Review Article

FISH LIPIDS FUNCTIONALITY IN HEALTH AND DISEASE

ABSTRACT

Aim: This literature review discusses the roles of fish lipids in health and disease.
METHODOLOGY **: Duration and location**: It was done between July 2021 and September 2022 by the author at the Department of Food Science and Nutrition, Karatina University, Kenya

Results: Adipocyte overabundance can result in cholesterol plaque deposition in arterial walls, which is a risk factor for diabetes, hypertension and cardiovascular diseases (CVD). Cholesterol is required for many cellular processes and its availability in oligodendrocytes may be the limiting factor in brain maturation, myelination and neurotransmission. The ω-3 and 6 fatty acids regulate cholesterol metabolism, blood clotting and control inflammation. They are important for brain activity, structure and function, form nerve cell membranes, and insulate neurons. Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) are associated with reduced risk of CVD, cardiac arrhythmias and sudden cardiac death by reducing small, dense, low-density lipoprotein (sdLDL) particles, which are more atherogenic and hence can shift some sdLDL to larger more buoyant LDL particles that are likely to reduce the risk of CVD. EPA is anti-atherosclerotic, anti-inflammatory, reduces platelet aggregation, increases vasodilation and lowers plasma triglycerides. DHA is necessary for cognitive development and visual function, while DPA reduces platelet aggregation, improves lipid metabolism, reduces endothelial cell migration and improves resolution of chronic inflammation. Regular intake of EPA and DHA is important for nursing or pregnant women, as a child needs DHA to form the brain and other parts of the nervous system up to about 2 years of age.

Conclusion: The roles of DPA vis-a-vis those of EPA and DHA require further investigation.??? While regular intake of the ω-3 FA seems beneficial for brain health and alleviation of major mental-depression, further research is needed to better understand their roles in brain health and in related dystrophies. ??????

Comment [I1]: THIS OBJECTIVE DOES NOT OBJECTIVELY DEFINE WHAT THE OBJECTIVE OF THIS WORK IS IN REALITY.
ACCORDING TO THE AUTHORS THE OBJECTIVE IS TO DISCUSS. EX. (Discuss the roles of fish lipids in health and disease.) WHY IS THIS DISCUSSION NECESSARY.)??????

Comment [I2]: 307 words. /300 words

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METHODOLOGY MISSING)
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Comment [I3]: WHAT ARE THE RESULTS OF THE RESEARCH IN REALITY??????

Comment [I4]: Abstract
The abstract should be concise and informative. It should not exceed 300 words in length. It should briefly describe the purpose of the work, techniques and methods used, major findings with important data and conclusions. Different sub-sections, as given below, should be used. No references should be cited in this part. Generally non-standard abbreviations should not be used, if necessary they should be clearly defined in the abstract, at first use.

Comment [I5]: WHAT WAS THE RESULT OF YOUR BIBLIOGRAPHIC STUDY ON THIS TOPIC OF FISH LIPIDS????
WHAT DATA INDICATE THE RESULTS CONSULTED IN THE LITERATURE ON THE TOPIC?????

WHAT APPEARS AS A RESULT IS NOT INDICATED AS A RESULT OF A REVIEW, BUT AS THE BASIC KNOWLEDGE THAT THIS FISH FATTY ACIDS HAS

Comment [I6]: CONCLUSIONS: THE ARTICLE'S CONCLUSIONS MUST REFLECT THE MAIN RESULTS ACHIEVED IN THE RESEARCH.

THESE CONCLUSIONS HAVE NO RELATION TO THE RESEARCH PERFORMED.

Conclusions
This should briefly state the major findings of the study.

Keywords: Marine omega-3 and 6 fatty acids, brain development, depression

Abbreviations

ALA---Alpha linolenic acid
ARA---Arachidonic acid
CHD---Coronary heart disease
CLA---Conjugated linoleic acid
CM---chylomicrons
CNS---Central nervous system
CVD---Cardiovascular diseases
DHA---Decosahexaenoic acid
DPA---Decosapentaenoic acid
EFA---Essential fatty acids
EPA---Eicosapentaenoic acid
FA---Fatty acids
FFA---Free fatty acids
GLA---Gamma linoleic acid
HDL----High density lipoprotein
LA---Linoleic acid
LDL---Low density lipoprotein
LDL-C---Low density lipoprotein cholesterol
MUFA---Mono-unsaturated fatty acids
NCD---Non-communicable diseases
PNS---Peripheral nervous system
PUFA---Polyunsaturated fatty acids
sdLDL---Small, dense, low-density lipoprotein
SFA---Saturated fatty acids
TAG---Triacylglycerol
TC-Total cholesterol
TFA---Trans fatty acids
TG----Triglycerides
UFA---Unsaturated fatty acids
VLDL---Very low density lipoprotein

Comment [I7]: Abbreviations

Non-standard abbreviations should be listed and full form of each abbreviation should be given in parentheses at first use in the text.

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INTRODUCTION

Oils and fats are a group of heterogeneous organic compounds that are made up of three fatty acids (FA) attached to a glycerol molecule, and can thus be described as triesters of FA with glycerol. Fats and oils differ in the nature of the FA on the chain and in the degree of unsaturation of the constituent FA. Fats are mainly composed of saturated fatty acids (SFA) and trans FA (TFA), while in oils, monounsaturated FA (MUFA) and polyunsaturated FA (PUFA predominate [AOCS, 1998-1]. FA may also be linear or branched and contain hydroxyl, methyl or cyclopropane groups.

