5g/d (Clarke et al., 1990) and 4.3g/d (DeCaterina et al., 1990) showed significant prolongation of bleeding time exceeding the normal

range. The study conducted by Clarke et al (1990) noted that in 3/11 children with hypercholesterolemia, intake of n-3 fatty acids at the level of 1.5g/d increased bleeding time. However, this study had a very small number of subjects on concomitant medications that could confound results, and was not controlled or blinded. DeCaterina et al. (1990) reported a 40% increase of bleeding times in patients underwent cardiovascular by-pass surgery compared with baseline at the intake level of 4.3g/d and in four out of 15 subjects, the bleeding time increased to more than 10 minutes after 28 days of fish oil consumption. However, the patients consuming the fish oil did not have significantly increased bleeding at or after surgery compared with matched control patients.

Studies also assessed end points other than bleeding time, such as bleeding frequency, platelet count, prothrombin time, thromboplastin time; results showed no or inconsistent effects of n-3 fatty acids on these parameters.

The results from these 23 studies suggested that findings of any effect of n-3 fatty acids on bleeding parameters are rare, inconsistently found, not associated with a dose-response and not linked to bleeding complications or clinically adverse outcomes. Recently, Salisbury et al (2012) assessed n-3 indexes (low = <4%, intermediate = 4-8% and high = >8%) and rates of serious bleeding (Thrombolysis In Myocardial Infarction [TIMI] major or minor) and mild to moderate bleeding (TIMI minimal) in 1523 patients at the time of acute myocardial infarction. Salisbury et al (2012) found no differences in bleeding across n-3 index categories, suggesting that concerns about bleeding should not preclude the use of n-3 supplements or increased fish consumption.

A number of literature reviews corroborate the conclusions reached from an analysis of the studies summarized above. Knapp (1997) reviewed the published research from 39 studies evaluating n-3 fatty acids and human thrombosis and hemostasis, concluded that “the prolongation of bleeding time is usually modest and there have been no reports of serious bleeding”.

In the 2004 Cochrane Review (Hooper et al. 2004), seven studies (949 subjects in the n-3 fatty acid group and 836 subjects in the control group) reporting a bleeding outcome were reviewed. The supplementation of DHA+EPA was in the range of 3-7 g/d. No significant differences in bleeding time between the DHA/EPA supplemented groups and control groups were found (Hooper et al. 2004).

Harris (2007) identified and reviewed 19 studies included a total of 4397 participants; EPA and DHA were supplemented using various sources in the different studies and with durations between 4-28 months. The intake levels of EPA+DHA ranged between 1.4-21 g/d. Among them intake levels of EPA+DHA in 15 studies fell between 3 and 6.9 g/d. Five studies used n-3 fatty acid drugs (EPA and DHA as ethyl esters), while the others used other n-3 ethyl esters (4 studies), n-3 contained in fish oil or capsules with n-3 as TAG (11 studies). Harris concluded that EPA and DHA do not increase the risk for adverse bleeding episodes and considered the evidence to be at "A" (well designed randomized controlled clinical trials). In another review, Bays (2007) concluded that "clinical trial evidence has not supported increased bleeding with omega-3 fatty acid intake, even when combined with other agents that might also increase bleeding (such as aspirin and warfarin)".

An authoritative review of relevant studies by the IOM concluded that collectively the results indicate no dose-response for EPA and DHA intake and the percent increase in bleeding time and none of the studies considered reported any increase in bleeding episodes (IOM, 2005).

Recently, the Norwegian Scientific Committee for food Safety (VKM 2011) evaluated the negative and positive health effects of n-3 fatty acids as constituents of food supplements and fortified foods. VKM (2011) indicated that "only a few controlled studies have assessed the effect of EPA and DHA (intake range 1.8 – 6.9 g/day) on bleeding time, bleeding tendency and international normalized ratio (INR). A significant increase in bleeding time has been observed at 6.9 g/day EPA and DHA in one study with coronary heart diseased patients on anticoagulant medication (note: refers to the study by Leaf et al., 1994). No significant impact on bleeding time was observed in two other studies in patients on anticoagulation medication using 3.4 and 5.4 g EPA and DHA per day (note: refers to studies by Eritsland et al., 1996 and Dehmer et al., 1988)."

BfR (Opinion No. 030/2009 of May 26, 2009) recommended a maximum intake of n-3 fatty acids of 1.5g/day. The BfR report cited two studies (Clarke et al. 1990; Emsley et al., 2008) with bleeding time as the end point and one meta-analysis studying the effect of fish oil supplementation on blood viscosity (Sommerfield et al. 2007). The study conducted by Clarke et al (1990) noted that in 3/11 children with hypercholesterolemia, intake of n-3 fatty acids at the level of 1.5g/d increased bleeding time. However, this study had a very small number of subjects who were on concomitant medications that could confound results, and was not controlled or blinded. Of the three patients experiencing epistaxis, the prolongation of bleeding time was

considered modest in two of them. These patients had no adverse changes in prothrombin, partial thromboplastin times or platelet counts. In addition, it is important to note that this finding could not be replicated in a later trial in children on dialysis (Goren et al., 1991), in which the highest intake of EPA+DHA used was 2.4g/d, which is higher than the intake reported by Clarke et al (1990). The clinical trial conducted by Emsley et al (2008) was double blinded and placebo controlled. Although an increase in bleeding time in psychiatric patients given 2g/d ethyl ester-EPA was seen compared to the placebo arm, values remained within the normal range and the increase in bleeding time did not persist. In the analysis by Sommerfield et al (2007) three studies reported outcome on blood viscosity. In two studies, a statistically significant reduction was found in the intervention group but no change was found in the control group. The other study found no significant change in blood viscosity in either the intervention or control groups. Since this hematological test was carried out at different shear rates, the results were not pooled for meta-analysis. It was noted that fish oil supplementation (1.8 g EPA or 3 DHA/EPA) was possibly related to reduction in blood viscosity. However, administration of additional substances to the subjects, the fact that the studies were not powered to evaluate this endpoint, and the short durations of administration with no long-term follow up in two studies and small daily intakes in one study prevented meaningful evaluation.

Confounding conditions may play a role in study interpretation. Hereditary or acquired defects such as von Willebrand disease involve increased bleeding risk such as increased risk for nosebleeds, menorrhagia, and gastrointestinal bleeding and may not be part of the exclusion criteria of studies. Additionally, background dietary levels of n-3 fatty acid intake, confounding medications such as aspirin and warfarin and underlying health conditions confound interpretation.

Conclusions

Twenty three studies identified from the literature in both healthy and health-compromised individuals evaluated the effect of intakes ranging from 1.08 to 36 g/day of n-3 fatty acids given for durations up to 1 year. Results from a detailed review of these studies suggest that findings of effect of n-3 fatty acids on bleeding parameters are rare, inconsistently found, not associated with a dose-response and not linked to bleeding complications or clinically adverse outcomes. The conclusions are supported by other authoritative reviews and meta-analyses.

Table 2. Effects of omega-3 fatty acids on bleeding complications

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
Bairati et al., 1992	Double blind, randomized, placebo controlled	Patients for PTCA. MaxEPA (n=59), corn oil placebo (n=60)	15 x 1g capsules per day of MaxEPA (TAG) (2.7 g/day of EPA, 1.8 g/day of DHA) or olive oil.	3 weeks before PTCA and continued for 6 months thereafter	Taken regularly (e.g., calcium channel blockers, β-blockers) or occasionally (e.g., aspirin).	Recurrence of Stenosis	Bleeding	4.5g/d		None of the patients reported bleeding, infection, or any major adverse outcome attributable to fish oil supplements.
Barber and Fearon 2001	Open label. dose escalation without a control group	Pancreatic cancer patients (*n*=5)	Diester (with propane-1,3-diol) of EPA, 4.5g/d x 2wk, 9g/d x 2wk, 18g/d x 2wk and then 36g/d x 2wk.	8 weeks	Pancreatin, or Domperidone or Diclofenac. Two patients had previously taken the mixed fish oil preparation MaxEpa® at a dose providing around 1 g EPA daily	Tolerance and incorporation	Bleeding, platelet count	36g/d		No adverse events related to bleeding were observed, and all clotting measurements remained within the normal range. No patient had any change in clotting or platelet count outside the normal range.
Bender et al., 1998	Double blind, randomized, placebo controlled parallel	Patients receiving chronic warfarin. Placebo (n= 6), medium dose (n=5), high dose (n=5)	Medium dose, 3x1g capsules of MaxEPA (TAG) (with 0.54 g n-3 fatty acids), high dose, 6x1g capsules of MaxEPA (with 1.08 g n-3 fatty acids), placebo capsules with materials not specified	4 weeks	Warfarin	Anticoagulation status	INR value, bleeding episodes, recurrent deep vein thrombosis	1.08 g/d		1.08g of omega-fatty acids (6g fish oil) was the highest dose tested. There were no major or minor bleeding events.
Cairns et al., 1996	Randomized, placebo controlled	Patients after PTCA. MaxEPA (n=325), corn oil placebo (n=328)	18 capsules/d of either MaxEPA (providing 3.24g EPA and 2.16g DHA) or corn oil placebo	18 weeks	Heparin (during PTCA), low-molecular-weight heparin, aspirin	Restenosis	Bleeding frequency	5.4g/d		Bleeding was less frequent in the fish oil group than placebo
Clarke et al. 1990	Open label, without a control group	Adolescent patients with FHL type II (n=11)	1g capsules of MaxEPA (TAG) containing 18% EPA + 12% other n-3 oil. Dose level of n-3 fatty acids started at 0.3g/d and increased by 0.3g/d monthly to 1.5g/d in 5th and 6th months.	6 months	Colestipol (2 adolescents), aspirin (1 adolescent)	Epistaxis	Bleeding time	1.2 g/d	1.5 g/d	At 1.5 g/d level, one patient experienced epistaxis associated prolongation of the bleeding time (9 min. vs. 3-5 min normal). Two other subjects experienced epistaxis associated with modest prolongation of the bleeding time (7 minutes); one of these had a history of aspirin ingestion at the time. All prothrombin and partial thromboplastin times and platelet counts were normal.
							All prothrombin and partial thromboplastin times, platelet counts	1.5g/d		

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						Primary	Secondary	NEL	EL	
DeCaterina et al., 1990	Open label study without a placebo. The control patients did not receive fish oil capsules.	Patients for CABG. Fish oil group (n=15), control group without fish oil intake (n=15).	Ten fish oil capsules per day. Each capsule contains 1 ml purified fish oil concentrate with 300 mg EPA and 130 mg DHA providing a total of 4.3 g/day of combined EPA and DHA.	At least 28 days before surgery.	Cardiovascular medications	Hemostasis, plasma lipid levels, and production of prostacyclin (PGI2)	Bleeding time, platelet functions		4.3g/d	Bleeding times increased 40% ($p < 0.01$) from baseline In four subjects, the bleeding time increased to more than 10 minutes after 28 days fish oil consumption. Despite the increase in bleeding time during the period of dietary supplementation with fish oil, the patients so treated did not have significantly increased bleeding at or after surgery compared with matched control patients. Almost all platelet functions evaluated were lower than baseline values after the treatment with n-3 PUFAs, including platelet adhesiveness, platelet aggregation, and release of thromboxane. Platelet-aggregate ratio was within the normal range. Platelet counts did not change.
							Platelet-aggregate ratio, platelet counts	4.3g/d		
Dehmer et al., 1988	Unblinded, randomized. The control patients did not receive fish oil capsules.	PCI patients with test article (n= 44; without test article (n=39)	Conventional antiplatelet regiment (325 mg aspirin and 225 mg dipyridamole per day, control group), similar regiment supplemented with 18 capsules per day of MaxEPA (TAG) containing EPA (3.2g) + DHA (2.2g); one dose level at 5.4 g/d	6-month study; 3-month period for bleeding test.	Aspirin and dipyridamole. No modification or control was made in patients' diets or medications, except that each was encouraged to stop smoking.	Rate of early restenosis	Bleeding time	5.4 g/d		Bleeding time in the supplement group increased from 6.8 min to 9.0 min after 1 month, which is not significant ($p<0.1$) and returned to 6.8 min after 3 months. Platelet count decreased after 3 months in the treatment group ($p<0.05$) but not in the control group. In the first 13 patients treated with the test article, no signification changes were seen in prothrombin time and activated partial thromboplastin time.
							Platelet count		5.4 g/d	
							Prothrombin time, thromboplastin time	5.4 g/d		
Donnelly et al., 1992	Double blind, randomized, placebo controlled crossover study	16 patients on chronic dialysis therapy	12x1g capsules per day of MaxEPA providing 2.16g EPA and 1.44g DHA or olive oil	4 weeks x 2 (no wash out period)	Abnormal platelet count, a prolonged bleeding time (>9 min), an abnormal prothrombin or partial thromboplastin time, the need to take medications that increased the risk of bleeding were included in the exclusion criteria.	Hemostasis, blood pressure, and lipid profile	Bleeding times, platelet aggregation, platelet count	3.6g/d		There were no episodes of bleeding or bruising; no patient underwent a traumatic or surgical hemostatic stress. Bleeding times were 4.8 ± 0.4 min on MaxEPA and 4.5 ± 0.3 min on placebo. The bleeding time was not statistically significantly different within the groups at the two points in the study (after fish oil or after olive oil) nor between the groups. The authors concluded that n-3 fatty acids do not introduce a clinically important risk of bleeding for patients with end-stage renal disease.

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						Primary	Secondary	NEL	EL	
Emsley et al., 2008	Double blinded (followed by open label), randomized, placebo controlled parallel	Psychiatric patients. Blinded trial (EPA arm n=39; placebo arm, n=33). Open label extension (n=23 from the EPA arm and 22 from the placebo arm)	2g/d encapsulated ethyl ester-EPA (Amarin) or placebo (liquid paraffin). 2g/d encapsulated ethyl ester-EPA for all patients in the open label extension phase	12 weeks blinded followed by 40 weeks open label extension	Antipsychotic medication; non-steroidal anti-inflammatory agents or aspirin	Safety factors including bleeding time	Bleeding time, platelet count	2g/d		Significant increase in bleeding time in the EPA arm was seen compared to the placebo arm (12-week study), although values remained within the normal range. However, no difference in bleeding time was seen during the 40 week extension test (week 52 vs. week 12). Platelet count did not change during either period.
Eritsland et al., 1996	Randomize controlled trial. Control patients were those who took aspirin or warfarin only	CABG patients; aspirin (n=148), aspirin with supplement (n=143), warfarin (n=145), warfarin with supplement (n=174)	Four 1-g Omacor capsules providing ethyl esters of EPA (2.04g) + DHA (1.28g)	12 months	Aspirin or warfarin. Patients were told to reduce their intake of saturated fatty acids and to refrain from cod-liver oil and other fish oil products during the study period	1-year graft potency	Bleeding time.	3.3 g/d		The bleeding time (sec) increased from 243 before intervention to 282 after intervention in the fish oil group. In the control group, bleeding time (sec) increased from 249 to 283. The differences between the fish oil and control groups in bleeding time were however not significant.
Gadek et al. 1999	Double blind, randomized, controlled trial	ARDS patients, EPA+GLA (n=51), control (n=47)	Test article is EPA fish oil plus γ-linolenic acid (GLA; borage oil) (providing 6.9 ± 0.3 g EPA and 2.9 ± 0.1 g DHA per day); an isonitrogenous, isocaloric standard diet as a control.	4-7 days		Pulmonary inflammation , oxygenation and clinical outcomes in patients with ARDS	Hematological or blood coagulation changes	9.8g/d		No hematological or blood coagulation changes in treatment group, vs. control (data not shown)
							Thrombocythemia, hemorrhage, prothrombin decrease	9.8g/d		
Goren et al., 1991	Open label without a control group	16 patients, 7 to 8 years of ages, who had end-stage renal disease and were receiving renal replacement therapy.	3-8x1g capsules (EPAGIS). Each capsule contains 0.18g EPA and 0.12g DHA	8 weeks	Dialysis schedule and the medications remained unchanged.	Treatment of Hyperlipidemia	Platelet counts, Epistaxis	2.4g/d		Platelet counts remained stable during the entire study period. Epistaxis was not seen.
Grigg et al., 1989	Double blind, randomized, placebo controlled	Patients after PTCA. MaxEPA (n=52), corn oil placebo (n=56)	Ten 1-g capsules/d of either MaxEPA (providing 1.8g EPA and 1.2g DHA) or olive/corn oils (1:1 ratio) as placebo	4 months	Aspirin, verapamil	Restenosis	Bleeding complications, platelet count	3g/d		Bleeding times were not measured. However, no patient suffered from bleeding complications during follow-up.
Keck Jr. et al., 2006	Double blind, randomized, placebo controlled parallel	Patients with bipolar depression (n=28 in EPA, n=29 in placebo); Patients with rapid cycling bipolar disorder (n=31 in EPA, n=28 in placebo)	Either EPA (ethyl esters) 6 g/d or matching placebo capsules (liquid paraffin),	4 months	Mood-stabilizing medications	Depression symptoms	Bleeding time	6g/d		Bleeding time data were gathered only at baseline and at the fourth visit (8 weeks). No significant difference was seen.

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						Primary	Secondary	NEL	EL	
Leaf et al., 1994	Double blind, randomized, placebo controlled	PCI patients; treatment (n= 275), placebo (n=276)	Ten 1-g gelatin capsules providing ethyl esters of EPA (4.1g) + DHA (2.8) or ethyl ester of corn oil (control)	6-month study; 3-month period for bleeding test.	Aspirin. Patients were instructed to follow a Step-One American Heart Association diet, although compliance varied among patients.	Rate of restenosis	Bleeding time, platelet count	6.9 g/d		Bleeding times in the test group increased from 6.22 min to 7.02 min after 3 months but were still within the normal range. No difference in clinically significant bleeding was noted. Bleeding time increase was also seen in the control group. Group difference was not statistically analyzed.
Lempert et al., 1988	Open label without a control group	11 stable continuous ambulatory peritoneal dialysis (CAPD) patients	25 ml per day of MaxEPA providing 3.976g EPA and 2.526g DHA with a total n-3 fatty acids of 7.623g	4 weeks	The seven patients participating in coagulation studies had not taken aspirin or other medications known to affect platelet function for at least 1 month.	Serum lipids and blood coagulation	Platelet aggregation, template bleeding times	6.5g/d		No significant changes occurred in template bleeding time (TBT), platelet count, hematocrit, or platelet aggregation response. Clinically important uremic bleeding was not apparent.
Nelson et al. 1997	Single blinded study. Control subjects were those who took low-DHA diet.	Healthy adult males; intervention (n=6), control (n=4)	Low-DHA diet providing <50 mg/d DHA (control), or with safflower oil replaced by 15 g DHASCO oil to provide 6g/d DHA (TAG form) (no EPA) (high-DHA diet)	90-day study after a 30 day stabilization in which both arms were on low-DHA diet		Platelet function *in vitro*, bleeding times, coagulation factors, and platelet fatty acid composition	Bleeding time, prothrombin time, activated partial thromboplastin time, and the anti-thrombin-III levels	6g/d		No statistically significant differences were seen in prothrombin time, activated partial thromboplastin time, and the anti-thrombin-III levels. The *in vivo* bleeding times did not show any significant difference before and after the subjects consumed the high-DHA diet (9.4 ± 3.1 min before and 8.0 ± 3.4 min after).
Nordøy et al., 2000	Double blind, randomized, placebo controlled parallel study	Patients with combined hyperlipidemia. Simvastatin plus Omacor (n=21), Simvastatin plus corn oil (n=20)	4g/d of Omacor providing 1.8 g EPA and 1.56g DHA ethyl ester), or 4g/d corn oil	5 weeks	Simvastatin	Hemostatic risk factors and postprandial hyperlipidemia		3.36g/d		There was a reduction in both the lipoprotein-free tissue factor pathway inhibitor antigen and postprandial factor VII_a levels in the treatment groups versus placebo, but the clinical significance of these findings is unclear; other hemostatic variables were unaffected by treatment.
Olsen et al., 1992	Randomized , placebo controlled	Healthy women in week 30 of pregnancy. Fish oil (n=266), olive oil (n=136), no oil (n=131)	4x1g capsules per day of fish oil providing 1.28g EPA and 0.92g (a total of 2.7g n-3 fatty acids) DHA or olive oil	Till delivery		Pregnancy duration, birth weight, and birth length	Blood loss at delivery	2.2g/d		Studies in pregnant women supplemented with n-3 fatty acids at 2.7 g/day (in which EPA=1.28g and DHA=0.92g) did not lead to increased blood loss at delivery
Rapp et al., 1991	Open label study. Control subjects did not take fish oil.	Patients for arterial endarterectomy. Fish oil group (n=11), control group without fish oil intake (n=18).	Fish oil consumption ranged from 48 to 64 g/day (16.0-21.3 g/day of n-3 fatty acids)	6-120 days before endarterectomy	Not listed	n-3 fatty acids in atherosclerotic plaques	Bleeding time, platelet count	16.0-21.3 g/day of n-3 fatty acids		There were no changes in mean platelet counts (280 ±59 to 269±45 $x10^3$) or bleeding times (7.3 ±1.8 to 7.3 ±1.4 minutes) due to n-3 fatty acid feeding.

Table 2. Effects of omega-3 fatty acids on bleeding complications

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						Primary	Secondary	NEL	EL	
Watson et al., 2010	Retro-spective review of the electronic medical records	Patients with cardiovascular disease. High dose fish oil plus aspirin and clopidogrel (n=182), aspirin and clopidogrel alone (n=182),	Most patients were taking high doses of fish oil, with 140 (77%) of the 182 patients taking >2 g daily.	33 months follow up	Aspirin and clopidogrel	Bleeding complication		2g/d as fish oil		More patients had minor bleeding complications in the control group than in the treatment group; however, the difference was not statistically significant (p = 0.5). In conclusion, high-dose fish oil is safe in combination with aspirin and clopidogrel and does not increase the risk of bleeding compared with that seen with aspirin and clopidogrel alone.
Westerveld et al. 1993	Double blind, randomized, placebo controlled parallel	Patients with NIDDM. Two EPA arms (n=8 in each) and one placebo arm (n=8).	6 capsules providing 1.8g/d MND21, or 0.9g/d MND21 plus 0.828g olive oil or 1.656g/d olive oil (placebo). MND21 contains 93.6% ethyl ester-EPA	8 weeks	Insulin, diabetic drugs or diet control	Safety factors including glycemic control, lipid, lipoprotein, platelet aggregation.	Bleeding time, platelet aggregation	1.8g/d		1.8g/d is the highest dose tested. The collagen-induced platelet aggregation did not change, nor did the Simplate bleeding time. However, platelet activating factor-induced platelet aggregation was reduced at both 0.9 and 1.8 g levels.
Yokoyama et al., 2007	Randomized open label study. blinded endpoint analysis	Japanese hypercholesterolemic patients. EPA group (n=9326), control (n=9319)	1.8g/d EPA with statin or stain only as a control. EPA capsules contained 300 mg of highly purified (>98%) EPA ethyl ester	5-year follow up	statin	Any major coronary event	Hemorrhage	1.8g/d		The total number of adverse events was higher in the EPA group versus placebo, including incidences of cerebral, fundal, epistaxis, and subcutaneous hemorrhage. However, the hazard ratios for total hemorrhagic incidents were not statistically significant for the EPA ethyl ester intervention versus control.

ARDS= Acute respiratory distress syndrome due to sepsis/pneumonia, trauma or aspiration injury; CABG= Cardiovascular by-pass surgery; FHL=Familial hyperlipoproteinemia; INR=International normalized ratio; NIDDM=non-insulin-dependent diabetes mellitus; PCI=Percutaneous coronary intervention, commonly known as coronary angioplasty; PTCA=Percutaneous transluminal coronary angioplasty, TAG=Triacylglycerol or triglyceride

CHAPTER 3: THE EFFECTS OF EPA- AND DHA-RICH OILS ON INFLAMMATION, LYMPHOCYTE HOMEOSTASIS AND IMMUNE RESPONSES

Background

The following review highlights the effects of EPA- and DHA-rich oils on inflammatory and immunological endpoints (Tables 3 and 4). Currently fish oils have been supplemented to both healthy and diseased subjects in an attempt to understand their beneficial and/or detrimental effects on homeostasis and disease. Importantly, the mechanisms that modulate inflammation, lymphocyte homeostasis, and immune responses are numerous and complex, especially in the context of diseased states, and due to the absence of large studies powered to understand the effects EPA- and DHA-rich oil consumption on validated inflammatory mediators and/or incidences of infection, it is difficult to establish a safe upper level of intake.

Inflammation is a normal and immediate physiological response to harmful stimuli, such as pathogens, damaged cells, or irritants. It is also an attempt made by the host to eliminate the insulting stimuli and promote healing. Inflammation can be acute or chronic, the former being characterized by redness, swelling, heat, and pain, which result from vasodilation, increased vascular permeability, and the extravasation of neutrophils, monocytes, macrophages, and lymphocytes into the infected or damaged tissue. Important mediators of the inflammatory process include histamine, leukotrienes (i.e., LTB_4), prostaglandins (i.e., PGE_2), complement (C3 and C5a), proinflammatory cytokines (interleukin-1 (IL-1), IL-6, tumor necrosis factor (TNF)-α), reactive oxygen species, and matrix proteases. The adhesion molecules intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule I (VCAM-1), E-selectin, and P-selectin also play important roles in inflammation because they mediate the adhesion and infiltration of the leukocytes into the infected and/or damage site (reviewed in Calder, 2006; Janeway et al., 2005). In healthy tissue ICAM-1, VCAM-1, E-selectin, and P-selectin are expressed at low levels on the surface of endothelial cells. During inflammation, however, their levels dramatically increase most likely in response to proinflammatory cytokines. Importantly, acute inflammation is resolved and homeostasis is re-established. Chronic inflammation, on the other hand, is uncontrolled or/and unresolved inflammation and can result in tissue destruction and diseased states.

The immune system and its ability to respond to invading pathogens are essential for survival. The immune system is subdivided into the innate and adaptive immune systems (for review see Janeway et al., 2005). The innate immune system is the first-line of defense and, during an infection, uses phagocytic cells, such as macrophages and granulocytes (i.e. neutrophils) to engulf, digest and eliminate the infecting agent, or cytotoxic cells, such as natural killer cells, to kill virally-infected or tumor cells. In many cases, the innate immune system is able to successfully eliminate the pathogen, however, in times when it is unable, the adaptive immune system takes over, developing a pathogen-specific response coordinated by T and B cells. Two types of adaptive immune responses exist, cell-mediated immune responses and humoral immune responses. Cell-mediated immune responses involve cytotoxic T lymphocytes, which eliminate intracellular pathogens by killing infected cells. Humoral immune responses involve B cells, which produce antibodies that bind and eliminate soluble pathogens by agglutinating and precipitating antigens, and activating phagocytic cells and complement. Importantly, the adaptive immune response also provides immunological "memory," which allows us to remember previous infections and respond appropriately.