Fats and oils are classified under lipids, although steroid hormones, waxes and compound lipids are also included. The latter are complexed with other compounds to form glycolipids, phospholipids and lipoproteins. Food lipids are important for human health and the general functioning of the body and to prevent disease. This review of the literature provides an overview of food lipids with a focus on fish lipids, and specifically on the merits and demerits of their consumption for human health and as potential disease-preventive and curative agents. The review will include brain health, with passing mention of the health maintenance and curative role of fish lipids on cardiovascular diseases (CVD) and diabetes mellitus, along with its less understood roles in decreasing mental depression.

Material and methods

Give adequate information to allow the experiment to be reproduced. Already published methods should be mentioned with references. Significant modifications of published methods and new methods should be described in detail. This section will include sub-sections. Tables & figures should be placed inside the text. Tables and figures should be presented as per their appearance in the text. It is suggested that the discussion about the tables and figures should appear in the text before the appearance of the respective tables and figures. No tables or figures should be given without discussion or reference inside the text.

METHODOLOGY ????????

WHERE IS THE METHOD USED????

CADE OF THE WORKPLACE??? DEPARTMENT???? UNIVERSITY OR SCIENTIFIC INSTITUTION???? CITY COUNTRY????? RESEARCH DATE?????

Results & Discussion

Comment [I8]: Introduction

Provide a factual background, clearly defined problem, proposed solution, a brief literature survey and the scope and justification of the work done.

Comment [I9]: WRONG. DO NOT FOLLOW THE JOURNAL GUIDELINES. IT MUST BE WRITTEN IN PARENTS [X], WITH A CONSECUTIVE ARABIC NUMBER ACCORDING TO THE LITERATURE IN THE ARTICLE .

Reference style

References must be listed at the end of the manuscript and numbered in the order that they appear in the text. Every reference referred in the text must also present in the reference list and vice versa. In the text, citations should be indicated by the reference number in brackets [3].

Comment [I10]: IT COULD BE DEFINED AS A WORK OBJECTIVE, BUT IT WAS NOT. YOU MUST CORRECT THIS ASPECT OF THE ARTICLE.

Introduction

Provide a factual background, clearly defined problem, proposed solution, a brief literature survey and the scope and justification of the work done.

Results should be clearly described in a concise manner. Results for different parameters should be described under subheadings or in separate paragraph. Table or figure numbers should be mentioned in parentheses for better understanding.

RESULTS ????????.

OVERVIEW OF THE FUNCTIONS OF LIPIDS

The major FA of dietary significance are SFA, MUFA and PUFA. Their nature, location on the triglyceride (TG) and amount determines the enzymatic pathways of hydrolysis, absorption and metabolic fate in the body [Decker, 1996]. Energy from their metabolism is required for all physical work and to keep all the moving parts of the body functioning smoothly. Adipocytes are specialized for fat storage and are able to expand almost indefinitely in size. An overabundance of adipose tissue can, however, result in undue stress on the body and can therefore be detrimental to health. Excess fat consumption can lead to the accumulation of cholesterol plaques in the arterial wall, which can thicken the wall over time and are a risk factor for diabetes, hypertension and several types of CVD [Frohnert et al., 2013]. Thus, while some body fat is important for normal body functioning and good health, too much consumption of the wrong types can be detrimental to health, especially when consumed in excess of metabolic requirements and especially as saturated animal lipids (Liu et al., 2017).

The ω-3 and 6 essential FA help regulate cholesterol metabolism [Gibbons, 2003], blood clotting [Sanders, 1996] and control inflammation in the joints, tissues, and blood stream [Calder, 2011]. Fatty acids take part in impulse transmission and signaling, gene transcription and expression and act as biomarkers. These latter functions will not, however, be discussed in this article. Evidence indicates that intake of marine ω-3 FA lowers serum triglycerides (TG) and that replacing SFA with PUFA reduces total plasma cholesterol and low density lipoprotein-cholesterol (LDL-C) [Conway et al., 2021].

Cholesterol, a much maligned molecule, is an important constituent for the normal functioning of the nervous system, and has an important role both during the developmental stage and in the adult. The brain is considered a cholesterol-rich organ as it contains approximately 25% of the body's total cholesterol [Bjorken & Meahoney, 2004]. Cholesterol is the most important component and fundamental functional unit of the mammalian cell membrane [Segatto et al., 2013]. Most of the body's cholesterol is found in the brain in the form of myelin, which contains almost 80% of the cholesterol found in an adult brain [Segatto et al., 2013]. Therefore, it is an important constituent of myelin in the central nervous system (CNS) and peripheral nervous system (PNS). In the CNS and PNS, myelin is synthesized by oligodendrocytes and Schwan cells, respectively [Saher et al., 2011]. As cholesterol is synthesized actively in the CNS in humans and rodents during the first few weeks post-birth, any interruption in its synthesis and availability at this neonatal stage can lead to the development of neurodegenerative disorders [Cunningham et al., 2015]. Cholesterol is also required for cellular processes such as glial cell proliferation, neurite growth, stability of microtubules, synaptogenesis and myelination [Goritz et al., 2015]. As revealed in a review of several studies, the limiting factor in brain maturation, myelination and neurotransmission, seemed to be cholesterol availability for oligodendrocyte functions [Liu et al., 2010]

Comment [I11]: Manuscript structure

The manuscript should be written in English with a simple layout. The text should be prepared in single column format. Bold face, italics, subscripts, superscripts etc. can be used.

The text, excluding the abstract, if required, can be divided into numbered sections with brief headings. Starting from introduction with section 1. subsections should be numbered (for example 2.1 (then 2.1.1, 2.1.2, 2.2, etc.), up to three levels.

Comment [I12]: TIP: NUMBER EACH SECTION TO EASIER READING.
EX. 2.0 - OVERVIEW OF THE FUNCTIONS OF LIPIDS
2.1-
2.1.1 ETC.

Comment [I13]: THROUGHOUT THE ARTICLE THE REFERENCES DO NOT FOLLOW THE GUIDELINES OF THE JOURNAL

References must be listed at the end of the manuscript and numbered in the order that they appear in the text. Every reference referred in the text must also present in the reference list and vice versa. In the text, citations should be indicated by the reference number in brackets [3].

Comment [I14]: THE NAME DOES NOT MATCH THE ARTICLE AND THE BIBLIOGRAPHIC REFERENCE: WHAT IS THE REAL NAME>
??? MEAHONEY OU MEANEY ???????

Omega-3 FA as Functional Lipids

FA composition (especially the levels of ω-3, 6 and 9 FA) and other minor lipid compounds (glycolipids, phospholipids, tocols, phytosterols, aroma compounds, and phenolics) showed health-promoting properties and positively affected the physiological functions` of the human body [Sanders, 1996; Gibbons, 2003, Druce & Bloom, 2005; Calder, 2011].Other functional lipids include the ω-3 FA-alpha linolenic acid (ALA), eicosapentaenoic acid (EPA), decosapentaenoic acid (DPA) and decosahexaenoic acid (DHA); and the ω-6 FA, gamma linoleic acid (GLA), linoleic acid (LA), conjugated linoleic acid (CLA), medium chain triglyceride oils and phytosterols. Fish oils are good sources of EPA, DHA and DPA [Prakash, 2021]. The human body can make most types of fats that it needs from other fats or related raw materials, but not the ω-3 FA. Therefore, the ω-3 FA are essential FA (EFA).as the body cannot make them, and so must be provided in the diet. Good sources of the ω-3 FA include fish, vegetable oils, nuts (especially walnuts), flax seeds and leafy vegetables [Prakash, 2021]. These FA are an integral part of cell membranes throughout the body and affect the functioning of the cell receptors in membranes. Hormones, which are chemicals compounds that are responsible for the regulation of blood clotting, contraction and relaxation of arterial walls and the mitigation of inflammation, are synthesized from FA [Rudkowska, 2013]. Fatty acids also bind to receptors in cells that regulate genetic functions [Freitas & Campos, 2019]. Due to these effects, the ω-3 FA help prevent heart disease and stroke, may help control lupus, eczema, and rheumatoid arthritis and have protective roles with cancer and some other conditions [Leaf, 2007]. As EPA, DPA and DHA are mainly found in fish lipids, they are commonly referred to as the marine ω-3 FA. Alpha linolenic acid, the most common ω-3 FA in most Western diets [Daley et al., 2010], is found in other foodstuffs mentioned above [Prakash, 2021] and some animal fats, especially those from grass-fed animals [Daley et al., 2010].

The strongest evidence for a beneficial effect of ω-3 FA is related to heart disease. These FA seem to help the heart beat steadily and not go into a dangerous or potentially fatal and erratic rhythm (arrhythmia) [GISSI, 1999; Daley et al., 2010]. They also lower blood pressure and heart rate, improve blood vessel function, and, at higher doses, lower TG and may ease inflammation, which has a role in the development of atherosclerosis [Daley et al., 2010]. Several large trials have evaluated the effect of fish or fish oils on heart disease. In the Italian Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardio (the GISSI Prevention Trial), heart attack survivors who took a 1 g capsule containing ω-3 FA every day for three years were less likely to have a repeat heart attack, stroke, or die of sudden death than those who took a placebo [GISSI, 1999]. It was apparent from the same study that the risk of sudden cardiac death was reduced by about 50%. In a more recent Japan EPA Lipid Intervention Study (JELIS), participants who took EPA plus a cholesterol-lowering statin were less likely to have a major coronary event (sudden cardiac death, fatal or nonfatal heart attack, unstable angina, or a procedure to open or bypass a narrowed or blocked coronary artery) than those who took a statin alone [Mozzafarian et al., 2005].

Most modern diets including for Kenyans, are likely to be composed of far more ω-6 than ω-3 FA (except for fishermen and their families living in coastal and lakeside areas or those engaged in fish culture and regularly eat fish and other aquatic organisms), because of the widespread practice throughout Kenya of cooking, and/or frying with sunflower, palm, soya, simsim or maize oils. It has been suggested that this high intake of ω-6 FA could be a factor for CVD, although this has not been supported by evidence with humans.

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?? MAZZAFORIAN OR MAZAFFARIAN????