Studies cited by VKM (2011), BfR (2009), the reviews by Frische (2006) and Calder (2006 and 2011), and found by searching public databases were included in this review. Studies assessing the effects of oils containing EPA and DHA in cancer patients or those on hemodialysis were excluded. Furthermore, any study not reporting pre-treatment levels of the specific endpoint were considered uninterpretable because the fatty acid composition of the placebos differed from that of the EPA/DHA rich oil.

Results

Long chain polyunsaturated fatty acids (PUFAs) are critical role players in inflammatory process for a variety of reasons: they, namely arachidonic acid (20:4(n-6)), are the basic building blocks of eicosanoids, which modulate the intensity and duration of the inflammatory process; bind receptors and transcription factors, which indirectly or directly upregulate pro- or anti-inflammatory gene expression; and are integrated into the cell membrane, which can directly affect the transmission of signals received by the cells (reviewed in Calder, 2010). EPA and DHA, however, are widely thought to be anti-inflammatory because in vitro and ex vivo studies have shown that they can reduce proinflammatory cytokine production by peripheral blood mononuclear cells, reduce leukotriene and prostaglandin production by neutrophils and

monocytes, reduce neutrophil and monocyte chemotaxis, and reduce neutrophil superoxide production (Meydani et al., 1991; Endres et al., 1989; Caughey et al., 1996; Sperling et al., 1993; Mori et al., 1992; Trebble et al., 2003; Meydani et al., 1993; Endres et al., 1989; Lee et al., 1985; Lee et al., 1985; Schmidt et al., 1989; Schmidt et al., 1991; Schmidt et al., 1992; Sperling et al., 1993; Endres et al., 1989; Luostarinen et al., 1992; Luostarinen and Saldeen, 1996; Hill et al., 2007). Also, the consumption of fish or oils rich in EPA and DHA have been reported to ameliorate various aspects of chronic inflammatory disorders such as rheumatoid arthritis (Goldberg and Katz, 2007; Kremer et al., 1990; Calder, 2002; Calder, 2010; Olveira et al., 2010; Fortin et al., 1995; Fritsche, 2006; MacLean et al., 2004). Importantly, the consumption of oils rich in these fatty acids do not appear to increase the amount of the proinflammatory cytokines IL-6 and TNFα, the inflammatory mediator C-reactive protein (CRP), or the eicosanoids leukotriene B_4, leukotriene E_4, and prostaglandin E_2 (PGE_2) in the blood or urine of healthy individuals, although the amount of EPA and DHA vary widely between studies(Table 3 and 4; von Schacky et al., 1993; Mori et al., 1992; Himmelfarb et al., 2007; Grundt et al., 2004; Mori et al., 1992; Bloomer et al., 2009; Himmelfarb et al., 2007; Thienprasert et al., 2009; Ottestad et al., 2011; Kirkhus et al., 2011; Papageorgiou et al., 2011). EPA- and DHA-rich oils also do not appear to increase the amount of these inflammatory mediators in patients with rheumatoid arthritis, type 2 diabetes, metabolic syndrome, systemic inflammatory response syndrome, sepsis, previous myocardial infarctions, chronic kidney disease, vascular disease, and hypertriglyceridemia (Table 4; Espersen et al., 1992; Barbosa et al., 2010; Sundrarjun et al., 2004; Mori et al., 2003b; Kabir et al., 2007; Satoh et al., 2007; Madsen et al., 2007; Woodman et al., 2003, Deike et al., 2011; Krysiak et al., 2011; Dewel et al., 2011, Moertl et al., 2011). Moreover, these results are supported by two reviews summarizing the effects of EPA and DHA-rich oils in healthy individuals and those with chronic inflammatory states (Myhrstad et al., 2011; Sijben and Calder, 2007). Myhrstad et al. found that the consumption of oils containing as high as 2.56 g of EPA plus DHA/d do not increase plasma IL-6 or CRP concentrations in healthy individuals and as high as 4 g of purified EPA or DHA/d do not increase plasma IL-6 or CRP concentrations in subjects with an increased risk of developing cardiovascular disease. Sijben and Calder found that four of five studies administering 3.2 to 7.1 g of EPA plus DHA/day had no effect circulating levels of TNFα and two studies administering 3.4 and 7.4 g EPA plus DHA/d had no effect on circulating levels of IL-6 in patients with rheumatoid arthritis.

Table 3: Highest no-effect levels for EPA- and DHA-rich oils on inflammatory mediators in healthy individuals found by Spherix Inc.

Inflammatory mediator[3]	Analyzed in	Intake (g/d)		Reference
		EPA[1]	DHA[1]	
IL-6	Plasma	0.2	1	Thienprasert et al., 2009
		0.45	0.41	Kirkhus et al., 2011
TNFα	Plasma	0.45	0.41	Kirkhus et al., 2011
CRP	Plasma	1.6	-	Ottestad et al., 2011
		0.45	0.41	Kirkhus et al., 2011
LTE_4	Urine	3.8	2	von Schacky et al., 1993
LTB_4	Plasma	0.45	0.41	Kirkhus et al., 2011
PGE_2	PBMC[2]	1.23	-	Meydani et al., 1993
8-iso-PGF_{2a}	Urine	0.45	0.41	Kirkhus et al., 2011

Notes:
1. The levels at which EPA and/or DHA do not increase the amount of the inflammatory mediator found by Spherix.
2. Spontaneous release from cultured peripheral blood mononuclear cells (PBMC).
3. Abbreviations: IL=Interleukin; TNFα=Tumor necrosis factor α; CRP=C-reactive protein; LTE_4= Leukotriene E_4; LTB_4=Leukotriene B_4; PGE_2= Prostaglandin E_2: PGF= Prostaglandin F_{2a}

Nine studies investigated the effects of the consumption of EPA- and DHA-rich oils on the circulating levels of ICAM-1, VCAM-1, E-selectin, and P-selectin in healthy individuals (Miles et al., 2001; Eschen et al., 2004; Seljeflot et al., 1998; Paulo et al., 2008;Cazzola et al., 2007; Moertl et al., 2011; Dewel et al., 2011; Kirkhus et al., 2011; Papageorgiou et al., 2011). The effects, however, were neither reproducible nor dose-responsive. Importantly, because the function of these molecules in their soluble form is not known, any conclusions about the physiological relevance of these findings are purely speculative. Moreover and as discussed previously, the consumption of levels less than or equal to 2.56 g of EPA + DHA/d by healthy individuals do not appear to increase the circulating levels of well-defined inflammatory mediators IL-6 and CRP (Myhrstad et al., 2011).

Oils containing EPA and DHA have also been shown to affect the intensity of lymphocyte responses in vitro and in animal models (reviewed in Fritsche, 2006; Sijben and Calder, 2007), but their effects on the human immune response in vivo are not well defined. Importantly, the consumption of oils containing up to 2.1 g of EPA + 1.1 g DHA/d do not appear to reproducibly affect the number of circulating T cells, B cells, monocytes, natural killer (NK) cells, and CD11b-expressing cells in humans (Hughes et al., 1996; Meydani et al., 1991; Meydani et al., 1993; Thies et al., 2001a; Thies et al., 2001b; Miles et al., 2004; Yaqoob et al., 2000; Kew et al., 2003), no studies were found that performed the same analysis on individuals

that consumed oils containing more than 2.1 g of EPA + 1.1 g DHA/d. Furthermore, no reports indicate that people consuming fish oils are more susceptible to infection. In fact, one clinical study has shown that Thai school children consuming 2 grams of fish oil/day (200 mg EPA + 1 g DHA/d) experienced fewer and shorter upper respiratory infections and bouts of diarrhea as opposed to those consuming 2 grams of soybean oil (Thienprasert et al., 2009), and another found that the number of respiratory illnesses was significantly lower in toddlers that consumed DHA-rich formulas (Minns et al., 2010). Meta-analyses and other reports have also reported lower incidences of infection in patients consuming EPA- and DHA-rich oils, although the patients were diseased or had previously undergone surgical procedures implying the presence of diseased states (Badia-Tahull et al., 2010; Wei et al., 2010; Gadek et al., 1999).

A variety of reports have suggested that the consumption of EPA- and DHA-rich oils may impact the innate immune response in humans (Yamashita et al., 1986; Yamashita et al., 1991; Purasiri et al., 1997; Thies et al., 2001b; Yaqoob et al., 2000, Sijben and Calder, 2007), but these studies have been conducted in vitro or using cells harvested from individuals that have consume EPA- and DHA-rich oils, making it difficult to assess their physiological relevance. The effects of EPA- and DHA-rich oils include reducing NK cell cytolytic activity, reducing neutrophil and monocyte chemotaxis, cytokine and arachidonic-derived leukotriene production, respiratory bursts, and increasing neutrophil and monocyte EPA-derived leukotriene production (Schmidt et al., 1989; Schmidt et al., 1991; Sperling et al., 1993; Healy et al., 2000; Endres et al., 1989; Kew et al., 2003; Rees et al., 2006; Miles et al., 2004; Sperling et al., 1993; Mori et al., 1992). Reductions in ex vivo stimulus-induced proinflammatory cytokine production have also been observed in peripheral blood monocytes harvested from individuals that have consumed EPA- and DHA-rich oils (Meydani et al., 1991; Endres et al., 1989; Caughey et al., 1996). As for the reductions in NK cell lytic activity, the effects of EPA-and DHA-rich oils appear to be related to the extended consumption of EPA because the consumption of an oil containing 0.72 g of EPA + 0.28 g DHA/d but not a oil containing 0.7 g DHA/d for 12 weeks reduced ex vivo NK cell cytolytic activity by approximately 45% (Thies et al., 2001b). The reductions NK cell lytic activity also appear to be related to the length of consumption because they were not observed when subjects consumed a similar amount of EPA and DHA for4 weeks (Yaqoob et al., 2000). It is also noteworthy that γ-linolenic acid (18:3(n-6)) can also reduce NK cell lytic activity in vitro but at much higher concentrations (Purasiri et al., 1997), suggesting that this effect may not entirely specific to n-3 long chain fatty acids. Importantly, although these types of ex vivo

studies provide clues to the functionality of these cells in vivo, they offer little insight when interpreted in the absence of "proof-of-concept" in vivo studies.

Many studies have also shown that the consumption of oils rich in EPA and DHA can also affect the function of human T cells in vitro and ex vivo (Hughes et al., 1996; Meydani et al., 1993; Meydani et al., 1991; Miles et al., 2004; Thies et al., 2001a; Thies et al., 2001b; Sijben and Calder, 2007). However, the effects are not entirely reproducible or dramatic and contrast the findings of other studies and meta-analyses reporting reduced incidences of infection in consumers of EPA- and DHA-rich oils (Thienprasert et al., 2009; Minns et al., 2010; Badia-Tahull et al., 2010; Wei et al., 2010; Gadek et al., 1999). Three studies have evaluated the effects of fish oil consumption on delayed-type hypersensitivity (DTH) responses. DTH responses are in vivo indicators of cell-mediated immunity and used clinically to determine whether or not an individual has been previously exposed to a particular antigen. The prototypical DTH test is the tuberculosis test. In 1993 Medyani et al. evaluated the effect of a low-fat diet supplemented with either 0.27 and 1.23 g of EPA+DHA/day (delivered in the form of 100 g of filet of sole or salmon given twice a week (0.27 g/day EPA+DHA) or 121-188 g of filet of sole or salmon given eight times a wk (1.23 g of EPA+DHA/day)) on the severity of DTH reactions to seven antigens and found that only the low-fat diet supplemented with 1.23 g of EPA+DHA/day significantly reduced the cumulative DTH-induced in duration by 2-fold. Subsequent studies have evaluated the effect of EPA and DHA supplementation (up to 2.1 g EPA+DHA/day) with uncontrolled diets and found that EPA/DHA supplementation had no effect (Kew et al., 2003; Miles et al., 2004).

One study reported that the consumption 3 g of fish oil/day (containing 0.93 g EPA +0.6g DHA) for 21 days by 6 individuals resulted in approximately 30% reductions in the levels of three subtypes of major histocompatibility complex II (MHC II) molecules, intercellular adhesion molecule 1 (ICAM-1), and leukocyte-function-associated antigen-1 (LFA-1) expressed on the surface of myocytes (Hughes et al., 1996). MHC II is a family of molecules expressed on the surface of cells that present potentially immunogenic substances to CD4+ T cells. If the T cells deem the substance to be foreign, the cells respond by secreting cytokines and expressing ligands and receptors to initiate the immune response. ICAM-1 and LFA-1, however, promote the adhesion of leukocytes to sites of inflammation and infection. Normally these molecules are expressed at low levels on the surface of endothelial cells, and are dramatically upregulated during an infection. Although this study suggests that fish oil consumption may dull the immune

response, the analysis was performed on cells that had been purified from the peripheral blood of healthy uninfected individuals and the reductions in MHCII, ICAM-1, and LFA-1 may or may not be relevant during an immune response. Another study has also assessed the expression of ICAM-1 and LFA-1 on peripheral blood mononuclear cells freshly isolated from healthy individuals consuming fish oil (2.1 grams EPA + 1.1 grams of DHA/d) and found no effect(Yaqoob et al., 2000).

The consumption of oils containing 2.1 g of EPA + 0.9 g DHA has also been reported to increase serum concentrations of IgE and IgG1 (Miles et al., 2004). Both IgE and IgG1 are secreted by B cells and involved in humoral immunity. IgE mediates immune responses to parasites and is a central player in allergic reactions. IgG1 develops in response to an infection, activates complement, and binds Fc receptors expressing on phagocytic cells to promote the clearance of foreign substances from the body. Currently the affects of fish oil consumption on the development of allergy are unclear (Kremmyda et al., 2011). Moreover, although the effects of fish oil consumption on human B cell function is unknown (Shaikh et al., 2012), the increased amount of circulating IgG1 suggests that individuals that consume fish oils may be less sensitive to recurrent infections by the same agent.

Conclusion

The consumption of oils containing up to 2.1 g EPA + 1.1 g DHA/d do not appear to perturb immune homeostasis or suppress immune responses. Furthermore, EPA- and DHA-rich oils do not appear to induce inflammation, although the studies reviewed were relatively small in size, evaluated their effects on only a limited number of inflammatory markers, and the amount of EPA and DHA administered varied greatly.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Papageorgiou et al., 2011	Randomized, parallel	66 healthy subjects	Treatment groups: 1. 50 mls extra virgin oil 2. 50 mls corn oil 3. 50 mls soy oil 4. 50 mls cod liver oil (0.0041 g EPA and 0.0039 g DHA/day)	3 hr		Plasma levels of VCAM-1, ICAM-1, and TNFα		0.0041 g EPA and 0.0039 g DHA/day (VCAM-1)	0.0041 g EPA and 0.0039 g DHA/day (↓ ICAM-1 and TNFα)	All comparisons were made to baseline None of the oils had any significant effects on the plasma levels of VCAM-1. Olive oil, soy oil, and cod liver oil decreased ICAM-1 Extra virgin olive oil, soy oil, and cod liver oil significantly reduced TNFα
Kirhus et al., 2011	Randomized, parallel group	159 healthy adults (18-70 years old)	Treatment groups: 1. 500 ml of n-3 long chain polyunsaturated fatty acid (LCPUFA)-enriched fruit juice 2. 34 g n-3 LCPUFA-enriched fish pate. 3. Fish oil (0.45 g EPA and 0.41 g DHA/day) 4. Untreated	7 weeks		1. Plasma CRP, IL-6, TNFα, monocyte chemotactic protein-1, IFNγ, E-selectin, P-selectin, ICAM-1, VCAM-1, LTB_4 2. Urinary 8-$isoPGF_{2a}$	1. Plasma fatty acid composition and vitamin E concentrations 2. Serum Lipids including apoA and apoB concentrations	0.45 g EPA and 0.41 g DHA/day (Plasma CRP, IL-6, TNFα, monocyte chemotactic protein-1, IFNγ, E-selectin, P-selectin, ICAM-1, VCAM-1, LTB_4 Urinary 8-iso-PGF_{2a})		Blood was collected after an overnight fast All three treatments significantly increased the amount of EPA and DHA in the plasma fatty acids, but only the LCPUFA fruit juice and fish oil significantly reduced the amount of arachidonic acid. DPA concentration were also significantly increased in the treated groups. Fish oil supplementation significantly increased total cholesterol and LDL cholesterol and decreased total triglycerides compared to baseline, but there were no significant differences compared to the untreated group. Only the Fish pate groups was the only treatment that significantly reduced plasma α-tocopherol levels. Although there were significant increases of fish oil supplementation on plasma levels of E-selectin and all treatments on IFNγ levels compared to baseline, there were no significant effects compared to the untreated group. Also, there were no significant effects on urinary F2-isoprostanes.

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Dewel et al., 2011	Randomized	100 adults with metabolic syndrome	Treatment group: 1. Low dose flaxseed oil (2.2 g a-linoleic acid/day 2. High dose flaxseed oil (6.6 g a-linoleic acid/day) 3. Low dose fish oil (0.7 g EPA and 0.5 g DHA/day) 4. High dose fish oil (2.1 g EPA and 1.5 g DHA/day) 5. Placebo (4 or 6 g soybean oil/day)	8 weeks		Plasma levels of monocyte chemoattractant protein-1 (MCP-1), IL-6 and ICAM-1	1. Plasma lipids, insulin, and glucose 2. Red blood cell fatty acids 3. Systolic and diastolic blood pressure	2.1 g EPA and 1.5 g DHA/day (MCP-1, IL-6 and ICAM-1)	2.1 g EPA and 1.5 g DHA/day (↓ blood pressure)	The fatty acid composition of the placebo differs from the fish oil beyond the absence of EPA and DHA and therefore is not an appropriate control and comparisons must be made to baseline. Blood was harvested from fasted individuals. Fish oil supplementation: 1. Significantly increased EPA and DHA concentrations in the fatty acids of red blod cells 2. Had no effect on plasma levels of MCP-1, IL-6 and ICAM-1 3. Had no effect on lipid parameters 4. Significantly decreased systolic and diastolic blood pressure in the high dose fish oil group.
Moertl et al., 2011	Prospective, randomized, double-blind, placebo-controlled	36 patients with chronic heart failure of non-ischemic origin	Treatment groups: 1. Placebo – gelatin preparation 2. 0.45 g EPA and 0.55 g DHA 3. 1.8 g EPA and 2.2 g DHA/day	12 weeks	Undergoing optimised heart therapy	1. Monocyte aggregates (cell surface expression of CD14+, CD42b+, and monocytic tissue factor expression) 2. Plasma levels of P-selectin, CD40L, fibrinogen, prothrombin fragment F1.2, monocyte tissue factor, IL-6, CRP, TNFα, and monocyte chemotactic protein-1.		0.45 g EPA and 0.55 g DHA, and 1.8 g EPA and 2.2 g DHA/day (CD40L, fibrinogen, CRP, TNFα, or monocyte chemoattractant protein-1)	1. 0.45 g EPA and 0.55 g DHA, and 1.8 g EPA and 2.2 g DHA/day (↓monocyte-platelet aggregates, and plasma levels of monocytic tissue factor) 2. 1.8 g EPA and 2.2 g DHA/day (↓plasma levels of P-selectin, F1.2, and IL-6 but only at the high dose.	Placebo was a valid control in this study because it did not contain any active ingredients. EPA/DHA supplementation: 1. Significantly and dose-dependently inhibited monocyte-platelet aggregates, and plasma levels of monocytic tissue factor. 2. Significantly decreased plasma levels of P-selectin, F1.2, and IL-6 but only at the high dose. 3. Had no effect on plasma levels of CD40L, fibrinogen, CRP, TNFα, or monocyte chemoattractant protein-1

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Deike et al., 2011	Randomized, double-blind, placebo controlled	50 non-dialysis patients with chronic kidney disease	Treatment groups: 1. Placebo (Safflower oil) 2. 2. Fish oil (1.4 mg EPA and 1.0 g DHA)	8 weeks		Plasma levels of IL-1β, IL-6, and TNFα		1.4 mg EPA and 1.0 g DHA (Plasma IL-1β, IL-6, and TNFα)		Because the fatty acid composition of the placebo differed from OMACOR, only comparisons to baseline could be made. Although there was a tendency for fish oil supplementation to increase the plasma levels of IL-1β, IL-6, and TNFα, the increases were not statistically significant
Mackay et al., 2012										Because the fatty acid composition of the placebo differed from OMACOR, only comparisons to baseline could be made and the study did not provide pre- and post-treatment values. Therefore this study is uninterpretable.
Krysiak et al., 2011	Randomized, double-blind, placebo controlled	Patients with hypertriglyceridemia (35-70 years old; bezafibrate n=37; omega-3 n= 37; placebo n=33)	Treatment groups: 1. Bezafibrate (400 mg/day/day) 2. Omega-3 (0.9g EPA and 0.75 g DHA/day) 3. Placebo ?	12 weeks		1. Plasma levels of C-reactive protein 2. IL-2, IFNγ and TNFα production from phytohemagglutinin (PHA)-stimulated lymphocytes	1. Total, LDL- and HDL-cholesterol 2. Fasting and post challenge glucose 3. Homeostasis model assessment index (HOMA)	0.9g EPA and 0.75 g DHA on plasma levels of C-reactive protein; IL-2, IFNγ and TNFαproduced by PHA-stimulated lymphocytes; and total, LDL- and HDL-cholesterol, fasting and post challenge glucose HOMA		Because the composition of the placebo is unknown, only comparison to baseline can be made and only the analysis of the patients receiving EPA and DHA has been evaluate Two patients in the fish oil group dropped out due to nausea and vomiting One patient in the placebo group dropped out due to dizziness Fish oil supplementation nad no significant effects on IL-2 IFNγ, TNFαby PHA-stimulated lymphocytes, plasma levels of CRP, total, LDL- and HDL-cholesterol, fasting and post-challenge glucose, or HOMA

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Barbosa et al., 2010	Randomized, single-blinded, placebo controlled	Patients with diagnosed systemic inflammatory response syndrome or sepsis (n=13).	Treatment Groups (delivered parenterally: 1. 50:50 mixture of oil rich in medium-chain fatty acids and soybean oil. 2. 50:40:10 mixture of medium-chain fatty acids, soybean oil, and fish oil (1.6 g EPA and 0.7 g DHA/day).	6 days		1. Caloric intake. 2. Full blood count. 3. Blood biochemistry. 4. Coagulation 5. Plasma IL-1β, IL-6, IL-10, TNFα, PGE_2, LTB_4.		1.6 g EPA and 0.7 g DHA (PGE_2 and $LTB_{4)}$	1.6 g EPA and 0.7 g DHA (↓IL-1β, IL-10, IL-6, and TNFα)	Fish oil supplementation: 1. Significantly increased EPA concentration in plasma phosphatidycholine but had no effect on DHA and arachidonic acid concentrations. 2. Had no effect on plasma PGE_2 and LTB_4 concentrations. 3. Significantly decreased the amount of IL-1β, IL-10, and TNFα.
Caughey et al., 1996	Supplementation (run-in with flaxseed or sunflower oil followed by fish oil	Healthy male subjects 22-44 years old (Flaxseed n=15; sunflower n=15)	Treatment Groups: 1. Flaxseed diet – flaxseed oil (56% a-linolenic and 18% linoleic acid) and spread consisting of 2:1 flaxseed oil and butter (23% a-linolenic and 8% linoleic acids. 2. Flaxseed diet +1.62 g EPA, and 1.08g DHA/day (triglycerides) 3. Sunflower oil and sunflower oil based spreads and salad dressings 4. Sunflower oil and sunflower oil based spreads and salad dressings+1.62 g EPA, and 1.08 g DHA/day	Subjects maintained their flaxseed or sunflower diets for 8 weeks. After the 1st four weeks, they supplemented their diets with 1.62 g EPA, and 1.08g DHA/day as triglycerides		1. Fatty acid composition of PBMC phospholipids. 2. TNFα, IL-1β, and eicosanoid production by LPS stimulated PBMCs.		NA	NA	Fish oil supplementation 1. Significantly reduced the concentration of arachidonic acid and increased the concentration of EPA and DHA in both the flaxseed diet and sunflower diet group. 2. Significantly reduced IL-1β, TNFα, and eicosanoid (Thromboxane B and Prostaglandin E) production from LPS-stimulated PBMCs.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Cazzola et al., 2007	Randomly allocated; double-blind, placebo controlled	Healthy young (18-42; n= 93) and old (53-70; n= 62) males	Test article was EPAX 4510TG (45% fatty acids as EPA and 9% fatty acids as DHA. Treatment Groups: 1. Low dose = 6 g corn oil and 3 g EPAX 4510TG (1.35 g EPA and 0.27g DHA/day) 2. Med dose = 3 g corn oil and 6 g EPAX4510TG (2.7 g EPA and 0.54 g DHA/day) 3. High dose = 9 g EPAX 4510TG (4.05 g EPA and 0.81 g DHA/day) All capsules contained 3.6 mg -tocopherol equaling 32 mg/day of -tocopherol	12 weeks		1. Plasma phospholipid fatty acid composition 2. Plasma lipid conc (total cholesterol, LDL-cholesterol, HDL-cholesterol, and triacylcholesterol 3. Plasma lipid-soluble vitamin conc. 4. Plasma soluble adhesion molecule adhesion conc.		NA	4.05 g EPA and 0.81 g DHA/day (↑ sE-selectin	The fatty acid composition of the placebo and EPA-rich supplementswere noted and it is apparent that subjects in the different groups were also consuming different amounts of other fatty acids, making it difficult to conclude anything about the specific contribution of EPA, DHA, and DPA to the observed effects based on comparisons to placebo. Blood was from fasted individuals. Fish oil supplementation: 1. Significantly reduced linoleic, di-homo-γ-linolenic acid, and docosahexanoic acid, and increased EPA, DPA, and DHA levels in both young and old subjects. 2. Increased plasma phospholipid peroxidation in the high dose young subject group and in the moderate and high dose groups in older subjects (compared to baseline). 3. Significantly increased PUFA/-tocopherol levels in all groups of young subjects and only the low older subject group (compared to baseline). 4. Reduced triacylglycerols in young subjects only (compared to baseline). 5. Significantly reduced lag time of lipoprotein peroxidation in older subjects (compared to baseline). 6. Significantly reduced GSH/glutathione ratio in older subjects (dose-dependent; compared to baseline). 7. Increased soluble E-selectin in young subjects (dose-dependent and high dose is significant; compared to baseline).