In a study of an African population in South Africa, the dietary intakes of 1751 apparently healthy adults, stratified according to gender and stratum of urbanization were assessed using a validated quantitative food frequency questionnaire. The mean energy and protein intakes for all strata were adequate. Mean intakes of micronutrients were low in comparison to reference standards, while mean energy distribution was 65% carbohydrate, 12% protein and 22% fat for the rural, farm, informal settlement and middle class urban strata and 57, 13 and 31% for the upper class urban strata, respectively. Intakes of the staple, maize meal, decreased between the urban middle and upper class strata, while fruit and vegetable consumption was low for all groups. Food intakes showed a shift from the traditional high carbohydrate, low fat diet to a diet associated with non-communicable diseases [Macintyre et al., 2002]. This study did not, however, provide the nature, sources and amounts of the different lipid classes in the dietary fat of the study population. Despite the negative publicity about the consumption of the "unhealthy" ω-6 FA, the global studies by Harika *et al.* [2013] concluded that "at present, no sound evidence to suggest that the ω-6 FA should be looked upon as harmful exists, and there is therefore no reason to worry about the proportion of calories they provide within a healthy diet." In the US Health Professionals Follow-up Study, the ratio of ω-6 to ω-3 FA was not linked to the risk of heart disease because both of these FA seemed beneficial [Leitzmann et al., 2004]. Although there may be no question that many consumers could benefit from increasing their intake of ω-3 FA, there is evidence that ω-6 FA also positively influence cardiovascular risk factors and reduce heart disease [GISSI, 1999]. Researchers also examined the possible effects of marine and plant ω-3 FA on prostate cancer. Results from the US-based Health Professionals Follow-up Study showed that men whose diets were rich in EPA and DHA (mainly from fish and other seafood) were less likely to develop advanced prostate cancer than those with low intake of EPA and DHA [Koralek et al., 2006]. Also, some, but not all studies showed an increase in prostate cancer and advanced prostate cancer among men with high intakes of ALA (mainly from supplements), though this effect was inconsistent. In the large US Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, e.g., there was no link between ALA intake and early, late, or advanced prostate cancer [Leyrolle et al., 2021]. Given the presumed importance and benefits of marine ω-3 FA, it is important to eat fish or other seafood 1-2 times/week, particularly fatty (dark meat) fish that are richer in EPA and DHA. This is especially important for women who are nursing babies, pregnant or hoping to become pregnant. From the third trimester until the second year of life, a developing child needs a steady supply of DHA to form the brain and other parts of the nervous system [Oken et al., 2003]. Many women shy away from eating fish because of concerns that mercury and other possible contaminants might harm their babies [Peet and Stokes, 2005], yet the evidence of harm from lack of ω-3 FA is far more consistent, and a balance of benefit vs. risk suggests the need for greater ω-3 FA consumption.

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Functions of EPA, DHA and DPA

Eicosapentaenoic acid, a highly active PUFA, is a precursor of a large variety of bioactive metabolites and has diverse physiological functions in the human body including treatment of various neuropsychiatric disorders-such as bipolar disorder, depression, and schizophrenia [Wani et al., 2015; Pawelczyk et al., 2015; Back, 2017]. Clinical trials of EPA have usually involved oral administration of its ethyl ester. Eicosapentaenoic acid-rich oils are not commonly found as the only omega-3 FA in microorganisms unlike arachidonic acid (ARA) and DHA, which have

been found as the sole PUFA in several microbial species. Eicosapentaenoic acid always occurs along with either ARA or DHA or sometimes both. Algae contain significant amounts of EPA [Borowitzka, 2013]. However, sustainable commercial production is based on genetic engineering using microalgae and bacteria. In addition to their cardiovascular benefits, these FA are sought after for health benefits associated with the brain and eyes, as well as general inflammation and multiple inflammatory conditions. Pregnant and lactating women also take fish oil to benefit their unborn foetuses and babies, respectively. The majority of these oils are sold in developed countries as dietary supplements, pharmaceuticals and in infant formula, for better nutrition and as functional foods. The numerous antiatherosclerotic effects of EPA include antiplatelet aggregation, vasodilation, anti-inflammation and lowering plasma TG [Valdivielso, 2009; Daley et al., 2010]. In a small study, ω-3 FA induced a significant reduction of apoB-48 when added to a fluvastatin treatment with type 2 diabetes mellitus and mixed hyperlipidemia [Hogue et al., 2008]. The effect of fluvastatin on apoB-48 has not been reported. Nevertheless, atorvastatin is able to reduce apoB-48 by increasing CM and CM-remnant catabolism [Mohebi-Nejad & Bikdeli, 2014]. This study was consistent with apoB-48 containing atherogenic lipoproteins that may be further reduced by ω-3 FA, even when added to a traditional statin therapy. The reduction of apoB-48 was over twofold greater with omega-3 FA than fluvastatin (80 mg) alone. Because patients were evaluated for 8 weeks, it is likely that the reduction in apoB-48 could have been even more pronounced over a longer period of time. ApoB-48 only decreased significantly when ω-3 FA were added to the fluvastatin treatment. Only the addition of omega-3 FA to the fluvastatin treatment significantly decreased ApoB-48. Supplementation with ω-3 FA reduced ischemic events and vascular death, even in populations that consume high amounts of fish [Oscarsson & Hut-Camejo, 2014]. A reduction of ApoB-48 containing particles could have been the cause of the benefit. Omega-3 FA may enhance CM clearance by reduced hepatic VLDL synthesis [Lepretti et al., 2018]. Furthermore, ω-3 FA may also reduce intestinal lipoprotein production, which is increased in insulin resistance and type 2 diabetes mellitus [Chewcharat et al., 2020]. In this group of diabetics with mixed hyperlipidaemia, the preliminary results suggested that supplementation with ω-3 FA (4 g) to a higher dose of fluvastatin was accompanied by a significant additional benefit of reducing ApoB-48 particles. This approach may represent a complementary therapy for a reduction of LDL-C, non-HDL cholesterol, and apoB-100 when used with statins.