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Damsgaard et al., 2007	Randomized unmasked 2x2 factorial, supplementation	Infants (9 – 12 months; n=83)	Treatment Groups: 1. Cow's milk 2. Formula without fish oil 3. Formula with fish oil (571 mg EPA and 381 mg DHA/day)	12 months		1. Fatty acid composition inerythrocytes 2. Plasma C-reactive protein CRP, IgE, or sIL-2R concentrations. 3. Fecal IgA 4. *Lactobacillus. paracasei*- and lipopolysaccharide (LPS) +phytohaemagglutinin (PHA)-induced IFNγ. IL-10, and TNFα.		0.571 g EPA and 0.381 g DHA/day (plasmaCRP, IgE, and fecal IgA)	NA	Blood was from fasted infants Fish oil supplementation 1. Increased n-3 PUFAs and decreased n-6 PUFAs in erythrocytes 2. Had no effect on plasma CRP, IgE, or sIL-2R concentrations at 12 months or fecal IgA at 10 months. 3. Significantly increased *L. paracasei*-induced IFNγ and tended to increase LPS+PHA-induced IL-10 but had no effect on TNFα, or LPS+PHA IFNγ, or *L. paracasei*-induced IL-10.
Endres et al., 1989	Dietary supplementation Supplementation	Healthy males (n=9; average age of 29 years)	Treatment: 2.75 g EPA and 1.8 g DHA/day	6 weeks		Plasma and mononuclear cell lipids. 1. Lipopolysaccharide (LPS)- and *Staphylococcus epidermidis*-induced IL-1β and TNFα production by PBMCs. 2. S. *epidermidis*-induced PGE_2production by PBMCs. 3. LTB_4 chemotaxis of neutrophils.		NA	NA	EPA/DHA-supplementation: 1. Significantly increased the plasma concentration of EPA. 2. Significantly reduced triglyceride levels but did not affect cholesterol levels, or leukocyte or platelet counts. 3. Significantly reduced the concentration of arachidonic acid and increased the concentration of EPA and DHA in PBMC phospholipids. 4. Reduced the production of IL-1β and TNFα production from LPS-induced PBMCs. The reduction was still apparent 10 weeks after the supplementation was terminated. At twenty-weeks post-treatment, the levels of IL-1β and TNFα were similar to pretreatment levels. Similar results were also found using *S. epidermidis* although the reductions were not statistically significant. 5. Significantly reduced *S. epidermidis*-induced PGE_2 production. 6. Significantly reduced the chemotactic response of neutrophils towards LTB_4.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Eschen et al., 2004	Randomized double-blind, placebo-controlled	Healthy subjects (n=60, 20 participants/group; men and women)	Treatment Groups: 1. Placebo - olive oil 2. 6.6 g n-3 PUFA (3.0 EPA and 2.9 g DHA (re-esterified triacylglycerol)) 3. 2.0 g n-3 PUFA (0.9 g EPA and 0.8 g DHA and olive oil).	12 weeks		1. Fatty acid composition of granulocytes 2. Serum levels of VCAM-1, ICAM-1, and P-selectin	Blood lipids	3.0 g EPA and 2.9 g DHA/day (serum VCAM-1, and ICAM-1)	3.0 g EPA and 2.9 g DHA/day (↑ serum P-selectin)	Blood was harvested from fasted individuals Olive oil is not an appropriate control because its fatty acid composition is dramatically different than fish oil. Therefore it is difficult to compare across the different group and only comparisons to baseline are relevant. Supplements were well tolerated and no adverse events were reported. 1. Although no data was provided, the authors state that the serum triglycerides were decreased in a dose dependent manner and there was no effect on total cholesterol, LDL-cholesterol or HDL cholesterol. 2. P-selectin levels significantly decreased in the group receiving 6.6 g PUFAs (men and women combined) 3. n-3 PUFA supplementation had no effect on the serum levels of the other adhesion molecules. 4. Lack of a difference in the other adhesion molecules was due to gender differences. Although the differences were slight, ICAM-1 levels were significantly decreased in women but not men and there was a significant increase in VCAM-1 in women but not men.
Espersen et al., 1992	Randomized, double-blind, placebo-controlled	Subjects with rheumatoid arthritis (n=32)	Treatment Groups: 1. Placebo – mixture of fatty acids typical of the Danish diet 2. EPAX 5500 (2 g EPA and 1.2 g DHA triglycerides/day)	12 weeks		Plasma TNFα, IL-1β, and complement (C3d)		2 g EPA and 1.2 g DHA triglycerides/day (TNFα and C3d)	2 g EPA and 1.2 g DHA triglycerides/day (↓IL-1β)	EPA and DHA supplementation significantly reduced the serum levels of IL-1β but had no effect on the serum levels of TNFα or C3d.

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Gadek et al., 1999	Double blind, placebo-controlled	Acute respiratory distress syndrome (ARDS) patients, EPA+GLA (n=51), control (n=47)	Treatment groups: 1. Placebo-isonitrogenous, isocaloric standard diet 2. Fish oil plus γ-linolenic acid - 6.9 ± 0.3 g EPA and 2.9 ± 0.1 g DHA/day	4-7 days		Pulmonary inflammation, oxygenation and clinical outcomes in patients with ARDS.	Inflammatory cells and neutrophils. Infection rate.	NA (sick patients)	NA	The composition of the control and test diets were different, making it difficult to make comparison between the two groups. Fish oil supplementation significantly decreased the number of inflammatory cells and neutrophils per volume of recovered alveolar fluid by day 4 and reduction in # concomitant infections per patient.
Healy et al., 2000	Randomized, placebo-controlled	Healthy males (n=8 subjects/group; mean age of 23 years)	Treatment Groups: 1. Placebo - 80% palm oil, 20% soybean oil) 2. Tuna oil - 6.4% EPA, 0.8% DPA, and 18.5% DHA; daily amounts of EPA, DPA, and DHA were 576 mg, 72 m, 1.62 g, respectively 3. 50:50 mix of tuna oil and placebo oil. 4. 25:75 mix of tuna oil and placebo oil 5. 12.5:87.5 mix of tuna oil and placebo oil 6. Linseed oil	12 weeks		1. Fatty acid composition of neutrophils. 2. Neutrophil chemotaxis toward N-formyl-methionine-leucine-phenylalanine (FMLP). 3. FMLP-induced superoxide production.		NA	NA	The placebo is not an appropriate control because its fatty acid composition differs beyond the absence of EPA, DPA, and DHA. Therefore comparison will only be made to baseline values in the Tuna oil group. Blood was harvested from fasted individuals. Tuna oil supplementation: 1. Significantly increased the amounts of EPA and DHA in the fatty acid composition of neutrophils. EPA and DHA levels reached maximum levels by 4 weeks. 2. Significantly reduced the amount of AA in the fatty acid composition of neutrophils. 3. Had no effect on neutrophil chemotaxis toward FMLP or FMLP-induced superoxide production.

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Hill et al., 2007	Randomized, double blind, parallel, placebo-controlled 2x2 factorial design	Overweight and obese adults with cardiovascular risk factors (25 -65 years old; n=15)	Treatment Groups: 1. Sunflower oil 2. DHA-rich tuna oil (1.56 g DHA and 0.360 g EPA/day)	12 weeks	Subjects were instructed to exercise for 45 minutes three times per week or not. Unhealthy individuals	1. Fatty acid composition of erythrocytes. 2. Neutrophil function (superoxide production, bactericidal activity, adherence, and chemotaxis). 3. Stimulus induced cytokine production from stimulated T cells.		1.58 g DHA and 0.36 g EPA/day (neutrophil adherence and chemotaxis)	NA	The exact composition of the two oils was not noted. Presumably the placebo is not an appropriate control because its fatty acid composition differs from DHA-rich oil beyond that of being devoid of DHA and EPA. Blood was collected from fasted individuals DHA-rich oil: 1. Significantly increased the amount of DHA in erythrocytes. 2. Significantly reduced PMA-induced superoxide production. Also modestly reduced bactericidal activity. 3. Had no effect on adherence or chemotaxis. 4. Both the sunflower oil group and DHA-rich group had similar cytokine responses to ConA, PHA, and LPS, indicating that the consumption of fatty acids in general can affect stimulus-induce cytokine production.
Holm et al., 2001	Randomized, double-blinded, placebo controlled	Heart transplant recipients (mean time after transplant of 6 years; range 1-12 years; n=45)	Treatment Groups: 1. Placebo - corn oil, 3.7 mg α-tocopherol 2. n-3 fatty acids (1 g fatty acids; 46.5% EPA and 37.8% DHA, and 3.7 mg α-tocopherol).	12 months	All received triple drug regimen maintenance immunosuppressive therapy with cyclosporine, azathioprine, and prednisolone. Thirty-three patients were also treated with anti-hypertensive medications (ACE inhibitors or calcium channel blockers. Medications remained unchanged in the last 3 months before the study and did not change throughout the study. Some patients were also receiving -blockers, diuretics, and/or statins	Plasma levels of TNFα and IL-10.	Plasma levels of vitamin A, E, and -carotene	NA (due to confounding medications)	NA (due to confounding medications)	The exact composition of the placebo was not noted. Therefore it is it difficult to draw conclusions based on comparisons to the placebo group. Two patients in the placebo group and one patient in the Ω-3 group died during the study. Details of blood harvesting were not noted. 1. n-3 fatty acids increased the levels of EPA and DHA levels in plasma phospholipids. 2. Serum TNFα levels were significantly increased by approx. 25% in those receiving n-3 fatty acids. 3. IL-10 levels were approximately 30% lower in those receiving n-3 fatty acids but were not statistically significant. 4. Heart transplant patients with diagnosed transplant coronary artery disease receiving n-3 fatty acids had significantly higher levels of TNFα than those with normal angiograms that received n-3 fatty acids. 5. n-3 fatty acids significantly reduced the levels vitamin e and -carotene, and did not affect the levels of α-tocopherol.

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Hughes et al., 1996	Supplementation	1. One male and three females consumed a normal diet 2. Supplemen tedgroup: Three males and three females (19-41 years old)	Treatment Groups: 1. Without supplement 2. With fish oil - 930 mg EPA and 630 mg DHA/day as triglycerides	21 days		1. Expression of Major histocompatibility complex (MHC) class II, intercellular adhesion molecule-1 (ICAM-1), and leukocyte-function-associated antigen-1 (LFA-1) on monocytes. 2. Interferon (IFN)γ-induced upregulation of MHC II, ICAM-1, and LFA-1 on ex vivo monocytes.		NA	0.930 g EPA and 0.630 g DHA/day (↓ reduced the levels of MHC II, ICAM-1, LFA-1 on monocytes)	Details of the blood draws were not provided and, importantly, comparisons were made within the different groups before and after treatment. 1. Fish oil supplementation did not affect the percentage of monocytes expressing MHC II, ICAM-1, or LFA-1 but did reduce the levels of the different markers by approximately 30%. The physiological relevance of these reductions are unclear because functional tests were not performed. 2. Significant reductions were also observed in both the percentage of cells MHC II HLA-DR and HLA-DP when the monocytes were stimulated with IFNγ. 3. Fish oil supplementation also reduced the levels of HLA-DQ, HLA-DP, ICAM-1, and LFA-1 expressed by IFNγ-induced monocytes.
Johansen et al., 1999	Placebo-controlled	Congestive heart disease patients, Omacor arm (n=23), control oil arm (n=31)	Treatment Groups: 1. Placebo -corn oil 2. Omacor - ethyl esters of 2.7 g EPA and 2.34 g DHA/day	6 months plus 4 weeks "study period" in which both arms received 5.04 g/d ethyl esters of EPA/DHA.	Some patients were also consuming aspirin, warfarin, statins, and ACE-1 inhibitors	Soluble markers of endothelial function		NA (confounding medications)	NA (confounding medications)	The exact composition of the corn oil and Omacor capsules was not noted. Presumably the variety of fatty acids in the corn oil capsules is different than those in the Omacor capsules, making if difficult to compare between the different groups. Blood was drawn from fasted individuals n-3 fatty acid supplementation: 1. Significantly decreased the concentration of arachidonic acid and increase the concentration of EPA and DHA in serum phospholipids. 2. Had no effect on total cholesterol but significantly reduced serum triglycerides. 3. Modestly increased HDL cholesterol but the increase was not statistically significant. 4. Significantly reduced tPAag and soluble thrombomodulin, and increased the soluble E-selectin and sVCAM-1 levels. 5. Significantly increased serum vitamin E levels and modestly increased serum TBARS.

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Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Kabir et al., 2007	Randomized, double-blind, placebo-controlled	Postmenopausal women with type 2 diabetes (average age 55 years; n=27)	Treatment Groups: 1. Placebo – paraffin oil 2. 1.08 g EPA and 0.72 g DHA	2 months	23 patients were taking oral hypoglycemic treatments; 5 patients were taking lipi-lower agents and 6 were receiving hormone replacement therapy	Multiple endpoints were measured – only those pertaining to inflammation have been noted here,i.e., plasma levels of IL-6 andTNFα.		1.08 g EPA and 0.72 g DHA (IL-6 and TNFα)	NA	Although the fatty acid composition of the placebo and EPA/DHA-rich supplements were not noted , they are presumably different. Therefore comparisons can only be made to baseline. Blood was harvested from fasted individuals. Fish oil supplementation had no effect on plasma IL-6 and TNFα(compared to baseline).
Kew et al., 2003	Randomized, double-blind, placebo-controlled	Healthy aged adults (25-72years old; n=150); approximately 30 subjects/group	Treatment Groups: 1. Placebo group – linoleic acid-rich margarine and capsules (average diet of the United Kingdom) 2. Low -linolenic – 5 g/d of -linolenic 3. High -linolenic – 10 g/d of -linolenic 4. Low EPA/DHA– 0.7 g/d EPA/DHA (composed of a spread of 0.18 g EPA and 0.28 g DHA/25 g body weight and capsules containing 0.31 g EPA and 0.48 g DHA) 5. High EPA/DHA – 1.5 g/d EPA/DHA	6 months	Moderately hyperlipidemia (fasting cholesterol of 4.5 mm/L and fasting triglycerides 0.8 and 3.2 mm/L)	1. Fatty acid composition of peripheral blood mononuclear cells (PBMC) 2. LPS-induced cytokine production (TNFα, IL-6, IL-1, and IL-10) from PBMC 3. Concanavalin A (ConA)-induced proliferation and cytokine secretion (IL-2, IFNγ, and IL-4) from PBMCs. 4. Delayed-type hypersensitivity (DTH) responses to tetanus toxoid, Strepococcus, mycobacterium tuberculosis, Candida albicans, trichophyton metagrophytes, and Proteus mirabilis		1.5 g EPA/DHA (circulating number of lymphocytes and monocytes)	NA	An analysis of the fatty acid composition of the different fat spreads and oil capsules shows that subjects in the low EPA+DHA and high EPA+DHA group consumed similar amounts of linoleic acid, arachidonic acid, and -linolenic acid as the placebo group. 1. The increase in EPA and DHA was significantly greater in the high EPA+DHA-supplemented diet than in the placebo group. 2. None of the diets significantly affected the distribution of CD4+, CD8+, B cells, and monocytes in the blood 3. There was no significant effect of the diets on E. coli. or PMA-induced respiratory burst in neutrophils or myocytes, or in their ability to phagocytose E. coli. 4. EPA+ DHA diets had no effect on LPS-induced cytokine production by PBMCs. 5. EPA+ DHA diets had no effect on ConA-induced proliferation or cytokine production by PBMCs. 6. EPA+ DHA diets had no effect on DTH responses.

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Lee et al., 1985	Supplementation	Healthy (22-53 years old; n=7)	Treatment: 3.2 g EPA and 2.2 g DHA triglycerides/day	3 and 6 weeks		1. Neutrophil fatty acid composition 2. Leukotriene B_4(LTB_4), 6-trans LTB_4, and 5-hydroxyeicoate traenoic acid (5-HETE) production from calcium ionophore-stimulated ex vivo neutrophils and monocytes. 3. Neutrophil chemotaxis towards LTB_4. 4. Neutrophil adherence to LTB_4-treated endothelial cells.		NA	NA	Fish oil supplementation: 1. Significantly increased the amount of EPA in the neutrophil fatty acids. There were no significant changes in amount of AA or DHA in neutrophil fatty acids over the course of the supplementation. 2. Had no effect on calcium ionophore-induced AA-derived 5 lipoxygenase products from ex vivo neutrophil harvested 3 weeks aftersupplementation began. 3. Reduced the production of LTB_4, 6-trans-LTB_4, and 5-HETE from ex vivo neutrophil stimulated with a calcium ionophore 6 weeks aftersupplementation began. Also reductions were dependent on the supplementation because responses returned to baseline after a 6-week washout period. Similar results were also seen in monocytes. 4. Reduced neutrophil chemotaxis to LTB_4. 5. Reduced neutrophil adherence to LTB_4-treated endothelial cells.
Linday et al., 2004	Randomized, dietary supplementation	Children (6 months to 5 years old	Treatment Groups: 1. Medical records control group 2. Supplementation group (1 teaspoon of reformulated cod liver oil (total daily doses 45 – 50 mg of a-linolenic, 460 – 500 mg EPA, and 500-550 mg DHA) and ½ tablet of a multivitamin)	200 days		1. The number of upper respiratory tract visits 2. Other illness visits		NA	NA	There were no adverse events reported over the course of the study. Fish oil supplementation significantly decreased the number of upper respiratory visits over the course of the study whereas there was no change in the medical records control group. There was no significant change in the number of other illnesses of the course of the study.

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Luostarinen et al., 1992	Double-blind crossover; supplementation	Healthy volunteers (n=12; mean age of 51)	Treatment groups: 1. Without 2. With fish oil supplements (40% n-3 PUFAs) with increasing doses ofa-tocopherol (0.3IU/g or 1.5IU/g). Daily intake of EPA and DHA was 5.4 and 3.2 g/day, respectively.	3 weeks	Three volunteers has had uncomplicated hypertension	1. Neutrophil chemotaxis towardformyl-methyl-leucyl-phenylalanine (FMLP). 2. Calcium ionophore-induced leukotriene B_4 (LTB_4) production by neutrophils 3. Serum vitamin E and malondialdehyde (MDA) concentrations.		NA	NA	The purpose of this study was to determine the effect of different levels of vitamin E on fish oil supplementation. Fish oil supplementation: 1. Reduced neutrophil chemotaxis to FMLP. There was no dose effect of the vitamin A. 2. Had no effect on calcium ionophore-induced neutrophil LTB_4 production. 3. The consumption of the oil containing low amounts of vitamin E increased the plasma concentrations of malondialdehyde but the oil containing higher amounts of vitamin E had no effect. These results suggest that serum MDA levels are the result of having low amounts of vitamin E during the fish oil supplementation.
Luostarinen and Saldeen, 1996	Supplementation	Healthy men and women (n=12; mean age of 48 years) with mildly increased serum triglycerides.	Treatment Groups: 1. Without supplement 2. With fish oil supplement (5.4 g EPA and 3.2 g DHA/day).	4 weeks		1. Neutrophil phospholipids 2. Superoxide production from ex vivo phorbol 12-myristate 13-acetate(PMA) or PMA+indomethancin-stimulated neutrophils. 3. Spontaneous and calcium ionophore-induced elastase release from ex vivo neutrophils.		5.4 g EPA and 3.2 g DHA/day (elastase release)	NA	Blood was harvested from fasted individuals Oil intake did not increase plasma malondialdehyde concentrations or decrease serum a-tocopherol levels Fish oil supplementation: 1. Significantly increased EPA and decreased linoleic and arachidonic acid concentrations in neutrophil phospholipids 2. Significantly decreased superoxide production (30%) from ex vivo PMA or PMA+indomethancin-stimulated neutrophils. 3. Had no effect on elastase release from ex vivo calcium ionophore-stimulated neutrophil. 4. Had no effect on spontaneous release of elastase.
Madsen et al., 2007	Randomized, double-blind, placebo-controlled	Patients with a previous myocardial infarction (mean age 63 years old; n=41)	Treatment Groups: 1. Placebo – olive oil 2. 5.2 g n-3 PUFA (4.3 g EPA and DHA)	12 weeks		1. Platelet fatty acid composition 2. Serum levels of C-reactive protein (CRP)		4.3 g EPA and DHA (serum CRP)		The fatty acid composition of the placebo and EPA/DHA-rich supplement are different. Therefore only comparisons to baseline are relevant. 1. EPA/DHA supplementation significantly increased the amount of EPA, DPA, and DHA in platelet membranes and decreased the amount of linoleic and arachidonic acid. 2. Although there was a small increased in serum levels of CRP in the group that received the EPA/DHA supplementation, the increase was not statistically significant.

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Meydani et al., 1991	Supplementation	Young (23-33 years old) and older women (51-68 years) n=6 for each group	Treatment: 1.68 g EPA, 0.72 g DHA, 0.6 g other fatty acids, and 6 IU vitamin E/day)	12 weeks		1. Lymphocyte proliferation 2. Stimulus-induced interleukin (IL)-1, 2 and 6 production from PBMCs 3. LPS-induced prostaglandin E_2 production 4. Complete blood count and white cell differential 5. Plasma tocopherol 6. Plasma fatty acid composition		1.68 g EPA and 0.72 g DHA/day (number of circulating mononuclear and polymorphonuclear cells)	NA	All comparisons were made to baseline (pretreatment) values. Volunteers reported no side effects and all measurements were compared to baseline values. EPA and DHA supplementation: 1. Had no effect on the total number and percentage of mononuclear and polymorphonuclear cells, nor did it affect the levels of plasma α-tocopherol. 2. Increased the amount of EPA and DHA in both younger and older women, although the increase was more significant in older women. Supplementation also significantly reduced the amount of AA in older women. The end result was significant reductions in the AA:EPA ratio in bother young and older women. 3. Reduced lipopolysaccharide-induced IL-1 and TNFα production in a time depended fashion in PBMCs harvested from both young and old women; and reduced Staphylococcus epidermidis-induced TNFα production in young and older women, although the reduction in younger women was not significant. The reduction in older women was approx. 5-fold. 4. Reduced Concanavalin A (ConA)-induced IL-6 production in older women (approx. 2 –fold) and IL-2 production in younger women (approx. 2-fold). 5. Had no effect on PHA-induced proliferation of PBMCs harvested from either young or old women. 6. Had no effect on LPS-induced PGE_2 production.

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Meydani et al., 1993	Two-phase diet study, overlapping baseline controlled	Males and females over 40 years old (n= 11 male and 11 females)	Diets: 1. Baseline diet (American diet) 2. Low fat, low fish - subjects consumed low fat, low cholesterol diet conforming to the NCEP Step 2 recommendations. Treatment groups: 1. Low fish - 0.27 g EPA and DHA/day 2. High fish - 1.23 g EPA and DHA/day	1. Baseline diet 6 weeks 2. Low fat, low fish and low fat, high fish diets were consumed for 24 wks. Delayed hypersensitivity responses (DTH) were determined at the end of the baseline diet and the end of the Low fat, low fish and low fat, high fish diets.		1. Plasma fatty acid composition 2. Plasma - tocopherol concentrations 3. Ex vivo cytokine and PGE_2 production. 4. Number of circulating $CD4^+$ and $CD8^+$ T cells. 5. Ex vivo mitogen-induced proliferation 6. Delayed-type hypersensitivityresponses		1.23 g EPA and DHA/day (lymphcyte numbers)	1.23 g EPA and DHA/day (skewed T cell populations ↓ CD8+, ↑ CD4+; ↓PGE_2,↓ DTH responses)	Because the subjects consumed fish, it is difficult to attribute affects to specifically EPA or DHA. Blood samples were collected after a 12 h fast during weeks 4, 5, and 6 of the baseline diet period and weeks 22, 23, and 24 of the treatment diets. Importantly, this study did not include a placebo and all endpoints were compared to baseline values. 1. With the exception of 22:6n-3 fatty acids, low fat, low fish and low fat, high fish diets significantly increased n-3 fatty acids and decreased 20:4n-6 fatty acids. A significant increases in 22:6n-3 was only seen with the low fat, high fish diet. 2. Both low fat, low fish and low fat and high fish diets reduced the arachidonic acid (AA)/EPA ratio. 3. Low fat, low fish diet reduced plasma tocopherol concentration/g plasma. Low fat, high fish increases plasma tocopherol concentration/ total cholesterol and triglycerided, and reduced the plasma tocopherol concentration/no of double bonds in plasma fatty acids 4. Although there were significant effects on ex vivo mitogen-induced IL-1, TNF, and GM-CSF production, the effects were not dose-dependent. Low fat, high fish diets, however, did reduce ConA-induced IL-6 production. PGE_2 production was lower in both unstimulated and PHA stimulated cultures. 5. The low fat, low fish and low fat, high fish diets did not significantly affect the total numbers of lymphocytes or total number of T cells ($CD3^{+)}$. However, low fat, low fish diet significantly reduced circulating $CD8^+$ T cell numbers whereas the low fat, high fish diet significantly reduced circulating $CD4^+$ and increased circulating $CD8^+$ T cell numbers. 6. The low fat, low fish diet significantly increased ex vivo mitogen induced proliferation by 30% whereas the low fat high fish significantly reduced lymphocyte proliferation by 20%. 7. The low fat, low fish had no effect on DTH responses and low fat, high fish diet significantly reduced DTH responses by just less than 2-fold. Importantly, positive DTH responses required a previous exposure to the inoculating antigen.

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Miles et al., 2001	Randomly allocated, double-blind, placebo controlled	Healthy young (< 40 years of age; n=16) and old (>55; n=12) males	Treatment Groups: 1. Placebo - palm oil mix (80:20 palm oil and soybean oil) 2. Fish oil (daily total of 1.2 g EPA+DHA)	12 weeks	Some consumed less than 10 cigarettes/day; on day of blood draw were not permitted to smoke prior to blood draw	1. EPA content of plasma phospholipids. 2. Plasma levels of sE-selectin, sVCAM-1, and sICAM-1.		NA	1.2 g EPA+DHA (↓sVCAM-1 in old males; ↑sE-selectin in young males; ↓sE-selectin in old males)	The specific fatty acid composition of the placebo and fish oil group was not noted, making it difficult to clearly define the contribution of EPA in modulating the observed effects based on comparisons to the placebo group. Blood was from fasted individuals. 1. Fish oil consumption significantly increased EPA content of plasma phospholipids in young and old males (compared to placebo). 2. Significantly reduced sVCAM-1 in old males (compared to baseline). 3. Significantly increased sE-selectin in young males and decreased sE-selectin in old males (compared to baseline).