Seafood, and especially fatty fish and various forms of ω-3 supplements, contain fairly high amounts of DHA. However, the amount of the FA in seafood and in supplements varies [Wang & Daggy, 2017]. Breast milk also contains DHA. It is found esterified into complex lipids within the bloodstream, in adipose stores and in cell membranes. The concentration of DHA in different compartments varies greatly [Wang & Daggy, 2017]. The brain and eye have high DHA contents compared to other organs [Walchuk & Suh, 2020]. The FA is especially concentrated in the grey matter of the brain and in the outer rod segments of the retina [Wang & Daggy, 2017]. In the brain, it is involved in neuronal signalling, while in the eye it affects the quality of vision [Eilander et al., 2007]. It is accumulated in the brain and eye late in pregnancy and in early infancy [Peet & Stokes, 2005; Wani et al., 2015]. A lower DHA content is linked to poorer cognitive development and visual function [Byelashov et al., 2015]. It affects cell and tissue physiology and function through various mechanisms, including alterations in membrane structure and function, in membrane protein function, in cellular signalling and in lipid mediator production [Wang & Daggy, 2017].

Comment [I17]: IN THIS REFERENCE, THE WAY TO REFERENCE THE ARTICLE HAS CHANGED. PEET AND STOKES, OTHER FREITAS & CAMPOS. MUST UNIFY

Decosapentaenoic acid is a part of healthy nutrition, since infants obtain almost as much DPA as DHA from human milk [Wang & Daggy, 2017]. For the general population, the primary DPA sources are fish oil supplements, oily fish, and beef from grass-fed ruminants [Wang & Daggy, 2017; Daley et al., 2010]. Although the DPA levels in fish oils are substantially lower than those of EPA and DHA, concentrated DPA products are now becoming commercially available, and DPA-based drugs are under development [Frigerio et al., 2018]. Epidemiological studies showed that similar to EPA and DHA, DPA is linked to various improvements in human health, perhaps owing to its structural similarity to EPA and DHA molecules. Studies in mammals, platelets, and cell cultures have shown that DPA reduces platelet aggregation [Kaur et al., 2011], and improves lipid metabolism, endothelial cell migration [Bäck & Hansson, 2019], and resolution of chronic inflammation [DiNicolantonio & O'Keefe, 2018]. Further, other *in vivo* and *in vitro* studies have shown that DPA can improve neural health [Kaur et al., 2011]. A human supplementation trial with 99.8% pure DPA suggested that it serves as a storage depot for EPA and DHA in the human body [Kaur et al., 2011]. Future randomized controlled human trials with purified DPA are required to clarify its effects on human health, and confirm the available evidence pointing to its nutritional and biological functions, either as overlapping or are unique from those of EPA and DHA.

Relationship of Omega-3 FA and Other Lipids and Lipid Components

The flesh of fatty fish such as herring, tuna, anchovies, mackerel and salmon is the source of fish oil. The livers of codfishes, Atlantic and Pacific cod are the most commonly used raw material for cod liver oil production. The fish get their omega-3 FA by eating phytoplankton, which absorb microalgae. Microalgae are the original sources of the ω-3 rich FA, found in fish and sea algae. Intake of fish and fish oil, containing ω-3 FA, EPA, DPA and DHA, has beneficial health effects as described by several authors [GISSI, 1999; Daley et al., 2010; Mozzafarian et al., 2005; Kaur et al., 2011]. A high ω-6:ω-3 ratio diet (~11:1), when compared with a diet enriched with EPA and DHA (an ω-6:ω-3 ratio of ~ 3:1) caused a reduction in fasting and postprandial TG as well as sdLDL [Bäck & Hansson, 2019]. Reducing the ω-6:ω-3 ratio with EPA and DHA ingestion also reduced VLDL, increased LDL particle size and increased HDL2. ALA did not confer these benefits. Moreover, increasing the intake of LA from 4.7 to 7% of total energy reduced the protective HDL2 (35.2 vs. 31.7%) (DiNicolantonio & O'keefe, 2018]. Reducing intakes of animal fats and by gradually reducing intakes of TFA, a reduction of cholesterol-raising FA by about one-third seems practical as this will reduce the contribution of these FA to total energy intake to ~ 7-8% [Griffin et al., 2006]. Such a reduction seems reasonable and practical for the general population. Furthermore, because of the potential harmful effects of high intakes of PUFA, their consumption should not exceed current intakes, i.e., ~ 7% of total energy [Griffin et al., 2006]. Thus, the ratio of cholesterol-raising FA to PUFA probably should be ~ 1:1, with total intakes of each being ~ 7% of total energy. The ideal diet of human ancestors which was approximately 1:1 would be desirable, although this may be difficult to achieve in modern diets. Also, MUFA should feature more prominently in diets for better outcomes for an appropriate LDL to HDL balance in humans and it is therefore apparent that a ratio of approximately 1:1.3:1 of SFA:MUFA:PUFA is appropriate [Grundy, 1997]. Avocados, unsalted nuts such as almonds, cashews, peanuts and olives; and cooking oils made from plants or seeds, including olive, canola, peanut, sunflower, soybean, sesame and safflower, are good dietary