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Miles et al., 2004	Randomly allocated, double-blind, placebo-controlled	Healthy males (21-44 years old; n=70)	Nine capsules (1 g each) containing individual or blend of palm, sunflower, EPA, borage, or echium oil. Only thecontrol and EPA supplemented group will be considered. Treatment Groups: 1. Placebo - stripped palm- oil sunflower (80:20; 800 g of palm oil 200 g sunflower oil/kg total oil) 2. 2.1 g EPA/day (436 g palm oil, 109 g sunflower oil, and 455 g EPA-rich oil/kg total oil). The EPA-rich oil also contained 10.9 g DHA/100 g total fatty acids. Given that EPA concentration was 25.7 g/100 g total fatty acids, the ratio of EPA to DHA equals2.35:1, which is equivalent to 0.893 g DHA/day as well.	12 weeks		1. Fatty acid composition of peripheral blood mononuclear cells (PBMCs). 2. Number of circulating natural killer cells. 3. Serum IgG2 and IgE concentrations. 4. *E. coli* and phorbol myristate acetate (PMA) –induced respiratory burst of circulating neutrophils and monocytes. 5. Lipopolysaccharide (LPS)-induced IL-1 and TNFα production by PBMC. 6. Concanavalin A (ConA)-induced proliferation and IL-2, IL-4, IL-10, and IFNγ production by PBMC. 7. Delayed-type hypersensitivity (DTH) responses to tetanus toxoid, Strepococcus, mycobacterium tuberculosis, Candida albicans, trichophyton metagrophytes, and Proteus mirabilis.		2.1 g EPA and approx 0.893 g DHA/day (circulating number of lymphocytes, serum IgE, and DTH responses)	2.1 g EPA and approx 0.893 g DHA/day (↑serum IgG1; ↓circulating number of natural killer cells but there elevated in the EPA/DHA group at baseline)	The composition of the capsules was noted and the EPA supplemented capsules contained approximately ½ the amount of palm oil and sunflower oil to accommodate for the EPA-rich oil. Thus the EPA-supplemented group did not receive comparable amounts of the other fatty acids as the placebo group, making comparisons to the placebo group difficult. Blood was drawn from unfasted individuals. The EPA-supplemented diet: 1. Significantly increased the amount of EPA fatty acid in PBMC. 2. Did not affect the number and distribution of circulating T cells (CD4+ and CD8+), and B cells. It also significantly reduced the amount of circulating natural killer cells (compared to baseline) but had no effect on when compared the levels detected in the placebo group. Importantly, the number of natural killer cells was elevated approximately 1.7-fold in the EPA group before the treatments began. 3. Significantly increased IgG2 and decreased IgE (compared to baseline) but did not significantly affect the concentration of circulating IgG2 and IgE comparing across the groups. 4. Had no effect across all the groups in the stimulus induced respiratory burst of neutrophils and myocytes. 5. Significantly increased ConA-induced IL-2, IL-4, and IL-10 production but had no effect on LPS-induced IL-1 and TNFα-production or ConA-induced proliferation of PBMCs (compared to baseline). 6. Increased the indurations and cumulative diameter of DTH-although the increases were not significant and they also increased in the placebo group.

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Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Minns et al., 2010	Randomized, double-blind, controlled, parallel-group	Healthy children (18-36 months; n=~29/group)	Cow's milk-bases ready-to-drink toddler formula. Treatment Groups: 1. 0 mg DHA 2. 43 mg DHA (27 mg/day) 3. 130 mg DHA (87 mg/day) DHA was from a single cell algal oil that contained approximately 40% DHA, 15% DPA, and 2.5 % EPA.	60 days		1. Fatty acid composition of red blood cells (RBC), RBC-phosphotidylcholine (PC), or RBC-phosphotidylethanolamine (PE), and plasma phospholipids. 2. Toddler formula intake. 3. Body weight. 4. Adverse events including infections and illnesses.		NA	130 mg DHA (↓# illnesses)	1. Formulas were well tolerated,groups consumed a similar amount during the study, and there was no significant difference in body weight. 2. DHA increased in a dose-dependent fashion in RBC, RBC-PC, PBC-PE, and plasma phospholipids of those supplemented with increasing amounts of DHA. 3. DHA supplementation decreased arachidonic acid concentrations in RBC but not in RBC-PC, PBC-PE, and plasma phospholipids. 4. The group consuming 130 mg of DHA had significantly less adverse events, which was due to significantly lower respiratory illnesses.
Mori et al., 1992	Double-blind	Patients with peripheral vascular disease (n=32; 47-71 years old)	Treatment groups: 1. Placebo - olive oil 2. Fish oil (daily intake 5.2 g n-3 including 2.8 g EPA and 1.8 g DHA)	4 weeks	Patients with peripheral vascular disease	1. Serum lipids 2. Platelet aggregation 3. Neutrophil leukotrienes and platelet activating factor in response to calcium ionophone stimulation 4. Plasma lyso-platelet activating factor (PAF) 5. Platelet phospholipid fatty acids 6. Serum thromboxane B_2 (TXB_2) 7. Urinary prostaglandin metabolites TXB_2 and 6-keto-$PGF_{1\alpha}$		2.8 g EPA and 1.8 g DHA/day (serum TXB_2 or lyso-PAF or urinaryTXB2 or 6-keto-PAF)	NA	Fish oil supplementation: 1. Significantly increased the amount of EPA and decreased the amount of arachidonic acid in platelet phospholipids. 2. Significantly increased total cholesterol and reduced total triglycerides 3. Increased LDL-cholesterol and HDL-cholesterol (increase was greater in LDL-cholesterol; increase in HDL-C was accounted for by an increase in HDL2 and a corresponding decrease in HDL3-C. 4. Significantly reduced platelet aggregation to collagen in vitro. Also significantly reduced platelet aggregation to PAF but only at a low dose. 5. Had no effect on serum levels of TXB_2. 6. Significantly reduced LTB_4 and increased LTB_5 production from calcium ionophore-induced polymorphonuclear leukocytes (PMNs) and had no effect on PAFs produced by PMNs. 7. Had no effect on serum levels of lyso-PAF or urinary excretion of 6-keto-$PGF_{1\alpha}$or TXB_2.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Mori et al., 2003a	Randomized, placebo-controlled	Hypertensive diabetic men (40-75 years; n=59)	Treatment Groups: 1. Placebo – olive oil 2. 3.84 gEPA ethy ester/day 3. 3.68 g DHA ethyl ester/day	6 weeks	All subjects were on hypertensive therapy also study included subjects if they were taking hypoglycemic agents but not insulin.	1. Plasma C-reactive protein (CRP), IL-6, and TNFα. 2. Urinary F_2-isoprostanes. 3. Platelet and plasma phospholipid fatty acid composition.		3.84 gEPA ethy ester/day or 3.68 g DHA ethyl ester/day (IL-6, CRP, or TNFα)		Olive oil is not an appropriate placebo because its fatty acid composition differs from the purified EPA and DHA supplements. Therefore only comparison to baseline can be made. Blood was obtained from fasted individuals 1. EPA supplementation significantly increased EPA and decreased arachidonic acid and DHA concentrations in platelets. 2. DHA supplementation significantly increased DHA and decreased arachidonic acid and EPA concentrations in platelets. 3. EPA supplementation significantly increased EPA and decreased arachidonic acid and DHA concentrations in platelets. 4. DHA supplementation significantly increased DHA and decreased arachidonic acid and EPA concentrations in platelets. 5. F2-isoprostanes were significantly reduced by EPA and DHA supplementation 6. Although there was a slight reduction in plasma levels of TNFα in both the EPA- and DHA-supplemented groups, they were not statistically significant. 7. EPA and DHA supplementation had no effect on plasma levels of IL-6 and CRP.

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Ottestad et al., 2011	Randomized, double-blind, placebo-controlled	Healthy men and women (18 – 50 years old; n=19/group)	Treatment Groups: 1. 8 g/day fish oil (1.6 g/day EPA/DHA 2. 8 g/day oxidized fish oil (1.6 g/day EPA/DHA) 3. 8 g/day sunflower oil	7 weeks		1. Plasma levels ofsecondary oxidation products4 HHE or 4-HNE. 2. Urine levels of 8-iso-$PGF_{2}\alpha$,, a biomarker of oxidative stress 3. Plasma levels of the antioxidants α-tocopherol or GSH. 4. Plasma levels of inflammatory biomarker C-reactive protein (CRP). 5. Plasma fatty acids.	Serum lipids	1.6 g/day DHA/EPA (GSH, CRP, 8-iso-$PGF_{2}\alpha$)	NA	Sunflower oil is not an appropriate placebo because its fatty acid composition is dramatically different than fish oil or oxidzed fish oil. Therefore it is difficult to compare across the different groups and only comparisons to baseline are relevant. Only native fish oil will be considered Blood was drawn following an overnight fast. Fish oil supplementation: 1. Had no effect on serum lipids 2. Significantly reduced plasma levels of 4-HNE. 3. Had no effect on urinary 8-iso-$PGF_{2}\alpha$, or plasma levels of theα-tocopherol or GSH, CRP, or 4-HHE. 4. Significantly increased the amount of EPA, DPA, and DHA and decreased the amount of AA in plasma phospholipids.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Paulo et al., 2008	Randomized, double-blind, placebo-controlled	Healthy men and women (20 - 40 years old; men (n=120); women (n=155)	Treatment Groups: 1. Placebo/ control = 5.6 mg of n-3 PUFA (vegetable oil; no detectable EPA/DHA) 2. 271 mg of n-3 PUFA/day (150 g 3. Cod Fish/3 times/wk; 53.6 mg EPA and 207 mg DHA/day) 4. 3003 mg of n-3 PUFA/day (150 g farmed salmon; 773 mg EPA and 1369 mg DHA/day) 5. 1417 mg n-3 PUFA/day (Fish oil; 633 mg EPA and 429 mg DHA/day) All subjects were instructed to follow a hypocaloric diet for eight weeks. Calories were restricted to 30% of estimated individual energy expenditure form the Harry-Benedict equation and OMS criteria for physical activity.	8 weeks		Plasma levels of soluble ICAM-1 and VCAM-1		0.6 g EPA and 0.4 g DHA/day (sVCAM-1)	NA	The fatty acid composition of the different diets and fish oil supplements were noted and it is apparent that subjects in the different groups were also consuming varying amounts of linoleic, -linolenic acid, and other fatty acids. Thus it is difficult to conclude anything about the specific contribution of EPA to the observed effects based on comparisons to the placebo group. Drop-outs were due to failure to lack of time to attend the clinic and/or inability to follow the restricted energy diet. Blood was from fasted individuals. Cod-based diet significantly decreased sICAM-1 and had no effect on sVCAM-1 (compared to baseline). Salmon and fish oil diets significantly increased sVCAM-1 but had no effect on sICAM-1 (compared to baseline). Treatments did not significantly affect sICAM-1 and sVCAM-1 (compared to placebo).

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Rees et al., 2006	Placebo-controlled, double-blind	Healthy young and old men (n=15-16/group)	Treatment Groups: 1. Placebo (corn oil) 2. 1.35 g EPA-rich oil/day 3. 2.7 g EPA-rich oil/day 4. 4.05 g EPA-rich oil/day	12 weeks		1. EPA concentration in plasma and peripheral blood mononuclear cells (PBMC). phospholipids 2. Neutrophil and monocyte phagocytosis, respiratory burst. 3. Lipopolysaccharide (LPS)-induced production of inflammatory cytokines by neutrophils.	Expression of CD54 and CD11b on monocytes	4.05 g EPA-rich oil (CD54 and CD11b)	NA	EPA-rich oil supplementation: 1. Increased the amount of EPA and DPA and decreased the amount of arachidonic acid in plasma phospholipids in a dose-responsive fashion. The amount of di-homo-g-linolenic acid also decreased dose-dependently in older men. In younger men the decrease was not dose-dependent. 2. Significantly increased EPA in PBMCs but had no effect on PBMC levels of DPA, arachidonic, or di-homo-g-linolenic acid. 3. Had no effect on CD54 or CD11b expression on freshly isolated monocytes. 4. Had no effect on neutrophil or monocyte phagocytosis 5. Significantly reduced E. coli-induced respiratory burst of neutrophils in older men but not in younger men. Also had no effect on PMA-induced respiratory burst. 6. Had no effect on LPS-induced cytokine or PGE_2 production.
Satoh et al., 2007	Randomized; single-blind	Japanese obese type 2 diabetic patients (n=44; average age of 51 years)	Treatment Groups: 1. Control – diet 2. Experimental – diet + 1.8 gEPA ethyl ester/day	3 months		A variety of endpoints were taken. Only the effects on C-reactive protein (CRP) are noted		NA	1.8 gEPA ethyl ester/day (↓CRP)	EPA supplementation: 1. Significantly increased the amount of EPA and decreased that amount of DHA in serum 2. Significantly reduced the amount of serum CRP
Schmidt et al., 1989	Supplementation	Healthy males (29 – 49 years old; n=12)	Treatment: Cod liver oil (5.3 g n-3 PUFAS = 2.5 g EPA/day)	6 weeks		1. Neutrophil chemotaxis to N-Formyl-Met-Leu-Phenylalanine (FMLP) and autologous serum. 2. Monocyte chemotaxis to FMLP and autologous serum.		NA	NA	Blood was drawn from fasted individuals. All measurements were compared to baseline. Cod liver oil supplementation: 1. Significantly decreased neutrophil migration by 33% to FMLP and autologous serum (40%). 2. Significantly decreased monocyte migration by 46% to FMLP but not to autologous serum.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Schmidt et al., 1991	Dietary supplementation	Hyperlipidemic patients (n=17); Healthy volunteers (n=10)	Treatment for hyperlipidemic patients: 10 ml daily of re-esterified fish oil triglyceride (6 g n-3 PUFA; 34% EPA, 3.5 % DPA, and 19% DHA equaling 2.04 g EPA. 0.2 g DPA/day, and 1.14 g DHA/day) Treatment for healthy volunteers: Same fish oil as above but used inhyperlipidemic patients but in encapsulated form. Dosages: 1.3 g, 4 g, 9 g/day.	6 weeks for hyperlipidemic patients; 3 x 6 week periods with a 4 week washout period in-between each treatment for healthy volunteers		1. Monocyte migration to autologous serum and N-formyl-Met-Leu-Phenylalanine (FMLP) in normal and hyperlipidemic individuals. 2. Neutrophil migration to autologous serum and FMLP in normal and hyperlipidemic individuals 3. Dose-response analysis of monocyte and neutrophil migration in healthy volunteers.	Plasma cholesterol levels	NA	NA	Blood was drawn from fasted subjects. n-3 PUFAs were well tolerated and no adverse effects were observed. In healthy volunteers, 18:2(n-6) concentrations diminished and EPA, DPA, and DHA concentration increased significantly as the amount of fish oil increased. Monocyte migration toward autologous serum was increased in type IIa hyperlipidemic subjects compared to normolipidemic controls. n-3 supplementation inhibited migration toward autologous serum in the type II hyperlipidemic subjects but not in normolipidemic or in type IV hyperlipidemic subjects. n-3 PUFAs reduced monocyte chemotaxis in healthy volunteers in a dose-dependent manner even at the low dose of 1.3 g/day. In type IIa and IV hyperlipidemic patients, neutrophil migration was inhibited by approximately 10% after receiving fish oil supplementation. n-3 PUFAs reduced neutrophil chemotaxis in a dose-dependent manner even at the low dose of 1.3 g/day Plasma cholesterol levels in the hyperlipidemic subjects were unaffected by fish oil supplementation.
Schmidt et al., 1992	Supplementation	Healthy (mean age of 24 years, n=24)	Treatment group: 4 g of n-3 PUFAs/day (Jahres Fabrikker AS)	6 weeks and9 months		Neutrophil and monocyte chemotaxis toward autologous serum and formyl-methyl-leucyl-phenylalanine (FMLP).		NA	NA	Blood was from fasted individuals Fatty acid composition of the n-3 PUFA supplement was not directly noted. Fish oil supplementation: 1. Was well tolerated. 2. Reduced spontaneous neutrophilmigration at 9 months; reduced migration toward autologous serum but not FMLP at 6 weeks. 9 months of supplementation significantly inhibited directed neutrophil migration towardsboth autologous serum and FMLP. 3. Significantly reduced directed monocyte migration toward autologous and FMLP at 6 weeks and 9 months.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Schmidt et al., 1996	Randomized, double-blind, placebo-controlled	Healthy (n=32; 17 women with a mean age of 41 and 15 men with a mean age of 38)	Treatment Groups: 1. Placebo - ? 2. EPAX 5500 - 34% EPA, 3.5 % DPA, and 19% DHA as re-esterified triglycerides. Assuming that each capsule weighs 1 g, the approximate daily doses of EPA, DPA, and DHA were 340 mg, 35 mg, and 190 mg/day, respectively.	12 weeks		1. Fatty acid composition of monocytes. 2. In vitro monocyte chemotaxis toward autologous serum 3. Stimulus-induced production of LTB_4, IL-6, TNFα, or IL-6β, or chemiluminescence from monocytes (a measurement of superoxide production).		NA	NA	The placebo is not an appropriate control because its fatty acid composition differs beyond the absence of EPA, DPA, and DHA. Therefore comparison will only be made to baseline values. n-3 supplementation: 1. Significantly increased the amount of EPA in monocytes. DPA and DHA concentration also increased but the differences were not significant. 2. Had no significant effects on monocyte chemotaxis or ex vivo stimulus-induced production of LTB_4, IL-6, TNFα, or IL-6β, or chemiluminescence.
Seljeflot et al., 1998	Randomly allocated using two-by-two factorial, double-blind placebo controlled	Healthy men (41-57 years old; n=42, 22 participants in the EPA/DHA group and 19 in the placebo group)	Treatment Groups: 1. Placebo - ? 2. 4.8 g of EPA and DHA/day, 60% ethy ester	6 weeks	Some consumed less than 10 cigarettes/day;	1. Plasma cholesterol, HDL-cholesterol and triglyceride levels 2. Plasma phospholipid fatty acid composition 3. Plasma P-selectin, E-selectin, VCAM-1, tPAag, vWF, sTM.		NA	4.8 g of EPA and DHA(↑ plasma E-selectin and VCAM-1)	Although the study noted the amount of EPA and DHA in the n-3 fatty acid supplement, the exact composition of both the n-3 and placebo capsules was not noted, thus making it difficult to conclude anything about the specific contribution of EPA and DHA to the observed effects. One patient failed to complete the study because of the onset of a non-fatal myocardialinfarction. Blood was from fasted individuals. EPA/DHA consumption: 1. Significantly lowered serum triglycerides but did not affect HDLs or serum cholesterol (compared baseline and placebo). 1. Increased total n-3 fatty acids(EPA and DHA) and reduced total n-6 fatty acids (linoleic and arachidonic acid) 2. Significantly reduced von Willibrand factor (compared to baseline). 3. Significantly reduced thrombomodulin (compared to placebo). 4. Significantly increased sE-selectin and VCAM-1 (compared to placebo).

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Sperling et al., 1993	Supplementation	Healthy volunteers (n=8; and mean age of 43 years)	Treatment Groups: 1. Without supplementation 2. With supplementation - 20 g of SuperEPA (9.4 g EPA and 5 g DHA/day)	10 weeks		1. Neutrophil and monocyte chemotaxis 2. Receptor affinity and density 3. Inositol phosphate (IP)and diglyceride formation by LTB_4- and platelet activating factor (PAF)-stimulated neutrophils and monocytes 4. Formation of 5-lipoxygenase pathway products by calcium ionophore- and zymosan-stimulated neutrophils.	Neutrophil phospholipid composition	NA	NA	Seven volunteers experience mold gastrointestinal symptoms, including eructation, diarrhea, and occasional steatorrhea. None of the patients withdrew from the study. EPA and DHA supplementation: 1. Significantly reduced arachidonic acid and increased EPA contents in all neutrophil phospholipid pools. DHA was only increased in the alk-enylacyl-phosphatidylethanolaminepool. 2. Had no effect on the binding ofLTB_4 or PAF binding. 3. Significantly reduced the formation of IP1, IP2, and IP3 by LTB_4-and PAF-stimulated neutrophils but had no effect on diacylglycerol formation. 4. Significantly reduced the chemotaxis of neutrophils to LTB_4 and PAF. 5. Had marginal effects on phosphoinositide and diacylglycerol formation in monocytes. 6. Significantly reduced the arachidonic-derived 5-lipoxygenase pathway products by calcium and zymosan-stimulated neutrophils. Modestly but significantly increased EPA-derived LTB_5 from calcium ionophore- and zymosam-induced neutrophils. Similar results were also observed from calcium ionophore- and PAF-stimulated monocytes.
Sundrarjun et al., 2004	Randomized, double-blind, placebo-controlled	Patients with active rheumatoid arthritis Treatment groups: 1. n=14 2. n=23 3. n=23	Treatment Groups: 1. Control (no intervention) 2. Placebo 3. Fish oil (1.88 g EPA, 1.48 DHA)	6 weeks	All patients were receiving anti-rheumatic drugs for 3-months prior to the study	1. Serum fatty acid composition of 2. Serum levels of IL-6, TNFα, and sTNF receptor p 55 (TNFR-p55)		NA	1.88 g EPA, 1.48 DHA(↓TNFR-p55, TNFα, and IL-6)	Fish oil supplementation: 1. Significantly decreased the amount of serum linoleic acid, and significantly increased that amount of EPA and DHA. 2. Significantly decreased the amount of serum TNFR-p55, TNFα, and IL-6.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Thienprasert et al., 2009	Randomized, double-blind, placebo-controlled	Thai school children (9-12 years old; n=180).	Treatment Groups: 1. Placebo: Chocolate milk supplemented with soybean oil (2 g/day) 2. Fish oil: Chocolate milk supplemented with fish oil (200 mg EPA and 1 g DHA/day).	Subjects consumed either placebo or fish oil treatments 5 days a week for 6 months.		1. Fatty acid profile of plasma phospholipid phosphotidyl choline. 2. Plasma concentrations of sIL-2R, IL-6, IL-10, and TGFβ. 3. Episodes and duration of illness.		0.200 g EPA/1 g DHA/d (IL-6 and IL-10)	NA	The exact composition of the two oils was not noted. Blood was drawn from fasted individuals. Fish oil supplementation: 1. Significantly decreased linoleic and dihomo-γ-linolenic acid in plasma phosphotidyl choline fatty acids. 2. Significantly increased the EPA, DHA, and DPA in plasma phosphotidyl choline fatty acids. 3. Significantly reduced the episodes of illness per subject and total days of illness per subject. 4. Significantly increases in TGFβ were seen in both the placebo and fish oil supplemented groups although the increase was not as dramatic in the fish oil group. 5. Had no effect on plasma levels of sIL-2R, IL-6, and IL-10.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Thies et al., 2001a	Randomized, double-blind, placebo-controlled,	Healthy subjects (55-75 years old; n=48)	Treatment Groups: 1. Placebo - 80% palm and 20 % sunflower seed oil 2. α-linolenic acid -2.0 g/day 3. γ-linolenic acid -700 mg/day 4. Arachidonic acid 700 mg/day 5. DHA - 700 mg/day 6. Fish oil - 720 mg EPA and 280 mg DHA/day *All capsules contained 300 g α-tocopherol and 180 g ascorbyl-palmitate/g oil, equaling 1.2 mg α-tocopherol/day.	12 weeks		1. Fatty acid composition of peripheral blood mononuclear cell (PBMC) phospholipids. 2. Number of circulating lymphocytes and subpopulations. 3. Ex vivo lymphocyte proliferation and cytokine production.		0.7 g DHA/day and 0.7g EPA and 0.28 g DHA (number of circulating lymphocytes)	NA	All diets, including the placebo diet, also contained varying degrees of myristic, palmitic, palmitolei, stearic, oleic, and linoleic acid making it difficult to conclude anything about specific contributions of EPA and DHA to the observed effects. One subject in the γ-linolenic group dropped out due to illness and one subject dropped out of the fish oil group due to stomach upsets. Details of blood harvesting were <u>not</u> noted. 1. With the exception of α-linolenic acid diet, all other diets significantly increased the concentration of the respective fatty acid in the (PBMC) phospholipid pool; increases were treatment dependent and washouts reduced the concentrations of the different fatty acids, respectively. 2. Diets did not significantly affect the number of circulating lymphocytes. 3. γ-linolenic and fish oil diets significantly reduced concanavalin A (ConA)-induced proliferation ex vivo but did not affect ConA-induced IL-2 and IFN production. DHA diet also reduced ConA proliferation but not IL-2 or IFN production but the reduction was not statistically significant. 4. The reduction of ConA-induced proliferation appeared to be treatment dependent because the proliferative response increased after a four week washout. 5. Importantly, the anti-proliferative effects of the fish oil diet appear to be mediated by EPA because a diet containing approximately 2.5 times more DHA had no effect on ConA-induced lymphocyte proliferation.

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Thies et al., 2001b	Randomly allocated, double-blind, placebo controlled	Healthy subjects (<55 years old; n=48)	Treatment Groups: 1. Placebo - 80% palm and 20 % sunflower seed oil 2. α-linolenic acid -2.0 g/day 3. γ-linolenic acid -700 mg/day 4. Arachidonic acid -700 mg/day 5. DHA - 700 mg/day 6. Fish oil - 720 mg EPA and 280 mg DHA/day *All capsules contained 300 g -tocopherol and 180 g ascorbyl-palmitate/g oil, equaling 1.2 mg -tocopherol/day.	12 weeks		1. Fatty acid composition of plasma phospholipids. 2. Plasma concentrations of thiobarbituric acid reactive substances. 3. Number of circulating lymphocytes and subpopulations. 4. Ex vivo natural killer cell activity.		0.7 g DHA/day and 0.7g EPA and 0.28 g DHA (number of circulating lymphocytes)	0.7g EPA and 0.28 g DHA (↓ NK cell lytic activity)	All diets, including the placebo diet, also contained varying degrees of myristic, palmitic, palmitoleic, stearic, oleic, and linoleic acid making it difficult to conclude anything about specific contributions of EPA and DHA to the observed effects based on comparisons with the placebo group. One subject in the γ-linolenic group dropped out due to illness and one subject dropped out of the fish oil group due to stomach upsets. Details of blood harvesting were <u>not</u> noted. 1. Treatments did not significantly affect the habitual intake of fatty acids. 2. Treatments did not significantly affect thiobarbituric acid-reactive substances. 3. Treatments did not significantly affect the distribution or number of circulating leukocytes, lymphocytes (CD45+CD14-), T cells (CD3+), or NK Cells (CD3-CD56+CD16+). 4. Fish oil, but not a diet containing 3x the amount of DHA, significantly reduced NK cell lytic activity compared to baseline and placebo in a time dependent fashion. Furthermore the reduction was reversible, i.e. 4 weeks of washout reversed the reduction. However, the results of the cell lytic activity experiment may have been over-interpreted. The expectation is that if cells have reduced lytic activity, then differences would be more apparent at lower effector to target ratios. The experiment should that the effects are most dramatic using high amount of effectors.