sources of MUFA. The ability of the ω-3 FA to increase LDL-C has led to questions on the heart-healthy properties of these FA. However, their ability to transform the atherogenic sdLDL (pattern B) particles to large-buoyant LDL (pattern A), likely outweighs any harm of a higher LDL-C level. It is apparent that the increased lipoprotein gene expression in the plasma is responsible for the increase in large buoyant LDL with marine ω-3 FA consumption [Bäck & Hansson, 2019].

The effect of ω-3 PUFA on the susceptibility of LDL oxidation is controversial. Some studies suggested that marine ω-3 FA, provided as fish, do not increase oxidised LDL with decreases being noted in urinary isoprostane excretion [Hayes, 2002]. Plasma F(2)-isoprostane and malondialdehyde, which are markers of oxidative stress, were significantly lower with fish oil supplementation versus sunflower oil [Mori et al., 2000]. Maximal rates of phosphatidylcholine hydroxyperoxide and cholesteryl linoleate hydroperoxide formation were also significantly lower with fish oil (3.4 g of EPA or DHA/day) compared with safflower oil (10.5 g of LA/day). The authors concluded that "compared to dietary oils that are rich in oleate and linoleate, supplementation of diets of menopausal women with fish oil, does not result in overall oxidation of LDL *ex-vivo*" [Higdon et al., 2000].

In a randomized double-blind cross-over study in familial combined with hyperlipidaemia giving 3.4 g of EPA or DHA/day (as Omacor) for 8 weeks, significantly lowered plasma TG and VLDL (27 and 18%, respectively) [Higdon et al., 2001]. Although there was a decrease in LDL-C by 21%, and a decrease in the denser, slow floating LDL-3 subclass, it was accompanied by an increase in the more buoyant, fast floating LDL-1 and LDL-2 subclasses. This study confirmed that EPA and DHA can increase LDL-C but at the same time reduce LDL density. The average LDL size was not significantly reduced with fish oil, but this was thought to be due to the baseline LDL size (25.0 nm) already being quite low [Higdon et al., 2001]. Thus, the marine ω-3 FA EPA or DHA are associated with slight increases in LDL-C, which may be because DHA downregulates the LDL-receptor, possibly decreasing LDL clearance [Calabresi et al., 2000]. However, DHA was observed to increase LDL size and buoyancy, which indicated less atherogenic LDL [Calabresi et al., 2000].

Another double-blind parallel design placebo-controlled trial with 42 adults found that 4 g/day of EPA and DHA for 12 weeks increased LDL-C by 13% ($p<0.01$). However, there were increases in both large (LDL1 (+2.2 mg/dL) and LDL2 (+2.6 mg/dL)) and sdLDL (LDL3 (+6.3 mg/dL) and LDL4 (+0.04 mg/dL)) [Oelrich et al., 2013]. Only the change in LDL-4 was not statistically significant, while the changes with LDL 1-3 subclasses were all statistically significant. The authors concluded that 'in this population of hypertriglyceridemic adults, dietary supplementation with fish oil resulted in an increase in total LDL-C which was distributed relatively evenly across the range of smaller and more atherogenic as well as larger and less atherogenic LDL particles' [Oelrich et al., 2013].

In a trial carried out for 12 weeks, fifty seven men with dyslipidaemia were randomly assigned to one of three diets enriched with flaxseed oil (~ 25 g of ALA/day), sunflower oil (~ 25 g of LA/day) or sunflower oil plus fish oil (~ 3 g of EPA or DHA/day) [Rodriguez-Levya et al., 2010]. All three diets reduced cholesterol levels. The reduction in TG level was most pronounced with the fish oil group (−23%, $p<0.01$), although it was also reduced in the flax and sunflower plus fish oil groups, but to a lower level. Moreover, the fish oil also group had a significant reduction in small-dense LDL (−22%, $p=0.01$) and a significant increase in HDL2. Additionally, only the fish oil group had a significant reduction in the TC/HDL ratio, which is a better predictor of CHD compared with LDL-C. Both the flax oil and sunflower oil groups caused a

decrease in HDL (−10.5 and −5.6%, respectively), whereas the group given fish oil had a slight ….increase in HDL (+3%). Although the proportion of sdLDL decreased in all groups, it was only significant with the fish oil supplemented diet. A shift in the LDL subclasses towards larger, lighter LDL particles was found with the group supplemented with fish oil. The reductions in TG and sdLDL that occurred after fish oil consumption correlated with an increase in membrane DHA levels, suggesting that if DHA levels are not increased, small-dense LDL may not be reduced. The authors concluded that: an ALA-enriched diet cannot reproduce the predictable changes in plasma lipids and small-dense LDL that are apparent with fish oil [Rodriguez-Levya et al., 2010]. Thus, the overall evidence suggests that marine ω-3 FA reduce sdLDL, which are more atherogenic and hence supplementing with marine ω-3 FA to shift the sdLDL pattern to a larger more buoyant LDL particle pattern is likely to reduce CVD risk. Indeed, at least seven randomized controlled trials have found that ω-3 FA increased LDL particle size or shifted LDL particle distribution from atherogenic pattern B to pattern A [Egert et al., 2009]. The benefit of supplementing with marine ω-3 FA seems therefore to depend on whether someone has pattern B LDL to begin with and if plasma TG levels are significantly low.

Omega-3 Lipids and Brain Health

The glycerophospholipids in the brain contain a high proportion of PUFA derived from the EFA, LA and ALA. The main PUFA in the brain are DHA and ARA [Paluchova et al., 2020]. Experimental studies in animals have shown that diets lacking omega-3 PUFA lead to substantial disturbances in neural function, which in most circumstances can be restored by the inclusion of omega-3 PUFA in the diet [Sinclair et al., 2007]. Epidemiological and cross-sectional studies have also identified a role for long-chain omega-3 PUFA, including DHA, EPA and DPA in the aetiology of depression [Reimers & Ljung, 2019]. In four out of the seven studies done with depressed individuals, using long-chain ω-3 PUFA as adjunct therapy, and in two out of the five studies with bipolar patients, a positive outcome following supplementation with ethyl-EPA or fish oil containing long-chain ω-3 PUFA was reported, although no effect was shown in other participants [Stahl et al., 2008]. The mechanisms to account-for the benefits of the long-chain ω-3 PUFA with depression, included reductions in prostaglandins derived from ARA, which lead to decreased brain-derived neurotrophic factor levels, and/or alterations in blood flow to the brain [Stahl et al., 2008]. In a meta-analysis, EPA, rather than DHA, influenced the final clinical efficacy when the ω-3 PUFA were used as an adjuvant rather than mono-therapy. However, no relation between efficacy and study quality, baseline depression severity, study size, trial duration and age of patients was found. Omega-3 PUFA seemed to be effective in randomized controlled trials on patients with bipolar disorder, whereas no evidence was found in those studies that explored their efficacy on depression symptoms in young populations with perinatal depression, or a primary disease, other than depression and healthy subjects [Grosso et al., 2014]. The main limitation of the meta-analysis was the inability to control the many potential sources of heterogeneity. Despite using a logical grouping of trials, a non-modifiable degree of heterogeneity, due to the specific characteristics of the different trials, still weakened the pooled analysis. However, the inclusion of the updated randomized control trials strengthened the conclusions of the effects of ω-3 PUFA intake on depressive disorders. Therefore, trials with individuals diagnosed with major depressive disorder provided evidence that ω-3 PUFA supplementation had beneficial clinical effects on depression. Evidence of their efficacy was also provided for patients with bipolar disorder [Grosso et al., 2014]. However, according to the

results in other randomized controlled trials in healthy subjects and patients with schizophrenia, Alzheimer's disease and CVD, ω-3 PUFA supplementation seemed to be ineffective [Grosso et al., 2014]. But in a stratified meta-analysis, the effects of trial duration, trial methodological quality, baseline depression severity, diagnostic indication, dose of EPA and DHA in ω-3 preparations, and whether ω-3 FA were given as mono-therapy or augmentation were studied. In 13 randomized, placebo-controlled trials examining the efficacy of ω-3 FA involving 731 participants, the meta-analysis showed no significant benefit of ω-3 FA treatment compared with placebo (standard mean difference, SMD=0.11, 95% confidence interval, CI: -0.04, 0.26). The meta-analysis showed significant heterogeneity and publication bias. Nearly all evidence of an omega-3 benefit was removed after adjusting for publication bias using the trim-and-fill method (SMD=0.01, 95% CI: -0.13, 0.15). However, in trials of lower methodological quality, trials of shorter duration, trials which utilized completers rather than intention-to-treat analysis, and those in which study participants had greater baseline depression severity, secondary analyses suggested a trend towards increased efficacy of ω-3 PUFA. Recent published trials suggested a small, non-significant benefit of ω-3 FA for major depression and therefore nearly all of the treatment efficacy observed in the literature may be attributed to publication bias [Bloch & Hannestad, 2012]. Studies done specifically on the association between ω-3 intake and depression reported contrasting results, suggesting that the preventive role of ω-3 PUFA may depend on other less understood factors [Grosso et al., 2014], therefore suggesting the need for further research in this area.