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Trebble et al., 2003	Randomly assigned, double-blind	Healthy men (n=16)	Treatment Groups: 1. 1st week – 1 g fish oil/ day (0.2 g EPA, 0.024g DPA, and 0.1g DHA/ day) 2. 2nd week - 3 g fish oil/ day (0.624 g EPA, 0.072 DPA, and 0.303 g DHA/ day) 3. 3rd week- 6 g fish oil/day (1.2 g EPA, 0.144 g DPA, and 0.606g DHA)/day) *Importantly, capsules also contained modest amounts of linoleic, α-linolenic, and arachidonic acids.	4 weeks as each dose	Have the subject received a antioxidant preparation of 200 g Se, 3 mg Mn, 30 mg vitamin E, 450 g vitamin A, and 90 mg vitamin C	1. Fatty acid composition of plasma and erythrocyte phospholipids. 2. Concancavalin A (ConA)-induced peripheral blood mononuclear cells (PBMC) proliferation, prostaglandin E_2 (PGE_2), and cytokine production (IFNγ and IL-4).		NA	1.2 g EPA, 0.144 g DPA(↓PGE_2)	Because capsules contained modest amounts of n-6 PUFA, it is difficult to determine the specific effects of EPA, DHA, and DPA. No major side effects associated with the treatments. There was no statistical significance between the placebo and antioxidant group. Therefore, they were combine and the effects of EPA and DHA on immune parameters was investigated. 1. Diets reduced the amount of arachidonic acid and increased the amount of EPA and DHA in plasma phosphatidyl choline. Similar results were also seen in erythrocyte phosphatidylethanolamine and phosphatidylcholine 2. Diets dramatically reduced PGE_2 production from unstimulated and stimulated PBMC. 3. Diets lead to increased ConA-induced proliferation approximately 2-fold and IFN production 3-fold compared to baseline although background proliferation also increased 2-fold. 4. IL-4 production from stimulated cultures increased 2-fold but the increase was not significant.
Vaisman et al., 2005	Randomized, double-blind, placebo-controlled	Children (n=21; 8-12 years old)	Treatment Groups: 1. Placebo - 1 g of canola oil. 2. EPA/DHA (180 mg EPA and 120 mg DHA + 700 mg of canola oil)	12 weeks		1. Serum concentrations of TNFα and IL-6. 2. Spontaneous and lipopolysaccharide (LPS)-induced IL-6, TNFα, IL-1β, IL-10, and IL-1RA production from peripheral blood mononuclear cells (PBMC).		180 mg EPA and 120 mg DHA/day (plasma IL-6 and TNFα)	NA	EPA and DHA supplementation had no effect on plasma TNFα and IL-6 concentrations (compared to placebo). IL-6, IL-1β, IL-10, and IL-1RA were elevated after 24 hr of culture but the levels were dramatically less that those obtained with LPS stimulation.

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Varming et al., 1995	Supplementation	Healthy men and women (n=22; mean age of 40 years)	Treatment Groups: 1. Without supplementation. 2. With supplementation of 4 g of fish oil/day (re-esterified triglyceride)in the form of fish oil.	6 weeks		1. Platelet fatty acid composition 2. Neutrophil superoxide production		NA	NA	Blood was drawn after an overnight fast. n-3 PUFA supplementation: 1. Significantly increased the amount of EPA and reduced AA in the platelet fatty acids. 2. Signficantly reduced neutrophil superoxide production by 37%.
Varming et al., 1995	Randomized, placebo-controlled	Healthy (n= 24)	Treatment Groups: 1. Placebo – corn oil 2. Fish oil - 0.65 g of n-3 PUFAs/day(re-esterified triglyceride)	6 weeks		1. Neutrophil fatty acid composition 2. Neutrophil zymosan-induced superoxide production		NA	NA	The placebo is not an appropriate control because its fatty acid composition differs beyond the absence of EPA, DPA, and DHA. Therefore comparison will only be made to baseline values. Blood was drawn after an overnight fast. n-3 PUFA supplementation: 1. Increased the amount of EPA and reduced AA in the neutrophil fatty acids although the differences were not statistically significant. 2. Reduced neutrophil superoxide production, although the reduction was not statistically significant.
von Schacky et al., 1993	Blinded, Randomized supplementation	Healthy (mean age 28 years old; n=14)	Treatment Groups: 1. Without supplementation 2. With supplementation - fish oil concentrate (7g/day: 54.7 % EPA, 28.7% DHA, 5.4 % DPA, 2.4 % C20:4n-3, 2.3% 18:4n-3, 2.1% C21:5n-3 and 4.4% all other fatty acids. Total daily intake - approximately 3.8 g EPA and 2.0 g DHA	6 weeks		1. Fatty acid composition in granulocytes 2. The amount of cysteinyl-leukotrienes LTE_4, LTD_5, and LTE_5 from ex vivo stimulated granulocytes 3. Urinary amounts of LTE_4		NA	3.8 g EPA and 2.0 g DHA/d (↓ urinary LTE_4)	Fish oil supplementation: 1. Significantly increased EPA and DHA but had no effects on AA concentration in granulocytes. 2. Increased the amount of LTD_5 and LTE_5 but had no effect on LTE_4 from ex vivo stimulated granulocytes. 3. Reduced the amount of LTE_4 in the urine.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Woodman et al., 2003	Double-blind, placebo-controlled	Hypertensive ; type 2 diabetic men and postmenopausal women (40- 75 years old) Treatment Groups: 1. n=14 2. n=11 3. n=11	Treatment Groups: 1. 4 g Placebo (olive oil; 75% oleic acid)/day 2. 4 g EPA ethyl ester (96%)/day 3. 4 g DHA ethyl ester (92%)/day	6 weeks	Some patients were taking lipid-lowering drugs or anti-oxidant vitamins	1. Platelet phospholipid fatty acid. 2. Platelet aggregation to platelet activating factor (PAF). 3. Platelet release ofthomboxane (TBX_2) in response to collagen. 4. Plasma levels of glucose, insulin, glycated hemoglobin (HbA1c), tissue-plasminogen activator, PAI-1, P-selectin,, and von Willebrand factor (cWf). 5. Vascular function of the brachial artery.		4 g EPA ethyl ester/day and 4 g DHA ethyl ester/day (tPA, PAI-1, vWF, or P-selectin)	NA	The placebo is not an appropriate control because the fatty acid composition differs from the EPA or DHA supplements. Therefore, only comparisons to baseline will be made. Blood was harvested from fasted individuals. 1. EPA supplementation increased platelet phospholipid fatty acid EPA (400%) and DPA (59%) levels and reduced DHA (26%) and arachidonic acid (18%). 2. DHA supplementation increased platelet phospholipid fatty acid DHA (121%) and EPA (53%) and reduced DPA (33%) and arachidonic acid (7%). 3. Collagen-induced platelet aggregation was significantly reduced in the DHA-supplemented group. 4. DHA supplementation resulted in an significant reduction in platelet-derived TXB2. 5. There were no significant effects of any of the supplementations on the plasma levels of tPA, PAI-1, vWF, or P-selectin. 6. There were no significant effects of any of the supplements on vascular function.

Table 4. Effects of n-3 fatty acids on immune and inflammatory parameters

Study	Study Design	Patients/ subjects (n)	Treatment and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/day)	Effect Level (g/day)	Additional Notes
Yaqoob et al., 2000	Randomized, double-blind, placebo-controlled	Healthy Caucasians (n=8/group)	This study evaluated the effects of a variety of oils. The effects of only fish oil will be evaluated. Treatment Groups: 1. Placebo - 3:1 mixture of coconut and soybean oil 2. Fish oil - 2.1 g EPA and 1.1 g DHA/day	4 weeks followed by an 8 week washout		1. Fatty acid composition of plasma phospholipids. 2. Cell surface expression of CD7, CD21, CD4, CD8, CD64, CD16, CD2, CD54, and CD11b. 3. NK cell killing activity. 4. ConA-induced proliferation of PBMC. 5. Concancavalin A (ConA)- and lipopolysaccharide (LPS)-induced cytokine production.		2.1 g EPA and 1.1 g DHA/day (cell surface expression ofCD7, CD21, CD4, CD8, CD64, CD16, CD2, CD54, CD11b, NK cell lytic activity)	NA	The placebo is not an appropriate control because its fatty acid composition differs from fish beyond that of being devoid of EPA and DHA. Furthermore, because fish oil contains other fatty acids it is difficult to conclude anything about the specific contributions of EPA and DHA. Fish oil supplementation: 1. Significantly reduced dihomo-γ-linolenic acid and increased the amount of EPA and DHA in plasma phospholipids. Arachidonic acid was reduced following fish oil supplementation but the reduction was not statistically significant. 2. Significantly increased plasma α-tocopherol concentrations but had no effect on TBARS and total plasma antioxidant activity. 3. Significantly reduced arachidonic acid and increase EPA and DHA levels in PBMCs. 4. Had no effect on the cell surface expression of any of the markers tested, NK cell lytic activity, ConA-induced whole blood and PBMC proliferation, or ConA- or LPS-induced cytokine production.

Notes:
1. NA = not applicable. Unless otherwise noted, ex vivo studies have not been included in the "No Effect Level" and "Effect Level" columns because their relevance to immune homeostasis, immune responses, and inflammatory responses is unknown.
2. Abbreviations: FMLP(N-Formyl-Met-Leu-Phenylalanine), LPS (lipopolysaccharide), LTB_4 (leukotriene B_4), 5-HETE (5-hydroxyeicoatetraenoic acid), PHA (Phytohaemagglutinin), PBMC (peripheral blood mononuclear cells), PGE_2 (prostaglandin E_2), PUFA (polyunsaturated fatty acids), TNFα (tumor necrosis factor α), IL (interleukin), TXB_2 (thromboxane B2), AA (arachidonic acid), EPA (eicosapentanoic acid), DHA (docosahexanoic acid), DPA (docosapentaenoic acid). tPA (tissue plasminogen activator), PAF (platelet activating factor), VCAM-1 (vascular adhesion molecule 1), ICAM-1 (intercellular adhesion molecule 1), LFA-1 (lymphocyte function-associated antigen 1), NK (natural killer) , ConA (concanavalin A), CRP (C-reactive protein), IFN (interferon), IL-1RA (interleukin 1 receptor antagonist), PMA (phorbol 12-myristate 13-acetate).

CHAPTER 4:THE EFFECTS OF EPA AND DHA CONSUMPTION ON DIABETES

Background

Diabetes Mellitus is a group of disorders that share a common phenotype, hyperglycemia (for review see Kasper and Harrison, 2005). The factors that contribute to hyperglycemia include decreased glucose utilization, increased glucose production, and reduced insulin secretion. Although the ability to effectively deal with high levels of blood glucose can be lost for a variety of reasons, people with diabetes are classified into two primary groups, based on the pathogenesis of disease. In type 1 diabetics, hyperglycemia results from insulin deficiency, usually due to the destruction of the β-cells of the pancreas. Type 1 diabetes is also known as insulin-dependent diabetes mellitus (IDDM). In type 2 diabetics, the cause of hyperglycemia is less defined and encompasses varying degrees of insulin resistance, impaired insulin production, and increased glucose production. Type 2 diabetes is also known as non-insulin dependent diabetes mellitus (NIDDM). Other types of diabetes are the result of pregnancy (gestational diabetes) or genetic abnormalities that impair insulin secretion or action, metabolic abnormalities that impair insulin secretion, mitochondrial abnormalities, and glucose tolerance. Importantly, diabetes is usually associated with other complications such as dyslipidemia, cardiovascular disease, and renal failure. Also, affected individuals are usually treated with one or more therapies to restore blood sugar and alleviate symptoms associated with the other complicating disorders. Thus, studies involving diabetic patients must be interpreted cautiously due to the presence of numerous confounding factors.

In the clinic, diabetes is diagnosed based on the presence of symptoms associated with diabetes plus a random blood glucose concentration of greater or equal to 11.1 mm/L (200 mg/dL), fasting plasma glucose (FPG) levels greater or equal to 7.0 mmol/L (126 mg/dL), and blood glucose concentrations greater that 11.1 mmol/L (200 mg/dL) after a glucose tolerance test. Although FPGs and glucose tolerance tests are not mutually exclusive, individuals with impairments in either are at a greater risk of developing type 2 diabetes. Elevated levels of glycated hemoglobin A1c (HbA1c) are also indicators of diabetes but their predictive capacity is less definitive because HbA1c can be elevated in subjects that have normal glucose control or mild glucose intolerance. HbA1c measurements can also vary from method to method, are affected by certain types of therapies, such as transfusions, and other complications such as anemias and reticulocytosis. Accurate HbA1c assessments therefore require baseline

measurements for comparison, careful consideration of confounding factors, and are more frequently used to evaluate glycemic control greater than 2 to 3 months.

Studies investigating the effects of fish oils and EPA/DHA on glucose metabolism-related endpoints in diabetic patients cited by VKM (2011) and BfR (2009) reports, the review articles and meta-analyses by Friedberg et al., 1998, Montori et al., 2000, Balk et al., 2004, MacLean et al., 2004, De Caterina et al., 2007, Hartweg et al., 2008, Hartweg et al., 2009, Hendrich et al., 2010, Martin de Santa Olalla et al., 2009, and found in publicly available databases are included in this review. Studies not in diabetic patients were excluded from this analysis.

Results

Twenty-eight studies were retrieved that assessed the impact of consumption of EPA- and DHA-rich oils on glucose homeostasis in type 1 and type 2 diabetics (Table 5) (Annuzzi et al., 1991; Boberg et al., 1992; Bonnema et al., 1995; Connor et al., 1993; De Luis et al., 2009; Friday et al., 1989; Garg et al., 2007; Glauber et al., 1988; Hendra et al., 1990; Kabir et al., 2007; Lungershausen et al 1997; Luo et al., 1998; McGrath et al., 1996; McManus et al., 1996; Morgan et al., 1995; Pelikanova et al., 1993; Petersen et al., 2002; Pooya et al., 2010; Puhakainen et al., 1995; Schectman et al.1988; Shimizu et al 1995; Sirtori et al., 1997; Sirtori et al., 1998; Stirban et al., 2010; Tariq et al., 1989; Westerveld et al., 1993; Zambon et al., 1992; Woodman et al., 2002).

Results from these studies indicate that intake of these fatty acids below 3 g/day consistently had no effect on fasting blood glucose levels (Annuzzi et al., 1991; Boberg et al., 1992; De Luis et al., 2009; Garg et al., 2007; Holman et al 2009: Kabir et al., 2007; Luo et al., 1998; McManus et al., 1996; Petersen et al., 2002; Pooya et al., 2010; Shimizu et al 1995; Sirtori et al., 1997; Sirtori et al., 1998; Stirban et al., 2010) and the consumption of ≥ 3 g/day increased fasting glucose, but the increases were not always statistically significant or completely reproducible (Bonnema et al., 1995; Conner et al., 1993; Glauber et al., 1988, Hendra et al., 1990; Lungershausen et al., 1997; McGrath et al., 1996; Morgan et al., 1995; Pelikanova et al., 1993; Puhakainen et al., 1995; Zambon et al., 1992; Schectman et al.1988; Woodman et al., 2002; Friday et al., 1989).The greatest increases in fasting blood glucose levels (approximately 20%) were seen in patients that consumed 5.5 and 7.5 g/day and were usually associated with glucose intolerance and/or increases in HbA1c levels (Friday et al., 1989;Glauber et al.,

1988;Schectman et al., 1988), although two studies found that the consumption of fish oil delivering 6 and 11 g/day of n-3 fatty acids had no effect on both fasting plasma glucose and HbA1c levels (Conner et al., 1993; Morgan et al., 1995). Importantly, all of the studies assessing the effects of consuming ≥ 3 g/day were confounded by n's≤40, and many involved patients taking anti-diabetic therapies.

Two of 12 reports found that the consumption of EPA- and DHA-rich oils by type 2 diabetics reduced meal-induced insulin levels without affecting fasting levels of insulin (Glauber et al., 1988;Zambon et al., 1992). However, these findings are not conclusive because adverse impacts on insulin sensitivity were not consistently or reproducibly found in the other ten studies reviewed. Also, both studies were confounded by n's ≤ 10 and involved patients taking anti-diabetic therapies.

Nine meta-analyses found that the consumption of EPA- and DHA-rich oils at intakes less than 10 g/day did not affect fasting glucose, HbA1c, plasma insulin, or insulin resistance (Friedberg et al., 1998, Montori et al., 2000; Balk et al., 2004; MacLean et al., 2004; De Caterina et al., 2007; Hartweg et al., 2008, Hartweg et al., 2009, Hendrich et al., 2010; Martin de Santa Olalla et al., 2009).

Conclusions

Consumption of EPA- and DHA-rich oils at intakes up to 3 g/day of n-3 fatty acids consistently had no effect on glucose control or insulin production and studies delivering less than 11 g/day of n-3 fatty acids does not appear to reproducibly impair glucose control or insulin production. However, due to the presence of multiple confounding variables across studies, additional work on larger groups of diabetic patients should be performed to evaluate the effect of n-3 fatty acids on glucose control.

Table 5.Effects of omega-3 fatty acids on glucose homeostasis in diabetic patients

Study	Study design	Patients / subjects (n)	Product form and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/d)	Effect Level (g/d)	Additional notes
Annuzzi et al., 1991	Double blind, randomized, placebo controlled crossover	16 patients with type 2 diabetes	10 g (1g per capsules) per day of either fish oil (MaxEPA contained 1.8 g EPA and 1.2 g DHA) or olive oil	2 weeks x 2 (no washout period)	Glibenclamide and metformin	Lipid and glucose metabolism	Fasting blood glucose	3g/d		Hypoglycemic therapy was continued throughout the study without dosage adjustments. Fish oil supplement did not induce significant changes in fasting blood glucose and average daily blood glucose. Glucose stimulated plasma insulin response was not significantly influenced by fish oil. Insulin sensitivity was also unchanged. No differences in lipid and glucose responses were observed between the subjects who started with fish oil and those who started with the placebo (two way analysis of variance).
							Insulin response & sensitivity	3g/d		
Boberg et al., 1992	Double blind, randomized, placebo controlled crossover	14 patients with type 2 diabetes under dietary treatment Treatment group ss: Placebo (n=9) MaxEPA (n=5)	10 g (1g per capsules) per day of either fish oil (MaxEPA contained 1.8 g EPA and 1.2 g DHA) or olive oil	8 weeks x 2 (no washout period)	13 patients were on hypoglycaemic drugs (unchanged throughout the study). Only, one patient was on dietary treatment only	Lipid and glucose metabolism and fibrinolysis	Fasting blood glucose, HbA1c	3g/d		Statistically significant increases in fasting blood glucose was seen in both the placebo and EPA/DHA treated groups. Although EPA/DHA treated group had a statistically significant increase in HbA1c, the increase was more dramatic compared to placebo. There were no differences in insulin usage or intravenous glucose tolerance tests between the placebo and EPA/DHA treated groups.
							Blood insulin and insulin sensitivity	3g/d		
Bonnema et al., 1995	Double blind, randomized, placebo controlled parallel	28 patients with type 1 diabetes. Fish oil (n=14), olive oil (n=14)	6x1g/day capsules of fish oil (Pikasol® providing 2.01g EPA and 1.32g DHA) or olive oil	6 months	Insulin	Peripheral vascular compliance	Fasting blood glucose		3.3g/d	Fish oil increased fasting blood glucose compared to olive oil (baseline; end of trial: fish oil: 8.8±4.5; 9.2±4.1, olive oil: 10.7±4.5; 8.6±4.9 (mmol/l±SD), *p<0.05).* HbA1c was lowered in both groups.
							HbA1c	3.3g/d		
Connor et al., 1993	Double blind, randomized, placebo controlled crossover	16 patients with type 2 diabetes and hypertriglyceridemia	15 g/d of either olive oil or fish oil (Promega®). The fish oil contains 4.1 g EPA and 1.9g DHA	6 months x 2 (no washout period)	Hypoglycemic agents and insulin	Plasma lipids, lipoproteins and glucose control	Fasting plasma glucose	6g/d		Although not mentioned, we assume that patients did not adjust medications. Fasting plasma glucose levels, glycosylated hemoglobin, and C-peptide concentrations were the same after fish oil and olive oil. Likewise, 24-hour urinary glucose and C-peptide were similar after fish oil and olive oil.
							HbA1c	6g/d		

Table 5.Effects of omega-3 fatty acids on glucose homeostasis in diabetic patients

Study	Study design	Patients / subjects (n)	Product form and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/d)	Effect Level (g/d)	Additional notes
De Luis et al., 2009	Open label without a control group	30 type 2 diabetic patients with hyper-triglyceridemia.	2 capsules per day to provide 0.93 g EPA and 0.75 g DHA.	12 weeks	40% patients were treated with insulin and 60% patients were on hypoglycemic drugs (unchanged throughout the study).	Triglyceride levels and inflammatory markers	Fasting blood glucose and insulin	1.68g/d		Triglycerides levels and non-HDL cholesterol decreased (326±113.5 vs. 216.4±57 mg/dl; p<0.05 and 103.87±44 vs. 89.6±14 mg/dl; p<0.05 respectively). HDL-cholesterol levels increased (39.6±10.7 vs. 46.4±8.7 mg/dl; p<0.05). C reactive protein decreased (5.98±3.9 vs. 3.9±1.6 mg/dl; p<0.05).There were no changes in fasting glucose and insulin
Friday et al., 1989	Open label without a control group	8 type 2 diabetic patients not treated with insulin or sulfonylurea	15 marine-lipid concentrate capsules (RES-Q1000). Each capsule contains 1g methyl ester fatty acids providing 0.2g DHA and 0.3g EPA	8 weeks	Not treated with insulin or sulfonylurea but are receiving medications for diabetes control	Lipoprotein and glycemic control	Fasting and meal-induced plasma glucose		7.5g/d	Mean fasting plasma glucose levels increased 22% (p=0.005) and meal-stimulated glucose increased 35% (p=0.036). No significant changes were seen in fasting or meal-stimulated plasma insulin, glucose disposal, insulin-to-glucagon ratios, or HbA1c levels.
							Plasma insulin, HbA1c	7.5g/d		
Garg et al., 2007	Open label without a control group	13 type 2 diabetic patients	All participants consumed 100 g/day of long-chain n-3 PUFA-enriched dip. This dip provided 1.3–1.4 g long-chain n-3 PUFA (~0.54 g EPA, 0.59g DHA and 0.075 DPA)	6 weeks	No medication changed	Plasma lipid profile	Fasting glucose and HbA1c	1.2g/d		Both LDL and HDL were increased, triglycerides were decreased. There was no effect on fasting glucose or HbA1c compared with baseline
Glauber et al., 1988	Open label without a control group	6 type 2 diabetic patients	18 g/d MaxEPA, providing 3.3g EPA and 2.2d DHA	4 weeks	No medication except for ferrous sulfate	Fasting and meal-induced plasma glucose levels, and fasting and meal induced insulin and glucagon levels			5.5g/d	After 1 month of supplementation, fasting glucose rose from 13.1 ± 1.3 to 15.3 ±1.3 mmol/L (P = 0.03) and glucose area during a mixed meal profile rose by 22% (P = 0.04). Basal hepatic glucose output rose from 97 ± 9 to 122 ± 8 mg/m^2 min (P = 0.004) but glucose disposal rates were unchanged. Fasting insulin levels were similar; peak insulin levels stimulated by meals or intravenous glucagon fell by 30% and 39%, respectively.
Hendra et al., 1990	Double blind, randomized, placebo controlled parallel	Eighty type 2 diabetic patients. MaxEPA arm (n=40), olive oil arm (n=40)	10x1g/day capsules of MaxEPA (providing 1.8g EPA and 1.2g DHA) or olive oil (control)	6 weeks	Sulfonylureas, biguinides	Hemostatic function and fasting lipid and glucose levels	Fasting blood glucose		3g/d	Fasting plasma glucose increased after 3 wk (P=0.01) but not after 6 wk (P = 0.17) treatment with MaxEPA. There were no significant changes in HbA1c in either treatment group
							HbA1c	3g/d		

Table 5.Effects of omega-3 fatty acids on glucose homeostasis in diabetic patients

Study	Study design	Patients / subjects (n)	Product form and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/d)	Effect Level (g/d)	Additional notes
Holman et al., 2009	Double blind, randomized, placebo controlled parallel	800 type 2 diabetic patients without known CVD and not taking lipid-lowering therapy. Atorvastatin and n-3 EE90 (n=200), Atorvastatin and placebo (n=201), placebo and n-3 EE90 (n=197), placebo and placebo (n=202)	2g/d n-3 EE90 (Omacor) to provide 0.92g EPA and 0.76g DHA or olive oil	4 months		Estimated cardiovascular disease risk	HbA1c	1.7g/d		Decreased triglycerides and no effect on HbA1c or cardiovascular disease risk compared with placebo
Kabir et al., 2007	Double blind, randomized, placebo controlled parallel	26 type 2 diabetic patients without hypertriglyceridemia. Fish oil (n=12), placebo (n=14)	3 g/d of either fish oil (providing 1.08g EPA and 0.72g DHA) or placebo (paraffin oil).	2 months	23 patients were taking oral hypoglycemic treatments, biguanides with or without sulfonylureas. No insulin was used.	Adiposity, insulin sensitivity, adipose tissue	Fasting glucose, HbA1c, insulin and insulin sensitivity	1.8g/d		Fasting plasma glucose, insulin, insulin sensitivity and HbA1c were not significantly influenced by fish oil treatment compared with placebo Plasma triacylglycerol (P=0.03), the ratio of triacylglycerol to HDL cholesterol (atherogenic index, P=0.03), and plasma plasminogen activator inhibitor-1 (*P*=0.01), were lower in the fish oil group than in the placebo group.
Lungershausen et al 1997	Double blind, randomized, placebo controlled parallel	Diabetics (type 1 or type 2) with microalbuminuria. Omacor arm (n=16), corn oil arm (n=16)	4x1g capsules per day of either Omacor (providing 2g EPA and 1.4g DHA) or corn oil	12 weeks	Diabetic medications. preexisting levels of medication remained constant throughout the study	Micro albuminuria	Fasting blood glucose, HbA1c	3.4g/d		Fasting glucose rose slightly (not significant) with the fish oil supplementation but not in the corn oil group. There was a slight rise (not significant) in plasma HbA1c concentration over the 12-week period in both groups.
Luo et al., 1998	Double blind, randomized, placebo controlled crossover	12 type 2 diabetic patients. It was assumed that 6 patients in each group	6x1 g capsules of either fish oil (providing 1.08g EPA and 0.72g DHA) or sunflower oil.	2 months x3 (separated by a 2 month washout)	Sulfonylurea, metformin, no insulin treatment.	Glucose metabolism and lipid profile	Fasting glucose, insulin and HbA1c	1.8g/d		2 months of fish oil supplementation compared with sunflower oil led to similar fasting plasma insulin, glucose, and HbA1c. Basal hepatic glucose production did not increase after fish oil. There was no difference in insulin suppression of hepatic glucose production or in insulin stimulation of whole-body glucose disposal.
McGrath et al., 1996	Double blind, randomized, placebo controlled crossover	23 type 2 diabetic patients	10x1g capsules per day of either MaxEPA (providing 1.8g EPA, 1.2g DHA and0.3g DPA) or olive oil	6weeks x 3. Six week washout period included	Diabetic control was achieved by diet alone or diet plus sulphonylurea or biguanide preparations or both.	Peroxidation of serum lipids	Fasting glucose, HbA1 and LDL	3.3g/d		Change in glycemic control as assessed by glycosylated hemoglobin and LDL was not seen in either group. A slight rise (failed to reach significance) in fasting blood glucose was seen in both groups compared with base line, but no difference was seen between two groups.

Table 5.Effects of omega-3 fatty acids on glucose homeostasis in diabetic patients

Study	Study design	Patients / subjects (n)	Product form and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/d)	Effect Level (g/d)	Additional notes
McManus et al., 1996	Double blind, randomized, placebo controlled crossover	11 type 2 diabetic patients	Olive oil (run in period to provide 35 mg/kg/d 18:1 fatty acid), linseed oil (providing 35 mg/kg/d 18:3 n=3), fish oil (providing 35 mg/kg/d EPA+DHA equivalent to 2.8g/d n-3 fatty acids)	3 months olive oil followed by 3 months x 2 of fish oil or linseed oil (cross over) with no wash out period	Four individuals were taking oral sulfonylurea agents at constant dosage	Fasting blood glucose		2.8g/d		HbA1c and lipid values were within the normal range at randomization. Repeated measures analysis of variance testing found no significant differences in weight; fasting glucose and insulin levels; HbA1c; total, LDL, and HDL cholesterol levels; insulin sensitivity, glucose effectiveness and acute insulin response to glucose with either active oil. Fish oil was associated with significant reductions in triglycerides and a trend toward decreased insulin sensitivity.
						HbA1c		2.8g/d		
						Insulin		2.8g/d		
Morgan et al., 1995	Double blind, randomized, placebo controlled parallel	40 type 2 diabetic patients and hyperlipidemia. There were 4 arms, 9g fish oil, 18g fish oil, 9g corn oil and 18g corn oil arms. 10 patients per arm.	Capsules to provide 9g or 18g of fish oil or 9g or 18g of corn oil per day. The fish oil contains 28.8% EPA, 27.3% DHA and 5.1% DPA.	12 weeks	Diabetic control was achieved by diet alone (n=4) or oral agents (n=14) or insulin (n=22)	Blood lipids and glycemic control	Fasting plasma glucose and HbA1c	11g/d		The level of oil supplements (9 g compared with 18 g) did not have a significant effect within each oil group on glycemic control and lipids. No significant differences between fish oil- or corn oil-supplemented diets were found in fasting plasma glucose and HbA1c.
Mostad *et al.*, 2006	Double blind, randomized, placebo controlled parallel	type 2 diabetic patients without hypertriglyceridemia. Fish oil arm (n=12), corn oil arm (n=14)	17.6 ml/d fish oil/d (providing 1.8g EPA and 3g DHA or 5.9g total n-3 PUFAs) or 17.8 ml corn oil/d	9 weeks	Continued on anti-diabetic medications except insulin	Insulin sensitivity, lipoprotein				This study is uninterpretable because the data do not match the conclusions drawn by the authors.
Pelikanova et al., 1993	Randomized, placebo controlled parallel	20 mildly obese men with type 2 diabetes, treated with hypoglycemic agents. Fish oil arm and saline solution arm. 10 patients per arm.	15 ml/day fish oil (Martens Oil to provide 3.1g n-3 fatty acids) or saline solution added to a basic diet.	3 weeks	Glybenclarnid (10 mg daily)	Fasting blood glucose, meal-stimulated glucose; insulin		3.1g/d		Fish oil did not alter fasting or mixed meal stimulated blood glucose, plasma insulin, and C-peptide concentrations. No changes in insulin action were noted

Table 5.Effects of omega-3 fatty acids on glucose homeostasis in diabetic patients

Study	Study design	Patients / subjects (n)	Product form and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/d)	Effect Level (g/d)	Additional notes
Petersen et al., 2002	Double blind, randomized, placebo controlled parallel	42 moderately hypertriglyceridemic type 2 diabetic patients Fish oil (n=20) or corn oil (n=22)	4x1g capsules of fish oil providing 1.608g EPA, 1.016g DHA and 0.244 DPA or corn oil	8 weeks	Diabetic treatments including tablets, insulin and diets	Fasting lipid profile, including LDL and HDL subclasses	Fasting blood glucose and HbA1c	2.9g/d		The authors observed no significant changes in blood glucose, HbA1c, or blood pressure.
Pooya et al., 2010	Double blind, randomized, placebo controlled parallel	81 type 2 diabetic patients. Fish oil group (n=40), sunflower oil group (n=41)	3 capsules per day of fish oil providing a total of 2714 mgn-3 fatty acids (1548 mg EPA and 828 mg DHA) or 2100 mg sunflower oil	2 months	Oral hypoglycemic agents, metformin and glibenclamide	Plasma homocysteine and malondialdehyde levels	Fasting glucose and HbA1c	2.4g/d		HbA1c decreased by 0.75% in the treatment group and increased by 0.26% in the control group. However, the changes in fasting blood sugar, malondialdehyde, C-reactive protein, total cholesterol and LDL-cholesterol levels were not significant.
Puhakainen et al., 1995	Double blind, randomized, placebo controlled crossover	9 patients who were obese and with type 2 diabetes	12 capsules (1g/each) per day of either MaxEPA (providing 2.16g EPA+1.44gDHA or a total of 3.84g n-3 fatty acids) or placebo (containing 6g corn oil and 6g olive oil)	6 weeks x 2 (no washout period)	No insulin treatment. 7 patients were taking oral anti-diabetic agents (glyburide with or without metformin)	Lipolysis, glycerol gluconeogenesis, and fatty acid oxidation.	Fasting plasma glucose	3.6g /d		Serum triglycerides decreased by 30% during n-3 fatty acid supplementation. Glycerol gluconeogenesis increased by 32%. However, overall glucose production, glycemic control, and fatty acid oxidation remained unchanged.
							Serum free insulin	3.6g /d		
							HbA1c	3.6g /d		
Schectman et al., 1988	Single-blind, randomized, placebo controlled crossover	13 type 2 diabetic patients.	Crossover phases (12x1g capsules of either MaxEPA providing 2.6g EPA and 1.4g DHA or safflower oil). All patients took 15x1g capsules of OmegaCaps providing 5g EPA and 2.5g DHA	1 month x 3 (one month washout) followed by 1 month high dose fish oil	Treatment for type 2 diabetes consisted of sulfonylureas in 9 patients, insulin in 2, and diet alone in 2.	Lipoprotein composition	HbA1c	4g/d	7.5g/d	7.5 g of EPA/DHA significantly increased fasting glucose levels compared to placebo. HbA1c levels were increased compared to baseline but were not different than the placebo group. 4 g of EPA/DHA reduced oral glucose tolerance test compared to baseline but there was no significant difference compared to placebo. The effect of 7.5 g of EPA/DHA on oral glucose tolerance was not tested.
							Fasting glucose levels and meal-induced glucose utilization	4g/d	7.5g/d	
Shimizu et al 1995	Randomized, controlled (did not specify if it was a placebo)	45 type 2 diabetic patients. Ethyl ester EPA group (n=29), control group (n=16)	Capsules providing either 0.9g ethyl ester EPA or control. Type of control was not mentioned	12 months	Treatment for type 2 diabetes consisted of sulfonylureas in 25 patients, insulin in 17, and diet alone in 3.	Albuminuria	Fasting plasma glucose	0.9g/d		EPA-E administration did not affect blood pressure levels, glycemic control and lipid metabolism in these patients.
							HbA1c	0.9g/d		

Table 5.Effects of omega-3 fatty acids on glucose homeostasis in diabetic patients

Study	Study design	Patients / subjects (n)	Product form and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/d)	Effect Level (g/d)	Additional notes
Sirtori et al., 1997	Double blind, randomized, placebo controlled parallel	935 Patients with hypertriglyceridemia with and without glucose intolerance or diabetes. Esapent arm (n=470, 44% with type 2 diabetes and 11% with glucose intolerance), olive oil arm (n=465, 45% with type 2 diabetes and 11% with glucose intolerance)	n-3 Ethyl esters. In the first 2 months, patients received Esapent (providing 1.53g EPA and 1.05g DHA) or olive oil. Dose reduced to 2 capsules/day of Esapent (providing 1.02g EPA and 0.7g DHA) or olive oil up to 6th month	6 months	Dose schedule of oral hypoglycemic or antihypertensive medications was allowed during the 6 month of controlled investigation. Type 2 diabetics treated with insulin were excluded.	Diabetic risks	Fasting blood glucose, HbA1c or insulin	1.72-2.58 g/d		This study did not find an effect of EPA+DHA on fasting glucose, HbA1c or insulin in patients with type 2 diabetes. Additionally, the total area under the oral-glucose-tolerance curve was not changed in patients having impaired glucose tolerance.
Sirtori et al., 1998	Double blind, randomized, placebo controlled parallel followed by open label	Patients with hypertriglyceridemia, associated with additional cardiovascular risk factors, i.e. glucose intolerance, type-2 diabetes (~44-43% of patients) and/or arterial hypertension. Double blind phase, ESAPENT (n=470), placebo (n=465). Open phase, ESAPENT (n=442), placebo (n=426).	Double blind phase: 3 capsules per day of ESAPENT (ethyl ester) providing 1.53g EPA and 1.05g DHA or olive oil for the 1st 2 months followed by 2 capsules per day of ESAPENT providing 1.02 EPA and 0.7g DHA or olive oil. Open label phase: all patients took 2 capsules per day of ESAPENT providing 1.02 EPA and 0.7g DHA	6 months x2	Some type 2 diabetic patients took pharmacological treatment excluding insulin	Lipid and glycemic alteration	Fasting blood glucose, HbA1c and insulin	2.6g/d		Type 2 diabetic patients did not display any changes of fasting glucose, HbA1c and insulinemia after 1 year of treatment with n-3 fatty acids (ethyl ester) or after 6 months in the group with prior placebo.
Stirban et al., 2010	Double blind, randomized, placebo controlled crossover	34 type 2 diabetic patients	2x1g capsules of ethyl esters of n-3 fatty acids (Omacor) providing 0.92g EPA and 0.76g DHA or olive oil (placebo)	6 weeks x 3 (there was a 6 weeks wash out period)	Oral anti-hyperglycemic agents, insulin	Postprandial macro- and microvascular function	Fasting HbA1c, postprandial AUC for glucose and insulin	1.68g/d		There was no difference in the postprandial AUC for glucose and insulin between treatments. Fasting HbA1c was not affected by either therapy.
Tariq et al 1989	Randomized, placebo controlled	8 patients (newly diagnosed). MaxEPA arm and olive oil arm. 4 patients per arm.	20g/d MaxEPA providing 3.6g EPA and 2.4g DHA or olive oil.	9 months	Insulin	Remission of type 1 diabetes	Mean blood glucose and HbA1c	6g/d		The authors suggested that long-term supplementation of a diabetic diet with MaxEPA does not affect the autoimmune process of newly diagnosed type 1 diabetics
Westerveld et al. 1993	Double blind, randomized, placebo controlled parallel	Type 2 diabetic patients. Two EPA arms (n=8 in each) and one placebo arm (n=8).	6 capsules providing 1.8g/d MND21, or 0.9g/d MND21 plus 0.828g olive oil or 1.656g/d olive oil (placebo). MND21 contains 93.6% ethyl ester-EPA	8 weeks	Insulin, diabetic drugs or diet control	Safety factors including glycemic control, lipid, lipoprotein, platelet aggregation.	Blood glucose,	1.8g/d		1.8g/d was the highest dose level tested.
							HbA1	1.8g/d		

Table 5.Effects of omega-3 fatty acids on glucose homeostasis in diabetic patients

Study	Study design	Patients / subjects (n)	Product form and Intake	Duration	Concomitant medications, confounders	Primary Endpoints	Secondary Endpoints	No Effect Level (g/d)	Effect Level (g/d)	Additional notes
Woodman et al., 2002	Double blind, randomized, placebo controlled parallel	Type 2 diabetic patients with treated hypertension. EPA arm (n=17), DHA arm (n=18), Placebo (olive oil) arm (n=16)	Capsules containing 4 g ethyl ester EPA (~96% pure), ethyl ester DHA (~92% pure) or olive oil.	6 weeks	Oral hypoglycemic medications. No insulin therapy. Patients who were taking lipid-lowering drugs, aspirin (on a regular basis), or antioxidant vitamins were not excluded.	Glycemic control, blood pressure, and serum lipids	Fasting blood glucose		4 g/d	In comparison with the change from baseline in fasting glucose in the olive oil group, fasting glucose in the EPA and DHA groups increased 1.40±0.29 mmol/L (P = 0.002) and 0.98±0.29 mmol/L (P = 0.002), respectively. Neither EPA nor DHA had significant effects on glycated hemoglobin, fasting insulin or C-peptide, insulin sensitivity or secretion, or blood pressure.
							HbA1c	4g/d		
							Fasting insulin or C-peptide	4g/d		
							Insulin sensitivity or secretion	4g/d		
Zambon et al., 1992	Randomized, crossover study. Patients without taking glyburide are considered as a control.	Ten men with type 2 diabetes treated with either sulfonylureas plus diet or diet alone.	15 marine lipid capsules (Super EPA) per day, providing 7.5g EPA+DHA (or 8g n-3 fatty acids). Each capsule contains 1g ethyl esters fatty acids (0.2g DHA and 0.3g EPA).	8 weeks (4 weeks with glyburide, 4 weeks without glyburide)	Patients treated with diet or diet plus sulfonylureas (discontinued during the trial) were recruited. Glyburide was taken during the trial.	Glucose and lipid metabolism	Fasting glucose		7.5g/d	Compared with glyburide alone, fasting plasma glucose concentrations increased with fish oil. Although glyburide with fish oil decreased fasting glucose concentrations, they did not return to baseline. Basal insulin concentrations were unaltered by fish oil without or with glyburide; however, postprandial insulin concentrations were decreased by fish oil.
							Baseline insulin	7.5g/d		

AUC=area under the curve; CVD= cardiovascular disease; MI= myocardial infarction

CHAPTER 5:EFFECTS OF DIETARY SUPPLEMENTATION WITH LCPUFAS ON INFANT AND CHILD GROWTH

Background

LCPUFAs are found in breast milk, but are not present in standard infant formula. Possible nutritional deficiencies of fatty acid metabolites needed for growth and development have been suggested for infants fed solely on formula diets. To improve composition of infant formula relative to nutrients in breast milk, LCPUFA rich oils that contain high levels of omega-3 and omega-6 fatty acids have been used as supplements to improve infant formula nutrient profiles. Long chain polyunsaturated omega-3 fatty acids eicosapentaenoic acid (EPA; 20:5(n-3)) and docosahexaenoic acid (DHA; (22:5(n-3)) are derived primarily from fatty fish, seafood and marine microalgae while omega-6 fatty acids such as arachidonic acid (AA; 20-4(n-6)) are derived from eggs or fungi.

The safety of LCPUFA supplementation for women's prenatal diets, as well as formulas intended for feeding preterm and term infants has been assessed in infant and child outcomes in several clinical trials and meta-analyses that compared infant/child growth and development in groups given supplemented or unsupplemented formulas as well as comparison to infants that were breastfed. Studies included for review in this section were randomized, placebo-controlled clinical trials of DHA supplementation that primarily evaluated infant and child growth parameters. Literature reviews and efficacy studies that evaluated DHA for possible beneficial effects on various infant/child health parameters or for improving neurodevelopmental endpoints of auditory, visual, speech, IQ and cognitive skills were generally excluded from evaluation in this hazard assessment if the study did not concurrently assess anthropomorphic endpoints.

Results

DHA Supplementation to Mothers: Attempts to improve nutritional status during pregnancy have included providing mothers DHA supplements derived from fish or algal oils and determining effects on growth and development of their infants and children. Children from pregnant women given 4.5 g of fish oil/day containing 1.4 g of EPA and DHA during pregnancy (Lauritzen et al., 2005) had no significant effects on growth up to 9 months but at 2.5 yr of age the children in the fish oil group had significantly larger body mass index (BMI) and head

circumference. At 7 yr follow up of the children from the same study, Asserhøj et al. (2009) found no statistically significant differences in BMI or head circumference after adjustment of data for covariants at birth indicating that earlier differences did not persist. The same authors reported that diastolic and mean arterial blood pressure was significantly higher ($p<0.01$)in boys (n=24) but not in girls (n=12) in the children from mothers given fish oil compared to the group (boys n=12; girls n=16) from mothers given olive oil. However, this same finding was not seen at the earlier 2.5 yr assessment of the children in comparisons of the same fish oil vs. olive oil groups that showed no significant differences in blood pressure parameters, heart rate variability or pulse wave velocity (Larnkjaer et al., 2006; Lauritzen et al., 2005).Other studies with term infants fed formula supplemented with DHA have shown either no effect of supplementation on blood pressure at 9 yr follow up (De Jong et al., 2011) or reports of decreases in blood pressure of 6 yr old children from groups supplemented with DHA as infants (when compared to unsupplemented control) (Forsyth et al., 2003). Although the Opinion of the BfR (2009) indicated that there is a question of whether maternal/childhood supplementation with n-3 fatty acids may have "a negative long-term effect on blood pressure", the children from the DHA groups in this study had similar blood pressure values seen in children in breastfed reference groups indicating a possible benefit rather than risk from supplementation with n-3 fatty acids.

Numerous other studies with women supplemented with DHA from fish oil during pregnancy or while breastfeeding have shown no statistically significant adverse effects on infant/child growth parameters (weight, length, BMI or head circumference) at DHA levels from 0.15% to 0.96% of total fatty acids (Table 6) (Asserhøj et al., 2009; Bergmann et al., 2008; Collins et al., 2011; Jensen et al., 2005; Helland et al., 200; Larnkjaer et al., 2006; Lauritzen et al., 2005). In one study of maternal supplementation with DHA, a slight (1cm) but statistically significant enhancement of infant growth was seen as an increase in body length of children at 18 mo. (Makrides et al. 2009). Increased body length was also observed in infants at delivery from primagravid compared to multigravid women given 400 mg DHA during pregnancy (Ramakrishnan et al. 2010). Follow up studies of the same groups of infants/children at 1, 3, 6, 9, 12 and 18 mo. of age (Stein et al., 2010) showed no adverse effects and residual enhancement of body length measurements and other growth parameters in comparisons of children from primagravid vs. multigravid mothers (Table 6). All of the studies reviewed support the conclusion that supplementation of nursing mothers with fish oil to attain concentrations of DHA

of up to ~1.0% in breast milk was safe and produced no adverse effects in their infants or children.

Supplementation of Term Infant Formula: Term infants given LCPUFA supplemented formula with various levels of DHA up to 0.96% of total fatty acids in combination with AA for periods of up to 1 year consistently showed an absence of significant effects on growth indices of weight, BMI, body length and head circumference at assessment times ranging from 2 weeks to 9 yr of age (Table 6)(Agostoni et al., 2006; Auestad et al., 1997; 2001; Birch et al., 2005; 2010; Burks et al., 2008; De Jong et al., 2011;Field et al., 2008; Hoffman et al., 2006; 2008; Makrides et al. 1999; Pastor et al., 2006).Meta-analyses of data on effects of LCPUFA dietary supplementation of term infants and children for up to 1 yr. with DHA/EPA and AA rich oils summarized in Table 6 also confirmed the lack of statistically significant effects of LCPUFA supplementation on growth parameters of infant and children (Makrides et al., 2005; Simmer et al., 2008b; Rosenfeld et al., 2009). DHA supplementation to term infants with up 0.96% DHA for 1 yr was safe and produced no adverse effects on typical growth parameters (Birch et al., 2010).

Supplementation of Pre-Term Infant Formula: Preterm infants given LCPUFA supplemented formula with various levels of DHA up to 1.1% of total fatty acids in combination with AA had no adverse effects on growth indices of weight, BMI, body length and head circumference at assessment times up to 1 year (Table 6) (Clandinin et al., 2005; Collins et al., 2011; Fang et al., 2005; Groh Wargo et al., 2005;Henricksen et al., 2008; Innis et al., 2002; Koletzko et al., 2003; O'Connor et al., 2001; Rosenfeld et al., 2009). DHA supplementation to preterm infants was safe and produced no adverse effects on major growth parameters assessed later in life.

Other concurrent endpoints: Assessment of other developmental indices for infants and children evaluated for growth in various studies showed no adverse effects on visual development, neurodevelopmental or other parameters from LCPUFA supplementation to mothers (Jensen et al., 2005; Makrides et al., 2009) or from infant formula fortified with DHA/AA rich oils. No statistically significant differences between DHA supplemented and control groups of infants and children have been seen in follow up studies of infant/child visual acuity and Bayley neurodevelopmental indices by several authors (Agostoni et al., 2006;Birch et

al., 2005, 2010;Clandinin et al., 2005;Fang et al., 2005; Henricksen et al., 2008; Innis et al., 2002; Jensen et al., 2005; Makrides et al., 2009; O'Connor et al., 2001). No statistically significant effects from supplementation of formula with DHA/EPA and AA were observed in assessments of immune cell distributions and cytokine levels from 0.20% DHA to term infants for 4 wks (Field et al., 2008) or for allergenicity reactions from 0.32% DHA supplementation for 4 mo. (Burks et al., 2008).Measurements of bone density and mineral content in preterm infants fed 0.26% DHA and 0.42% AA for 12 months (Groh Wargo et al., 2005) similarly showed lack of statistically significant differences from controls. DHA supplementation to term and preterm infants was concluded to have no significant adverse effects on neurodevelopmental endpoints in meta-analyses of results from major clinical trials (Simmer et al., 2008 a).

Conclusion

No adverse effects from DHA supplementation on infant and child growth have been seen in numerous studies that evaluated nursing mothers given fish oil supplements sufficient to attain DHA levels up to 1% DHA in breast milk, term infants given formula supplemented with up to 0.96% DHA for intervention periods from 4 weeks up to 1 yr or preterm infants fed formula with up to 1.1% DHA (of total fatty acids) to term. The conclusion of safety and lack of adverse health effects from DHA when given together with AA in the current assessment of the literature on pregnant and nursing mothers, term and preterm infants is consistent with conclusions of comprehensive safety reviews of DHA supplements by several authoritative bodies including the U.S. FDA, U.S. National Academy of Science, European Food Safety Authority, European Society for Pediatric Gastroenterology, Codex Alimentarius Commission, the Commission of the European Communities and the World Association of Perinatal Medicine (Summarized in Stevens and Halcine, 2010).

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Supplementation to Mothers										
Asserhøj et al., 2009	7-years follow up study based on Lauritzen et al., 2005	Supplementation of pregnant mothers; follow up of 98 children	See Lauritzen et al., 2005	Follow up at 7 yr after 4 mo. postnatal supplementation to mothers		Blood pressure and body mass index and head circumference in children at 7 yr		1.4g total n-3 PUFAs/day to mothers (0.62g EPA/0.79g DHA), as percent of total fatty acids		In the follow-up of children after 7 years, there was no difference in BMI; head circumference was not significantly different from controls ($p>0.05$) after adjustment for covariants determined at birth. Blood pressure in 7 yr old boys but not girls was significantly higher ($p<0.05$) in groups from mothers given fish oil versus olive oil; this difference was not apparent at 2.5 yr in the original study by Lauritzen et al., 2005 (suggesting possibility of confounding effects in the 4.5 yr interval).
Bergmann et al., 2008	Randomized, double blinded, placebo controlled	Pregnant mothers in 3 groups (n=48 in each) and new born babies	Mothers were given either a basic vitamin-mineral supplement (Vit/Min);Vit/Min plus 4.5g fructo-oligosaccharide (FOS); or Vit/Min plus 4.5g FOS plus 200 mg DHA.	Supplementation (21-37 weeks of gestation and from the 2nd week after delivery to the 3rd month of lactation)		Growth (weight, length and head circumference)		200 mg DHA/day to mothers		There were no statistical differences ($p>0.05$) in weight, length or head circumference at birth. In a follow-up study, they found a lower BMI in infants at the age of 21 months from mothers receiving DHA.
Collins et al. (2011)	Double blind, randomized, placebo controlled trial	Lactating mothers High DHA group (n=136); Control group (n=133)	High DHA group: 3.0 g DHA/day (in 6 x 500 mg tuna oil capsules) resulting in 0.85% DHA in breast milk; Control group: 3.0 g soya oil/day (in 6 x 500 mg capsules) resulting in 0.25% DHA in breast milk; ~0.40% AA concentrations in high DHA and control breast milk samples	Intervention from day 2-5 from birth to expected delivery day; Assessments of infants/children at 4, 8 and 12 mo. corrected age.		Growth (weight, length, head circumference		3.0 g fish oil/day to attain: 0.85% DHA and 0.40% AA, as a percentage of total fatty acids in breast milk		No significant effects on age, weight, length or head circumference up to 12 mo. in premature infants from mothers given 3 g DHA/day to attain 0.85% DHA in breast milk.

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Helland et al., 2001	Randomized, double blind, placebo controlled	Pregnant mothers (weeks 17-19) given either Cod liver oil (n=301) or placebo (corn oil; n=289)	10 mL/day of either cod liver oil (providing 2.63g ω-3 fatty acids) or corn oil (providing 0.35 g ω-3 fatty acids)	Weeks 17 to 19 of pregnancy until 3 months after delivery		Birth weight, gestational length; Growth (length and head circumference)	Cognitive functions at 1 yr.	2.63g ω-3 fish fatty acids/day to mothers		No significant differences (p>0.05) observed for gestational length or birth weight between the mothers given cod liver oil group or corn oil. Infant birth length, head circumference, and placental weights were also not statistically different in the 2 groups. There were no significant differences (p>0.05) in infant growth during the first year between the 2 groups.
Jensen et al., 2005	Double blinded, placebo controlled	Breastfeeding mothers given DHASCO oil (n=114), or placebo (soy/corn oil; n=113)	Breastfeeding women given 1capsule/d of either DHASCO oil (algal TAG) (providing ≈200 mg/DHA/d) or soy/corn oil (50:50 mixture, no DHA)	4 months after delivery for oil consumption and up to 30 months for evaluation of infants.		Visual function and neuro-development	Growth (weight, length and head circumference)	≈200 mg DHA/day DHA to mothers		Neither neurodevelopmental indexes of the infants at 12 mo of age nor the visual function at 4 or 8 mo of age differed significantly between groups (p>0.05). In contrast, the BPI but not the MDI, of the supplemented group was higher ($p \leq 0.01$) at 30 mo of age. There were no significant differences (p>0.05) in growth parameters between the groups at any assessment period.
Larnkjaer et al., 2006	Same protocol as Lauritzen et al., 2005	Follow up of supplementation to pregnant mothers: See Lauritzen et al., 2005	See Lauritzen et al., 2005	2.5 yr follow up of blood pressures of children from mothers supplemented for 4 months post delivery		Blood pressure	Blood pressure parameters	1.4g total n-3 PUFAs/day to mothers (0.62g EPA/0.79g DHA), as percent of total fatty acids		Maternal supplementation with fish oil for 4 months had no significant effect (p>0.05) on blood pressures, heart rate variability or pulse wave velocity of their children at 2.5 yr of age.

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Lauritzen et al., 2005	Randomized, double blind, placebo controlled trial	Breastfeeding mothers given fish oil (n=62) or placebo (olive oil; (n=60)	Mothers that consumed diets with ω-3 PUFAs of <0.40 g/d were given either 4.5g/d of fish oil (containing 1.5g ω-3 PUFAs that inc. 0.62g EPA/0.79g DHA) as a percent of total fatty acids; Control: olive oil	Follow up of growth parameters of children at 2, 4, 9 and 2.5 yr from mothers supplemented for 4 months post delivery		Growth (head circumference, weight, length, skin fold thickness, and waist circumference)		1.4g total n-3 PUFAs/day to mothers (0.62g EPA/0.79g DHA), as percent of total fatty acids		Growth measured by changes in weight, length, and head circumference did not differ between the randomized groups up to 9 months ($p>0.05$). But at 2.5 years, children in the fish oil group had larger waist circumference, and statistically elevated body mass index and head circumference ($p<0.05$) compared to the olive oil group
Makrides et al., 2009	Randomized, double blinded, placebo controlled	Breastfeeding mothers given high DHA (n=322) or a soy oil(placebo; n=335)	Mothers given 6 capsules/d providing 3.0 g/d tuna oils high in 1%DHA (to achieve breast milk with DHA of total fatty acids) or soy oil; Infants assigned to DHA groups were given formula with 1.0% DHA/0.60% AA if mothers chose to stop or could not breastfeed.	Intervention from delivery up to time of term; Follow up at 18 months		Neuro-development	Growth (weight, length and head circumference)	1% DHA of fatty acids in breast milk (or formula with 1.0% DHA + 0.60% AA)		At 18 mo., no significant differences were seen between groups for weight or head circumference ($p>0.05$) but infants from mothers given fish oil had enhancement of body length (~1cm) ($p<0.01$). DHA at approximately 1% total fatty acids in the diet of mothers did not increase overall 18 mo. MDI scores of breast-fed preterm infants born earlier than 33 weeks but did improve the MDI scores of girls ($p=0.03$).

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Ramakrishnan et al., 2010	Randomized, double blinded, placebo controlled	1094 Pregnant women (18-35 yr): 547 in treatment and control groups, respectively	Pregnant women were given 400 mg DHA (algal; 200 mg capsules twice/day) Placebo capsules contained olive oil	Mid-pregnancy (weeks 18-22) until delivery		Gestational age, weight, length and head circumference of infants	Incidence of adverse events	400 mg DHA (supplement to 55 mg DHA from diet		No statistical differences ($p>0.05$) between control and DHA groups in mean gestational age, weight, length or head circumference at birth. In comparisons of subsets of infants from primagravidae vs. multigravidae women given DHA, primagravid infants had significant increases ($p<0.05$) in weight (+99.4g) and head circumference (+0.5 cm), suggesting a possible beneficial effect of DHA in this group of women. There were no significant differences in adverse events in supplemented vs. placebo groups.
Stein et al., 2010	Follow up study of infants from intervention trial by Ramakrishnan et al., 2010	Supplementation to pregnant women: follow up of 739 children (76% of birth cohort in Ramakrishnan et al., 2010)	400 mg DHA or placebo (Ramakrishnan et al., 2010)	Follow up measurements of Ramakrishnan et al., 2010 study in children at 1, 3, 6, 9, 12 and 18 mo. of age		Weight, length, BMI and head circumference (plus comparisons of age adjusted comparisons for each value)		400 mg DHA (supplement to 55 mg DHA from diet		No significant ($p>0.05$) differences in length, weight or head circumference in treatment vs. placebo groups (values adjusted for maternal height) at 18 mo. of age. Significant enhancement of growth was seen as increases were seen in comparisons of body length of infants from primagravid women compared to multigravid women at 3, 9, 12 and 18 mo. ($0.01< p <0.05$).

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Supplementation to Term Infants										
Agostoni et al. (2006)	Prospective, double blind, randomized study with dietary intervention until 20-weeks of age; follow up at one year of age.	Term infants of normal birth weight with type I hyperphenyl-alaninaemia identified by neonatal screening; 39 of 42completed study; Test group (n=21):Control group (n=18)	Test group received standard formula supplemented with 0.30% DHA and 0.70% AA of total fatty acids Control group received standard phenylalanine-free infant formula;	Intervention to 20 wk of age; follow up at 1 yr		Cognitive and psychomotor development	Visual evoked potential	0.30% DHA/ and 0.70% AA of total fatty acids		There were no significant differences (p>0.05) in DHA/AA groups from controls in cognitive or motor development or in evaluations of visual evoked potential as an indicator of effects on the CNS.
Auestad et al. (1997)	Longitudinal, prospective randomized study	Term infants; DHA group (n=43); DHA+AA group (n=46); Control group (n=45) Non randomized, breast-fed infants served as reference group (n=63)	Test groups received 0.23% DHA + 0% AA; or 0.23% DHA+ 0.43% AA, as percentage of total fatty acids Control group received standard formula w/o DHA and AA.	Feeding from <7 days of age to 12 months		Growth (weight, length and head circumference) and visual acuity		0.23% DHA + 0.43% AA, of total fatty acids.		No significant differences in growth parameters or visual acuity in any comparisons of the 4 groups.
Auestad et al. (2001)	Double blind, parallel, randomized prospective feeding study	Term infants; Test group 1 (n=80); Test group 2 (n=82); Control group (n=77) Breast-fed reference group (n=82)	Treatment group 1: 0.13%DHA+0.46% AA (fish/fungal); Treatment group 2: 0.14%DHA+0.45% AA (egg-derived triglycerides); Control: standard formula (0% DHA/AA) Breast milk: 0.12% DHA+0.51% AA Percentages based on total fatty acids	12 months; test formula fed for minimum of 4 mo. and as exclusive milk source for 1 year		Growth (weight, length and head circumference) and visual acuity		Up to 0.14% DHA; 0.46% ARA		No overall or gender specific differences were found for increases in weight, length, or head circumference among groups during the 12-month study. There were no significant differences in visual acuity in comparisons of the treatment and control group.

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Birch et al. (2010)	Double blind, randomized, placebo-control parallel dose response study	Term infants; Test groups (n= infants enrolled/ completed): 0.32% DHA (83/64); 0.64% DHA (84/59); 0.96% DHA (87/65) Control (85/56)	3 Test groups received term formula supplemented with either: 0.32%, 0.64% or 0.96% DHA; 0.64% AA added in all groups; Control was a bovine milk-based term formula	12 months; Anthropomorphic evaluations performed at birth, 1.5, 4, 6, 9 and 12 mo. of age		Growth parameters (weight, length, weight-for-length and head circumference); visual acuity		0.96% DHA + 0.64% AA, of total fatty acids		No significant differences in any anthropomorphic growth parameters in statistical pairwise comparisons to control values. Infants fed control formula had significantly poorer visual acuity ($p<0.001$) in comparison to groups given ≥0.36% DHA + 0.64% AA.
Birch et al. (2005)	Double blind, randomized, placebo controlled trial	103 Term infants; n=52 in control and n=51 in test group; Follow up at 52 weeks, n=44 in control and n=42 in test group.	Test group given formula with0.36% DHA and 0.72% AA; Control (placebo) group given unsupplemented infant formula (Control and test formulas also provided ~15% linoleic acid and 1.5% α-linolenic acid)	39-week intervention and follow up until 52 weeks		Growth (weight, length, weight/length, and head circumference)	Visual acuity	0.36% DHA and 0.72% AA of total fatty acids		No significant differences ($p>0.05$) in body weights and all other anthropomorphic measures in the DHA/AA test and control groups. Sweep visual evoked potential (VEP) acuity in the LCPUFA supplemented group was significantly better than that in the non-supplemented control group at all time points measured ($p<0.001$ to 0.01).
Bouwstra et al. (2005)	Prospective, double-blind, randomized placebo controlled study with18 month follow up	Term infants: a). 146 infants given LCPUFA supplemented formula (n=146); b). 169 infants given un-supplemented formula (n=169); c). 159 infants breast fed (reference group)	Test group: LCPUFA supplemented formula with 0.30% DHA and 0.45% AA of total fatty acids); Controls: unsupplemented infant formula (placebo); breast fed infants (reference group)	2 months supplemented formula followed by control formula to 6 mo. of age); neurological scoring at 18 mo. of age		Neurological development (Bayley MDI, PDI)		0.30% DHA and 0.45% AA of total fatty acids		Multivariate statistical analyses showed no significant ($p>0.05$) differences in infants given DHA/AA supplemented or control diets for 2 mo. or breast fed infants for Psychomotor Developmental Index (PDI) or motor development index (MDI) evaluated at 18 mo. of age.

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Burks et al. (2008)	Study 1: double-blind, randomized placebo controlled parallel-design prospective 120 day study; Study 2: double-blind, placebo-controlled food challenge	Study 1: 164 healthy, term infants Study 2: 32 healthy term infants and children (8 months to 2 years of age) with confirmed allergy to cow's milk	Test group received formula supplemented with 0.32% DHA and 0.64% AA based on %total fatty acids; Placebo group received unsupplemented formula	Study 1: 120 days; Study 2: 7 day allergenicity feeding challenge		Growth (weight, length and head circumference)	Allergenicity	0.32% DHA and 0.64% AA of total fatty acids		No significant differences observed (p>0.05) in weight, length, or head circumference between DHA/AA test and control groups. No allergic reactions were seen in any subject.
De Jong et al. (2011)	9 yr Follow up of a prospective, double-blind, randomized placebo controlled study by Bouwstra et al. (2005)	Term infants: a). 146 infants given LCPUFA supplemented formula (n=146); b). 169 infants given un-supplemented formula (n=169); c). 159 infants breast fed (reference group)	Test group: LCPUFA supplemented formula with 0.30% DHA and 0.45% AA of total fatty acids); Controls: unsupplemented infant formula (placebo); breast fed infants (reference group)	As infants: 2 months supplemented formula followed by control formula to 6 mo. of age); 9 yr follow up study		Growth (height, weight, head circumference)	Blood pressure	Given as infants: 0.30% DHA and 0.45% AA, of total fatty acids		No statistical differences (p>0.05) were observed for any anthropomorphic or cardiovascular parameter in comparison of 9 yr old children given DHA/AA or control diets as infants.
Field et al. (2008)	Randomized, placebo controlled comparative trial	Term infants given supplemented formula (n=16) or breast milk (n= 14; placebo) starting at 2 wk of age	Test group: fed standard infant formula supplemented with DHA (0.20%) and AA (0.34%) of total fatty acids. Control group received human milk (placebo)	4 weeks with measurements at age 2, 4 and 8 wk of age		Growth (weight, length and head circumference)	Immune effects	0.20% DHA and 0.34% AA, of total fatty acids		No significant differences (p>0.05) in weight, length, or head circumference at age 2 weeks or 6 weeks between DHA/AA test and control groups. Immune cell distribution and cytokine profile was similar in both DHA/AA and control groups.

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Forsyth et al. (2003)	Randomized, multi-center, placebo controlled trial	Term infants received a formula with LCPUFA (n=71), or a formula without LCPUFA (placebo; n=76). Breastfed infants (n=88) as a reference group.	In the LCPUFA group, infant formula contains 0.15-0.25% DHA and 0.3-0.4% AA per total fatty acids. The standard formula contains no DHA and <0.1% AA per total fatty acids.	Follow up at age 6 of a 4 month intervention study.		Blood pressure		0.15-0.25% DHA and 0.3-0.4% AA, of total fatty acids in formula		The children in the LCPUFA supplemented group had significantly lower mean blood pressure (p=0.02); diastolic (p=0.018); systolic (p =0.132) after 6 years in comparison to the non-supplemented control group. Diastolic and systolic pressures of the breastfed children were also significantly lower than that of the non-supplemented group and values were similar to those in the LCPUFA formula group (no stat. comparison possible). The authors indicated that the similarity of blood pressures in the LCPUFA and breastfed groups suggested a prolonged benefit of supplementation into adulthood.
Hoffman et al. (2006)	Randomized, double-blind, placebo controlled prospective trial	Term infants; 39/group given high or low LCPUFA supplemented infant formula	High LCPUFA 17 mg DHA, 34 mg AA, 85 mg ALA and 860 mg LA based on 100 kcal; Low LCPUFA: 8 mg DHA, 21 mg ARA,110 mg ALA and 1000 mg LA based on 100 kcal (ALA= alpha-linolenic acid; LA= linoleic acid)	120-days; evaluations at 14, 30, 60, 90, and 120 days of age		Growth (weight, length and head circumference)	Growth rate	17 mg DHA, 34 mg AA, 85 mg ALA and 860 mg LA based on 100 kcal diet		Growth rates (rate of weight gain), body weights length and head circumference were qualitatively similar in the two groups (no stat. comparison).

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Hoffman et al. (2008)	Randomized, double-blind, placebo controlled, parallel group study	Term infants (n=179): Test group (n=86): Control group (n=93)	Test group received infant formula supplemented with 0.17% DHA and 0.34% AA based on total fatty acids; Control group received infant formula (placebo)	120-days; evaluations at 14, 30, 60, 90, and 120 days of age		Growth (weight, length and head circumference)	Growth rate during study	0.17% DHA and 0.34% AA, of total fatty acids		No significant differences observed (p>0.05) in weight, length, head circumference or growth rates between DHA/AA test and control groups at any observation period.
Makrides et al. (1999)	Randomized, double-blind, placebo controlled, study	Term infants; DHA (n=27); DHA+AA (n=28); Placebo (n=28)	DHA group: 0.35% DHA alone; DHA+AA group: 0.34% DHA/0.34% AA; Placebo: standard formula 0% AA+DHA	12 months intervention; evaluations at 6. 16, 34 weeks and 1 and 2 yr		Growth parameters of weight, length and head circumference		0.34% DHA + 0.34% AA, of total fatty acids		No significant differences in growth parameters among groups assessed up to 2 yr of age. Adjustments for gender, postnatal age or gestational age still showed no treatment-related effects on growth.
Makrides et al. (2005)	Meta-analysis of 14 trials with supplementation and evaluation periods from 3 to 12 months.	Term infants (n=1846).	Infants in various trials received formula with 0.1% to 1.0% of n-3 LCPUFA and 0% to 0.72% n-6 LCPUFA in terms of total fatty acids	Eligible trials based on intervention commencing within14 d of birth, and intervention for at least 12 wk.		Growth parameters of weight, length and head circumference		Up to 1.0% n-3 + 0.72% n-6 LCPUFA, of total fatty acids		No significant differences (p>0.05) in weight, length or head circumference between the groups were observed at either 4 or 12 months of age or in subgroup analyses with regard to infant sex and different sources of LCPUFA from fish, egg, algal or fungal oils.

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Pastor et al. (2006)	Multi-center, prospective, open-label, 12-month observational study.	Term non-breast-fed infants (n=1342)	Test group formula supplemented with 0.32% DHA and 0.64% AA based on total fatty acids; Control Groups: low DHA/AA formulas: a.) No added DHA or AA; b.) 16 mg DHA + 6 mg of AA/100 kcal; c) 8 mg of DHA + 13 mg of AA / 100kcal .	12 months		Growth (weight, length and head circumference)	Growth rate	0.32% DHA an 0.64% AA, of total fatty acids		Growth rates (rate of weight gain), body weights, length and head circumference were similar ($p>0.05$) for DHA/AA test and control groups.
Rosenfeld et al. (2009)*	Individual patient data (IPD) meta-analysis of data pooled from 4 placebo controlled, randomized trials of term and pre-term infants	Term infants (n= 526) were given formula supplemented with LCPUFA; Control group (n=537) was given unsupplemented formula (placebo)	Term infants received 0.30 to 0.32 DHA/100 g fat	Term infants 2 to 6 mo.		Growth (BMI, weight, length, and head circumference at 18 mo.		0.30-0.32 g DHA/100 g fat in formula		IPD meta-analysis indicated no beneficial or adverse effect of LC-PUFA supplementation on growth parameters at 18 months of age.
Simmer et al. (2008b)	Meta-analysis of data from 14 randomized, controlled trials	Term infants (n=1719)	Formulas supplemented with LCPUFA from various sources including egg yolk, milk fat, vegetable oils, fish or fungus oil; some studies involved DHA supplementation only.	DHA/ARA formula supplementation periods from 2 months to 1 year;		Visual acuity (first 3yr, 9 studies) and growth indices (weight, length and head circumference; up to 3yr, 12 studies)	Neurodevelopment (up to 2 yr, 11 studies)	Not stated		LCPUFA supplementation did not produce beneficial or adverse effects on weight, length and head circumference or on vision, cognitive function or growth of term infants irrespective of the source of LCPUFA used in the studies used in the meta-analysis.

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Supplementation to Preterm Infants										
Clandinin et al. (2005)	A prospective, randomized double-blind study	Preterm infants (n= 361) ≤ 35 weeks; Test group 1 (n=112); Test group 2 (n=130); Control group (n-119); Reference Group: term infants (n=105) breast-fed for ≥ 4 months	Test group 1: Formula with 17 mg algal DHA + 34 mg AA/100 kcal; Test group 2: Formula with 17 mg fish oil DHA + 34 mg AA/100 kcal Control: unsupplemented infant formula; Reference group: breast-fed term infants	Supplemented diet fed from birth to 92 weeks "post-menstrual age" (PMA) with follow up assessment at 118 weeks PMA		Growth (weight, length, head circumference)	Neurological development	17 mg DHA and 34 mg AA per 100 kcal diet		No significant differences in mean weight, length and head circumference or respective growth rates at 40 wk PMA; during weeks 66 to 118 weeks PMA significant differences were seen in body weights in treated groups ($p<0.05$) bit did not differ from values in term infants by 118 weeks PMA. Bayley MDI and PDI scores were higher in both test groups compared to the control.
Collins et al. (2011)	Double blind, randomized, placebo controlled trial	Preterm infants (n=657) <33 weeks; High DHA group (n=322 infants); "Standard" DHA group (n=335 infants)	High DHA formula: 1.11% DHA+0.69% AA; "Standard" DHA formula: 0.42% DHA+0.69% AA. (%s of total fatty acids)	Intervention from day 2-5 from birth to expected delivery day; Assessments at 4, 8 and 12 mo. corrected age.		Growth (weight, length, head circumference		1.1% DHA + 0.69% AA, of total fatty acids		No significant effects of up to 1.1% DHA at any age on weight, length or head circumference up to 12 mo. Infants given formula with 1.1% DHA has slight, significant ($p<0.02$) increases in body length at 18 mo. Authors concluded that DHA up to 1% to preterm infants "does not adversely effect growth".
Fang et al. (2005)	Double blind, randomized, placebo controlled comparative study	Preterm infants (n=28); 30-37 wk gestation	Control group given standard infant formula; Test group: formula supplemented with 0.05% DHA + 0.10% AA in diet supplying 70-110 kcal/kg/day	6-months		Growth (weight, length, head circumference); Cognitive development	Visual acuity	0.05% DHA and 0.10% AA, of total fatty acids		No significant differences I\in DHA/AA supplemented vs. controls ($p>0.05$) were observed in growth parameters assessed monthly during the study. No significant differences ($p>0.05$) in visual acuity or Bayley's MDI or PDI scores between supplemented and control groups.

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Groh-Wargo et al. (2005)	Placebo controlled, double blind study	Preterm infants <33wk (n=60); Control group (n=22); Test group 1 (n= 18); Test group 2 (n=20)	Control: milk formula; Test group 1: milk formula supplemented with0.26% DHA+ 0.42% AA (fungal/fish source); Test group 2: milk formula supplemented with 0.26% DHA + 0.42% AA (egg source)	Preterm infants fed test or control formulas for 12 mo. with assessments at 35 and 40wks and at 4 and 12 mo.		Growth (weight, length, and head circumference)	Bone density and mineral content	0.26% DHA/0.42% AA, of total fatty acids		No significant differences in comparison to unsupplemented controls were seen among the three groups at any time point in weight, length, or head circumference (p>0.05). Bone mineral content and bone mineral density did not differ among treated and control groups.
Henriksen et al. (2008)	Randomized, double blind, placebo-controlled study	Preterm infants (n=141) with birth weights of <1500 g	Intervention group received human milk supplemented with 32 mg of DHA + 31 mg of AA/100 mL; Control (placebo) group received human milk	8 week intervention; assessment at 6-mo. of age		Growth (weight, length, head circumference)	Neurological development	32 mg DHA and 31 mg AA in 100 mL human milk		No significant differences (p>0.05) in growth were found in the DHA/AA supplemented or control groups. The DHA/AA supplemented group was associated with significantly better recognition memory (p<0.01) and higher problem-solving scores (p<0.02) at 6 months

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Innis et al. 2002	Randomized, prospective, double blind, multi-center placebo-controlled study	Preterm infants; Test group 1: DHA (n=56) Test group 2: DHA +AA (n=59); Control group (n=53); breast fed reference group (n=90)	DHA group received formula with 0.34% DHA; DHA+AA group received formula with 0.33% DHA + 0.60% AA; (% based on total fatty acids in formula) Control: unsupplemented premature infant formula	Minimum 28 days intervention followed by unsupplemented term formula for 57 weeks Assessment times at ages 40, 48 and 57 weeks.		Growth (body weight (bw), length, and weight:length ratio)	Visual acuity	0.33% DHA and 0.60% AA, of total fatty acids		BW of infants fed DHA alone were similar to controls at all observations; DHA+AA groups had significantly higher bw in comparison to control and had similar weights and weight ratios to term breastfed infants at 48 and 57 weeks PMA. There were no significant differences in body measurements of treated vs. control groups at any observation time except for increased weight: length ratio at 48 and 57 weeks (but similar to breastfed infants). There were no treatment-related differences in adverse events or in visual acuity scores.
Koletzko et al. (2003)	Double blind, stratified, placebo controlled study.	Preterm infants; DHA+AA (n=15); Control (n=15); human milk (n=19)	Test group: 0.57% DHA+0.10% AA; of total fatty acids. Control: unsupplemented formula; Reference group: mature human milk:0.20% DHA+0.40%AA	28 days intervention		Growth (weight, length, head circumference)	Tolerance of supplemented formula	0.57% DHA+0.10% AA, of total fatty acids.		No significant differences in growth parameters or gastric tolerance assessment between groups.
O'Connor et al. (2001)	Randomized, double blind, placebo-controlled study	Preterm infants (n=470); DHA+AA groups (n=283); Control (n=144); 43 infants were fed human milk as a reference group	Test groups received 0.16-0.26% DHA + 0.42-0.44% AA, as % total fatty acids; Control groups received standard formula (0% DHA + AA)	12 months intended intervention.		Growth (weight, length, head circumference) Anthropomorphic gains quantified for study day 1-term, 1-4 mo. and 1-12 mo.	Visual acuity	0.26% DHA+0.44% AA, of total fatty acids		No significant differences in growth parameters or anthropomorphic gains among treatment and control group at any study interval. No statistically significant effect found among groups for visual acuity.

Table 6.Effects of omega-3 fatty acids on infant/child growth

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No adverse effect level (NEL) or adverse effect level (AEL)		Additional notes
						Primary	Secondary	NEL	AEL	
Rosenfeld et al. (2009)*	Individual patient data (IPD) meta-analysis of data pooled from 4 placebo controlled, randomized trials of term and pre-term infants	Preterm infants: supplemented groups (n=526); Placebo groups (n=537)	Treated groups received 0.17% to 0.50% DHA as percentage of total fatty acids; Control group given standard formula	Preterm infants 3 week minimum up to 9 mo		Growth (BMI, weight, length, and head circumference at 18 mo.)		Up to 0.5% DHA of fat in formula		IPD meta-analysis indicated no beneficial or adverse effects of LC-PUFA supplementation on Growth at 18 months of age.
Simmer *et al.* (2008a)	Meta-analysis of data from 15 randomized studies	Preterm infants <37 weeks gestation.	Formulas supplemented with LCPUFA from various sources including egg yolk, milk fat, vegetable oils, fish or fungus oil; some studies involved DHA supplementation only.	Evaluation at 6 weeks from start of intervention up to 52 weeks; BSID evaluations from four studies at 12 months (N = 364) and three studies at 18 months (N = 494)		Visual development and growth indices; weight, length and head circumference	Neurodevelopment	Not evaluated		Pooling of data from15 trials did not indicate effects from LCPUFA supplementation on visual development, neurodevelopment or growth of preterm infants

AA = arachidonic acid; MDI = Bayley Mental Development Index; PDI = Bayley Psychomotor Development Index.
* Study pertinent to growth evaluations for both term and preterm infants.

CHAPTER 6: GASTROINTESTINAL AND TASTE RELATED EFFECTS

Background

Gastrointestinal disturbances reported in intervention studies with n-3 fatty acids include nausea, vomiting, diarrhea, increased fecal frequency, epigastria and defecation, belching, flatulence, eructation, dyspepsia, fishy aftertaste, loose stools, gastrointestinal discomfort, constipation, upset stomach and abdominal cramps. Several studies identified from a literature search of publicly available databases that assessed the effects of fish oils and EPA/DHA consumption on gastrointestinal- and taste-related effects were not included in the BfR (Opinion No. 030/2009 of May 26, 2009) or VKM (2011) reports. These studies were retrieved and evaluated. Three studies were conducted in healthy adults (Arterburn et al., 2007; Harris et al., 2008; Innis and Hansen 1996), one study was conducted in pre-term infants (Makrides et al., 2009), and the rest were conducted in health-compromised individuals.

Results

In the vast majority of studies reviewed (20 studies identified), the gastrointestinal disturbances are not specifically associated with the intake of n-3 fatty acids, but merely with the intake of relatively large amounts of oil or an oily substance; additionally, no difference in tolerance was seen between intake of these fatty acids in the ethyl ester compared to TAG form (Table 7) (Arteburn et al. 2007; Bays et al. 2011; Bromfield et al. 2008; DeTruchis et al. 2007; Emsley et al. 2008; Frangou et al. 2006; Freund-Levi et al. 2006; Hallahan et al. 2007; Harris et al. 2008; Keck et al. 2006; Kromhout et al. 2010; Leaf et al. 1994; Makrides et al. 2009; Marangell et al. 2003; Maresta et al. 2002; Peet and Horrobin 2002; Pontes-Arruda et al. 2006; Puri et al. 2005; von Schacky et al. 1999; Zhu et al. 2008).Among these 20 studies, 19 studies identified from the literature in both healthy and health-compromised individuals evaluated the effect of intakes ranging from 0.2 to 6 g/day of n-3 fatty acids given for durations up to 2 years. One study (Pontes-Arruda et al., 2006) provided 7.1g/day n-3 fatty acids to patients with severe sepsis or septic shock with n-3 fatty acids by enteral feeding. It is valuable to note that recent developments in micro-emulsification technology have allowed the fortification of foods with long-chain n-3 polyunsaturated fatty acid (PUFA) without the undesirable fish odor/taste and with reasonable shelf life (Garg et al., 2007).

However, in two studies the gastrointestinal disturbances were significantly more frequent in the groups given n-3 fatty acids than in the groups given placebo. In one double-blind randomized controlled trial of 32 healthy adults, although no apparent adverse effects were found, eructation was reported by 1/8 (mild symptoms), 4/8 (mild symptoms), 7/8 (mild symptoms), and 6/8 (five with mild symptoms and one with moderate symptoms) in groups received 0g DHA, 0.6g DHA, 1.7g DHA and 2.9g DHA, respectively. The differences were statistically significant (P <0.01) (Innis & Hansen, 1996).

Reis *et al*. investigated 222 patients after percutaneous coronary intervention. Seventy four patients were randomized to ethyl ester of fatty acids extracted from fish oil, 76 to purified fish oil containing the fatty acids as TAG, and 72 patients to placebo (olive oil). The treatment groups received 6 g/day marine n-3 fatty acid supplementation (Reis *et al.*, 1989). In this study both ethyl esters and TAG fish oil preparations were used, and the authors reported no differences in gastrointestinal disturbances between the two formulations. In both treatment groups, 48% of patients noted GI side effects compared with 22% in the placebo group (p<0.001) and18% of patients in the treatment groups noted bad taste compared with 4% in the placebo group (p<0.01).

In seven studies a placebo was not used or not defined, limiting the conclusions that can be drawn with regard to the effect of EPA and DHA on gastrointestinal disturbances (Barber and Fearon 2001; Bellamy *et al.*, 1992; Burns *et al.*, 1999, 2004; Dehmer et al., 1988; Eritsland *et al.*, 1996; Gadek et al.1999).

In an open label, dose escalation study, patients with pancreatic cancer were provided with diester (with propane-1,3-diol) of EPA (Barber and Fearon 2001). All patients managed to tolerate a intake providing 18 g EPA per day, with intakes between 9 and 27 g daily being taken for at least a month. Dosage was limited by a sensation of fullness, cramping abdominal pain, steatorrhea, and nausea. All such symptoms were controlled by dose reduction or pancreatic enzyme supplements.

In a 6-month study of 120 post-percutaneous coronary intervention patients with 3.0 g/day EPA and DHA as TAG, the only adverse events reported were 4 patients with nausea and one with diarrhea (Bellamy *et al.*, 1992).

In a clinical phase 1 study of 22 patients with neoplastic disease the maximum tolerated amount of fish oil as ethyl ester was determined to be 0.3 g/kg bw/day (Burns *et al.*, 1999). Side

effects included diarrhea, cramping, fecal incontinence of oil, belching, flatulence, nausea and vomiting. In a phase 2 clinical study, 43 patients with cancer cachexia were given 0.15 g fish oil/kg bw/day. For a 70 kg patient this would provide 4.7 g EPA and 2.8 g DHA (as a part of 8.5 g n-3 fatty acid). Effects such as edema, emesis and dyspepsia were reported in addition to the adverse effects reported in the phase 1 study (Burns *et al.*, 2004).

In a 6-month intervention study with EPA and DHA at a level of 5.4g/d in PCI patients, mild gastrointestinal side effects (belching, dyspepsia, flatulence) occurred in 7 out of 44 patients (Dehmer et al., 1988). Control patients did not receive fish oil. By comparison, 3 out of 39 control patients had dyspepsia. Because no placebo was used, the association of gastrointestinal side effects with EPA and DHA rather than consumption of fish oil could not be evaluated.

In a 12 month study with 610 post-cardiovascular by-pass surgery patients receiving 3.3 g/day EPA and DHA as ethyl ester, the investigators reported that "generally, the fish oil supplementation was well tolerated. Adverse effects attributed to fish oil, mainly gastrointestinal complaints, were usually mild, although in some cases the supplementation had to be withdrawn" (Eritsland *et al.*, 1996). However, no placebo was given and the gastrointestinal effect could not be linked specifically to EPA and DHA.

In a double blind, randomized, controlled trial that compared the effects of a specialized enteral diet which was supplemented with fish oil compared to a control formula in 98 patients with ARDS, Gadek et al. (1999) reported that patients receiving fish oil supplemented diet experienced fewer adverse events compared with the control group.

Conclusion

In summary, gastrointestinal disturbances were frequently associated with intake of an oily substance, but the effects were not specifically associated with the intake of n-3 fatty acids. Nineteen studies identified from the literature in both healthy and health-compromised individuals evaluated the effect of intakes ranging from 0.2 to 6 g/day of n-3 fatty acids given for durations up to 2 years. Results from a detailed review of these 19 studies suggest that intake of n-3 fatty acids was not associated with adverse gastrointestinal disturbances. No difference in tolerance is seen between intakes of these fatty acids in the ethyl ester compared to TAG form. Additional 7 studies identified showed no association of n-3 fatty acids with adverse gastrointestinal at intakes of up to 18 g/day. However, because of the lack of placebo used or defined, no conclusions were drawn from these 7 studies.

Table 7. Gastrointestinal and taste-related effects resulted from the consumption of omega-3 fatty acids

Study	Study design	Patients / subjects (n)	Product form and intake	Duration	Concomitant medications, confounders	End points		No effect level (NEL) or effect level (EL) (g/d)		Additional notes
						Primary	Secondary	NEL	EL	
Arterburn et al., 2007	Randomized, placebo controlled parallel	Healthy subjects. Eight groups (n=12 in each group)	Two different algal DHA oils in capsules (DHASCO-T and DHASCO-S) at doses of 0.2, 0.6 and 1.0g DHA/d, one DHASCO-S fortified snack bar (providing 0.465g DHA/d), and a corn/soy oil placebo	28 days	Not mentioned	Bioequivalence	Adverse effects including eructation	1g/d		Significant more eructation compared with placebo was only observed with DHASCO-T at doses of 0.2 and 0.6g/d. No subject discontinued supplementation due to an adverse effect. All adverse effects were evaluated by the investigator as being "mild" to "moderate" in severity
Barber and Fearon 2001	Open label. dose escalation without a control group	Pancreatic cancer patients (*n*=5)	Diester (with propane-1,3-diol) of EPA, 4.5g/d x 2wk, 9g/d x 2wk, 18g/d x 2wk and then 36g/d x 2wk.	8 weeks	Pancreatin, or Domperidone or Diclofenac. Two patients had previously taken the mixed fish oil preparation MaxEPA® at a dose providing around 1 g EPA daily	Tolerance and incorporation	Sensation of fullness, cramping abdominal pain, steatorrhea and nausea	18g/d		All patients managed to tolerate a dose providing 18 g EPA per day, with doses between 9 and 27 g daily being taken for at least a month
Bays et al., 2011	Double blinded, randomized, placebo controlled parallel	Patients with very high triglyceride levels. 4g/d AMR 101 (n=77), 2g/d ARM 101 (n=76), placebo (n=76)	AMR101 is an n-3 fatty acid agent containing >96% EPA ethyl ester and no DHA. Patients received either 4g/d or 2g/d EPA or a placebo	12 weeks safety and efficacy trial followed by a 40-week open-label extension	Medications, including anti-hypertensives, anti-diabetes mellitus drug therapies, tamoxifen, estrogens, and progestins were permitted	Change of triglyceride level	Diarrhea, nausea, and eructation. Eructations	4g/d		The most common treatment emergent adverse events were gastrointestinal (i.e., diarrhea, nausea, and eructation), with the greatest numerical incidence in the placebo group. Eructations were not reported in the AMR101 4-g/day group but were reported by 1 patient in the AMR101 2-g/day group and 3 patients in the placebo group.
Bellamy et al., 1992	Blinded, randomized, controlled (not placebo)	Post PCI patients. With treatment (n=60), without treatment (n=60)	Normal diet with or without supplementation with n-3 PUFAs (fish oil capsules, MaxEPA, providing 1.8g/d EPA and 1.2g/d DHA as TAG)	6 months	Heparin (during PCI). Post PCI medications include β blocker, calcium antagonist, nitrate, aspirin, dipyridamole, diuretic	Coronary angioplasty restenosis rate	Adverse effects including nausea and diarrhea	3g/d		Only adverse events reported were 4 patients with nausea and indigestion and one with diarrhea in the treatment group. The study did not use a placebo.
Bromfield et al., 2008	Double blinded, randomized, placebo controlled parallel	Adults with uncontrolled epilepsy. PUFA arm (n=12), placebo arm (n=9)	2.2g (EPA:DHA ratio 3:2) or mineral oil	12 weeks.	Medications treating intractable epilepsy. None had taken PUFA supplements previously	Refractory epilepsy	Adverse effects including nausea, diarrhea, fishy taste	2.2 g/d		Nausea or diarrhea occurred with the same frequency in the test group and placebo group. Only one in each group complained of a fishy taste

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						Primary	Secondary	NEL	EL	
Burns et al. 1999	Open label, dose escalation	Patients with neoplastic disease (n=22)	Ethyl esters of fish oil (EPA + DHA) capsules contained 378 mg EPA/g and 249 mg DHA/g. Doses were not specified.	Not specified	Patients were excluded if they took steroids, dronabinol, megestrol acetate, or diuretics.	Tolerated dose and dose-limiting toxicities	Diarrhea, fecal incontinence of oil, constipation, passage of orange stool, cramping, nausea, vomiting, "Unable to tolerate in esophagus or stomach"	11.6 g/d		Capsules contained 378 mg EPA/g and 249 mg DHA/g. Maximum tolerated dose of 0.3 g capsules/kg bw per day for more than 2 months, which corresponds to 18.6 g capsules/d for the median baseline patient weight (62 kg). 18.6 g capsules would contain 7 g EPA and 4.6 g DHA (total = 11.6 g EPA + DHA)
Burns et al., 2004	Open label with dose reduction	Patients with cancer related cachexia and with moderate or severe malnutrition (n=43)	Ethyl esters of fish oil (EPA + DHA). Patients received a daily dose of fish oil of 0.15g/kg bw. For a 70 kg patient, this dose would consist of 11 x 1g capsules, providing 4.7 g EPA and 2.8 g DHA (as a part of 8.5g n-3 fatty acid).	1.2 months median	Anti-diarrhea medications, anti-emetics, and analgesics were allowed only if clinically necessary.	Body weight loss and quality of life	Adverse effects including nausea, abnormal taste, belching, flatulence, emesis, diarrhea	varied		There was considerable variability in the ability of the patients to tolerate the fatty acids, and some patients had little or no side effects. Nausea (n=11); Abnormal taste in mouth (n=10); Abnormal taste of food (n=9); Excessive belching (n=9); Excessive flatulence (n=9); Emesis (n=8); Diarrhea (n=7); Dyspepsia (n=2).
Dehmer et al., 1988	Unblinded, randomized. The control patients did not receive fish oil capsules.	PCI patients with test article (n= 44; without test article (n=39)	Conventional antiplatelet regiment (325 mg aspirin and 225 mg dipyridamole per day, control group), similar regiment supplemented with 18 capsules per day of MaxEPA (TAG) containing EPA (3.2g) + DHA (2.2g); one dose level at 5.4 g/d	6-month study; 3-month period for bleeding test.	Aspirin and dipyridamole. No modification or control was made in patients' diets or medications, except that each was encouraged to stop smoking.	Rate of early restenosis	Belching, dyspepsia, flatulence	5.4 g/d		There were mild gastrointestinal side effects (belching, dyspepsia, flatulence) in 7 patients in the treatment group, but this did not necessitate alterations in therapy. Three of the control patients had dyspepsia.
DeTruchis et al., 2007	Double blind, randomized, placebo controlled parallel study	HIV-Infected Patients. n-3 PUFAs arm (n=60), placebo arm (n=62)	Six 1-g capsules of MaxEPA fish oil (providing 1.08g EPA and 0.72 g DHA) or paraffin oil (as control)	8 weeks	Multiple antiretroviral therapy	Triglyceride level	Gastrointestinal disorder	1.8g/d		No enhanced minor gastrointestinal disorder, particularly moderate diarrhea.
Emsley et al., 2008	Double blinded (followed by open label), randomized, placebo controlled parallel	Psychiatric patients. Blinded trial (EPA arm n=39; placebo arm, n=33). Open label extension (n=23 from the EPA arm and 22 from the placebo arm)	2g/d encapsulated ethyl ester-EPA (Amarin) or placebo (liquid paraffin). 2g/d encapsulated ethyl ester-EPA for all patients in the open label extension phase	12 weeks blinded followed by 40 weeks open label extension	Antipsychotic medication; non-steroidal anti-inflammatory agents or aspirin	Safety factors including GI side effects	Adverse GI effects including diarrhea, constipation, abdominal pain, loose stool	2g/d		EPA 2 g/day is generally well tolerated

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Eritsland et al., 1996	Randomize controlled trial. Control patients were those who took aspirin or warfarin only	CABG patients; aspirin (n=148), aspirin with supplement (n=143), warfarin (n=145), warfarin with supplement (n=174)	Four 1-g Omacor capsules providing ethyl esters of EPA (2.04g) + DHA (1.28g)	12 months	Aspirin or warfarin. Patients were told to reduce their intake of saturated fatty acids and to refrain from cod-liver oil and other fish oil products during the study period	1-year graft potency	GI complaint. Primary end point was 1-year graft potency	3.3g/d		The fish oil supplementation was well tolerated. Adverse effects attributed to fish oil, mainly gastrointestinal complaints, were usually mild, although in some cases the supplementation had to be withdrawn. Statistical significance analysis was not conducted.
Frangou et al., 2006	Double blind, randomized, placebo controlled parallel study	Patients met criteria for bipolar disorder I or II. 1g/d EPA (n=24), 2g/d EPA (n=25), placebo (n=26)	2g/d oil providing either 0g, 1g or 2g/d EPA (ethyl esters supplied as LAX-101 with purity >95%). In placebo group (0g EPA), all oils are paraffin oil.	12 weeks	There were no restrictions to the type and dose of psychotropic medication that they were receiving upon study entry.	Bipolar depression	Unpleasant taste and GI Side effects including loose stools, gastrointestinal discomfort, constipation, nausea, flatulence.	2g/d		This study was not powered to detect changes between the three treatment groups. Both EPA doses were well tolerated with no significant differences among three groups in terms of side effects.
Freund-Levi et al., 2006	Double blind, randomized, placebo controlled parallel study	Patients with Alzheimer disease. Fish oil (n=103), placebo (n=101)	Four 1-g capsules/d of EPAX1050TG (providing 1.7g/d DHA and 0.6g/d EPA in TAG form) or corn oil (placebo)	6 months	Acetylcholine esterase inhibitor	Rate of cognitive decline	Drop-out rate	2.3g/d		The dropout rate was 15% (14 patients in the treatment arm and 16 patients in the placebo arm). One of the reasons for leaving the study were gastrointestinal tract symptoms such as diarrhea
Gadek et al. 1999	Double blind, randomized, controlled trial	ARDS patients, EPA+GLA (n=51), control (n=47)	Test article is EPA fish oil plus γ-linolenic acid (GLA; borage oil) (providing 6.9 ± 0.3 g EPA and 2.9 ± 0.1 g DHA per day); an isonitrogenous, isocaloric standard diet as a control.	4-7 days		Pulmonary inflammation , oxygenation and clinical outcomes in patients with ARDS	Gastrointestinal-related adverse events	9.8g/d		A remarkably low percentage of gastrointestinal-related adverse events were reported in the study. There were no significant differences in the adverse events related to cardiac, hematologic, respiratory, and skin and appendage disorders between the groups
Hallahan et al., 2007	Double blind, randomized, placebo controlled parallel study	Patients with self-harm. Active arm (n=22), placebo arm (n=27)	4 capsules/d of Epax providing 1.22g EPA and 0.91g DHA (active arm) or 4 capsules/d of 99% corn oil and 1% EPA/DHA mixture	12 weeks.	All were on antidepressants, with two thirds taking prescribed benzodiazepines	Recurrent self-harm	Taste and gastric discomfort	2.1 g/d		Similar mild gastric discomfort and description of "fish like taste" in both groups. No patients discontinued the study because of adverse events.
Harris et al., 2008	Double blind, randomized, placebo controlled parallel study	Overweight healthy volunteers. Three groups and n=11 per group	GMO soybean oil (24 ml/d providing ~3.7d stearidonic acid) or regular soybean oil with or without EPA ethyl esters (~1g/d)	16 weeks		Omega-3 Index	GI side effects (e.g., diarrhea, abdominal cramps)	1g/d		Non-serious adverse events related to gastrointestinal distress (e.g., diarrhea, abdominal cramps) were evenly distributed across all three groups

Table 7. Gastrointestinal and taste-related effects resulted from the consumption of omega-3 fatty acids

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Innis and Hansen 1996	Double blind, randomized, placebo controlled parallel study	Healthy adults. 4 groups (n=8 in each group)	Blends of Canola oil with a microalgal oil (DHA) and fungal oil (AA oil) to provide 28.8g fat/d containing 0g AA/0g DHA (group 1), or 0.8g AA/0.6g DHA (group 2), or 2.2g AA/1.7g DHA (group 3) or 3.6g AA/2.9g DHA (group 4). All in TAG.	14 days	Subjects consumed a fish free diet for 7 d before the study	Plasma fatty acid responses, metabolic effects and safety	Adverse effects including eructation	2.9g/d		No apparent adverse effects were found. However, eructation was reported by one (mild symptoms), four (mild symptoms), seven (mild symptoms), and six (five with mild symptoms and one with moderate symptoms) of the eight subjects in Groups 1, 2, 3, and 4, respectively. And the differences were statistically significant (P <0.01).
Keck Jr. et al., 2006	Double blind, randomized, placebo controlled parallel study	Patients with bipolar depression (n=28 in EPA, n=29 in placebo); Patients with rapid cycling bipolar disorder (n=31 in EPA, n=28 in placebo)	Either EPA (ethyl esters) 6 g/d or matching placebo capsules (liquid paraffin),	4 months	Mood-stabilizing medications	Depression symptoms	GI disturbance	6g/d		The frequency of GI side effects did not differ between the EPA (6g/d) and placebo.
Kromhout et al., 2010	Double blind, randomized, placebo controlled parallel study	Patients had a myocardial infarction. EPA+DHA+ALA arm (n=1212), EPA+DHA arm (n=1192), ALA arm (n=1197), placebo arm (n=1236)	A margarine supplemented with EPA (targeting 400 mg/d intake), a margarine supplemented with ALA (targeting 2g/d intake), a margarine supplemented with EPA+DHA and ALA, or a placebo margarine	40 months	Antihypertensive, antithrombotic, and lipid modifying therapy	Rate of major cardiovascular events	GI disturbance	0.38/d		The rate of self-reported gastrointestinal symptoms did not differ significantly among the groups
Leaf et al., 1994	Double blind, randomized, placebo controlled parallel study	PCI patients; treatment (n= 275), placebo (n=276)	Ten 1-g gelatin capsules providing ethyl esters of EPA (4.1g) + DHA (2.8) or ethyl ester of corn oil (control)	6-month study; 3-month period for bleeding test.	Aspirin. Patients were instructed to follow a Step-One American Heart Association diet, although compliance varied among patients.	Rate of restenosis	GI symptoms	6.9 g/d		Gastrointestinal symptoms were reported in 8% of the corn oil recipients and 7% of the fish oil recipients. Statistical significance analysis was not conducted.

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Makrides et al., 2009	Double blind, randomized, placebo controlled parallel study	Pre-term infants. High DHA diet group (n=322), standard DHA diet group (n=335)	In the high DHA group, mothers were given 6 capsules/d providing 3g/d tuna oils high in DHA (to achieve breast milk with DHA 1% of total fatty acids). If supplementary formula was required, infants were given a high DHA preterm formula (1% DHA/0.6%AA). In the standard DHA group, mothers were given 6 capsules/d providing 3g/d soy oil. If supplementary formula was required, infants were given a standard preterm formula (0.35%DHA/0.6%AA)	Up to 18 months		Neuro-development of preterm infants	Adverse effects including diarrhea, constipation, nausea, or vomiting	3g/d high DHA tuna oil) for mothers with ~0.9g DHA		There were no differences between the groups (for infants) in maternal reports of diarrhea, constipation, nausea, or vomiting
Marangell et al., 2003	Double blind, randomized, placebo controlled parallel study	Depressed patients. DHA group (n=18), placebo (n=17)	2g/d DHA or placebo (with no further details)	6 weeks	Inclusion criteria include "dietary intake of no more than one serving of fish per week"	Major depression	Adverse effects including "fish" aftertaste, lightheadedness, loose stools, headache and insomnia	2 g/d		Adverse events in the DHA group included a "fish" aftertaste (N=14), belching (N=3), lightheadedness or dizziness (N=3), loose stools (N=2), headache (N=2), and insomnia (N=1). The adverse events in the placebo group included fatigue (N=3), insomnia (N=1), and loose stools (N=1). None withdrew because of adverse events.
Maresta et al., 2002	Double blind, randomized, placebo controlled parallel study	PTCA, Esapent arm (n=125), olive oil placebo (n=132)	Six 1-g capsules of Esapent providing ethyl esters of 3g EPA and 2.1 g DHA or olive oil (placebo)	8 months	Either aspirin (100 to 500 mg/d) or indobufen (200 mg Ibustrin tablets twice daily) beginning at least 48 hours before PTCA	Restenosis prevention	Dyspepsi, Epigastralgia, gastric discomfort	5.1 g/d		Patients received 6g/d 1 month before and after PTCA and 3 g/d for additional 6 months. 4 patients reported GI symptoms were equally distributed between the 2 treatment groups.
Peet and Horrobin, 2002	Double blind, randomized, placebo controlled parallel study	Patient with depression. 1g/d EPA arm (n=17), 2g/d EPA arm (n=18), 4g/d EPA arm (n=17), placebo (n=18)	8 capsules/d. Each capsule contains 500 mg of either EPA (ethyl esters) or paraffin. Placebo (4g paraffin), test 1 (1g EPA+3g paraffin), test 2 (2g EPA + 2 g paraffin), test 3 (4 g EPA).	12 weeks	Selective serotonin reuptake inhibitor, tricyclic antidepressant, and others	Depression rating scales	GI disturbance	4g/d		GI disturbance events were 4, 7, 8 and 5 in placebo, 1g/d EPA, 2g/d EPA and 4g/d EPA, respectively. The evens were linked to 4g of an oily substance, no to EPA as ethyl ester.

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Pontes-Arruda et al., 2006	Double blind, randomized, placebo controlled parallel study	Patients with severe sepsis or septic shock, treatment (n=55), control (n=48)	Tube-fed enteral diet enriched with EPA fish oil plus γ-linolenic acid (GLA; borage oil) (providing 4.9 ± 0.14 g EPA/d and 2.2 ± 0.06 g DHA/d). An isonitrogenous, isocaloric standard diet as a control	28 d or until death		All-cause mortality	Diarrhea and dyspepsia	7.1g/d		Diarrhea (n=9 in test versus 7 in control); dyspepsia (n=1 in test versus 0 in control)
Puri et al., 2005	Double blind, randomized, placebo controlled parallel study	Patients with Huntington disease. EPA arm (n=67), placebo arm (n=68)	2 g/day ethyl-EPA (code name LAX-101, purity >95%) or four 500-mg capsules/d (liquid paraffin).	12 months		Total Motor Score 4 subscale	GI side effects	2g/d		No significant difference in diarrhea and loose stool between EPA (as ethyl ester) and placebo.
Reis et al., 1989	Double blind, randomized, placebo controlled parallel study	Patients after PCI. Ethyl ester fish oil (n=74), TAG fish oil (n=76), placebo (n=72)	Twelve 1-g capsules/d of SuperEPA (providing 6g/d n-3 fatty acids, ethyl ester form), or Promega (providing 6g/d n-3 fatty acids, TAG form),or olive oil	6 months	Aspirin, dipyridamole and calcium-channel blockers,	Prevention of restenosis	Gastrointestinal discomfort		6g/d	Side-effects, primarily GI disturbances, were common in two fish oil groups. 48% in the treatment groups noted GI side effects compared with 22% in the placebo group (p<0.001); 18% in the treatment groups noted bad taste compared with 4% in the placebo group (p<0.01).
von Schacky et al., 1999	Double blind, randomized, placebo controlled parallel study	Patients with angiographically proven coronary artery disease. Fish oil arm (n=111), placebo arm (n=112)	First 3 month, six 1-g capsules/d; next 21 months, three 1-g capsules/g. Each capsule contains an oil mixture with 35.4% EPA, 21.5% DHA and 9.7% DPA (fish oil arm) or an oil mixture with no marine n-3 fatty acid.	24 months		Coronary atherosclerosis	Gastrointestinal discomfort	4g/d (3 mo), 2.0g/d (21 mo)		Mild gastrointestinal discomfort was reported in 3 placebo recipients and four fish oil recipients.
Zhu et al., 2008	Double blinded, placebo controlled	Patients with NAFLD associated with hyperlipidemia. Seal oil arm (n=72), placebo arm (n=72)	6g/d n-3 PUFAs from seal oil or control. No detailed information was provided for seal oil and placebo.	24 weeks		Symptom scores, liver alanine aminotransferase (ALT) and serum lipid levels	Gastrointestinal discomfort	6g/d		Gastrointestinal complaints of increased fecal frequency, epigastria, and defecation were occasionally noted in 8 of the 134 patients; but these adverse effects were not significantly different in the two groups.

AA=arachidonic acid, ARDS= Acute respiratory distress syndrome due to sepsis/pneumonia, trauma or aspiration injury, NAFLD = nonalcoholic fatty liver disease, PTCA=percutaneous transluminal coronary angioplasty; PCI=percutaneous coronary intervention, commonly known as coronary angioplasty;

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