Recommended Dietary Marine Omega-3 FA Intake

Due to the importance and benefits of marine omega-3 FA, it is important to eat fish or other seafood 1-2 or more times/week, particularly fatty (dark meat) fish that are richer in EPA and DHA [Calder, 2006]. This is especially important for women who are pregnant or hoping to become pregnant and nursing mothers. A developing child needs a steady supply of DHA to form the brain and other parts of the nervous system beginning in the third trimester until the second year of life [Mallick et al., 2019]. A 70% decrease in total mortality was observed in a secondary prevention of CVD study, with a ratio of 4:1 (of ω-6:ω-3 FA) (Simopoulos,2002). A ratio of 2.5:1 reduced rectal cell proliferation in patients with colorectal cancer [Simopoulos, 2002], whereas a ratio of 4:1 with the same amount of ω-3 PUFA had no effect. In women with breast cancer the lower ω-6:ω-3 FA was associated with decreased risk [Simopoulos, 2002]. The lower ω-6:ω-3 FA A ratio of 2-3:1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5:1 had a beneficial effect on patients with asthma, whereas a ratio of 10:1 had adverse consequences [Simopoulos, 2008]. These studies indicated that the optimal ratio may vary with the disease as chronic diseases are multigenic and multifactorial [Simopoulos, 2002, 2008]. Therefore, it is possible that the therapeutic dose of ω-3 FA will depend on the degree of severity of a disease resulting from a genetic predisposition. In developed and modernizing societies, a lower ratio of ω-6:ω-3 FA seems more desirable in reducing the risk of many of the chronic diseases of high prevalence. It is generally apparent that a ratio of ~ 1:1.3:1 of SFA: MUFA: PUFA is appropriate for a desirable LDL/HDL balance in humans [Grundy, 1997], which is close to the palaeontological dietary intake of 1:1 [Simopoulos, 2002]. Thus excessive amounts of ω-6 PUFA and a higher ω-6:ω-3 ratio, as is found in today's diets, promote the pathogenesis of many diseases, including CVD, cancer, inflammatory and autoimmune diseases.

However, higher levels of ω-3 PUFA (a lower ω-6:ω-3 ratio) have suppressive effects. Dietary ARA and LA increase the risk for CVD in those with the variants, whereas dietary intake of EPA and DHA, the major FA in fish lipids decrease the risk [GISSI, 1999].

Demerits of Eating Excessive Amounts of Fish Oils

As much as fish oil has been shown to be beneficial to human health and even some disease conditions, deleterious effects of either too much or too little consumption do occur.

Both fish oil and cod liver oil are generally considered safe, but caution and medical advice is called for before taking them. Both of them might not be safe for all people and may also cause minor side effects. The most common use of fish oils has been for children to relieve colds and headaches. However, medical advice is still necessary, especially for people with fish and shellfish allergies, and those with heart and blood conditions.

Cod liver oil has been associated with belching, nosebleeds, heartburn and blood thinning [Clarke, 1990; Akintoye et al., 2018], although a recent systematic review by Bergtrup *et al.* [2017] refutes the bleeding claim. Fish oils may also contain high levels of vitamins A and D, which when taken in excess may cause avitaminosis [Schmitt et al., 2020]. Fish oils normally contain high amounts of vitamins A and D, which when taken in excess may cause avitaminosis [Schmitt et al., 2020]. Vitamin D toxicity can lead to a buildup of calcium in the blood (hypercalcaemia), which can cause nausea, vomiting, weakness, and frequent urination [Schmitt et al., 2020]. Nausea and vomiting, weakness, and frequent urination can result from a buildp of calcium in the blood (hypercalcaemia), which is due to Vitamin D toxicity [Schmitt et al., 2020]. Vitamin D toxicity may sometimes progress to bone pain and kidney problems, such as the formation of calcium stones. Intake of excess Vitamin A on the other hand can lead to increased intracranial pressure (pseudotumour cerebri), dizziness, nausea, headaches, skin irritation, pain in the joints and bones, coma, and even death [Penniston & Tanumihardjo, 2006]. Consuming too much vitamin A over a long period of time can cause coarse hair, partial loss of hair (including the eyebrows), cracked lips, and dry, rough skin, while chronic consumption of large doses of the vitamin can cause liver damage and birth defects in a foetus [Penniston & Tanumihardjo, 2006]. Those taking excessive amounts of supplements of these vitamins are therefore advised to exercise caution. Pregnant women should also be cautioned as excess fish oil intake may cause blood clotting or nosebleeds, nausea, loose stools, rash, indigestion and fish-tasting burps, reduced vitamin E levels and even a spontaneous abortion [Schmitt et al., 2020], and interactions with contraceptive medications, weight loss drugs containing orlistat and blood medications.

Fish oils contain high levels of EPA and DHA as well as vitamins A and D, some of which may confer the anti-inflammatory properties of fish oils [Valdivielso et al., 2009; Daley et al., 2010]. The presence of vitamins A and D may also contribute to other beneficial effects of fish lipids, for optimal human health. The functions of fish lipids in CHD, ocular and retinal health, bone health, and dementia have been documented [Daley et al., 2010; Bloch & Hannestad, 2012]. But they also continue to be implicated as important remedies in similar disease conditions [Shahidi & Ambigaipalan, 2018].

CONCLUSION

Comment [I18]:
Conclusions

This should briefly state the major findings of the study.

IT MUST DEFINE WHICH RESULTS ARE THE PRODUCT OF THE BIBLIOGRAPHIC REVIEW AND THUS DEFINE THE CONCLUSIONS, WHICH HAVE TO DO WITH THE OBJECTIVES OF THE RESEARCH.

Lipids have numerous functions in human metabolism and health. Cholesterol availability in oligodendrocytes seems to be the limiting factor in brain maturation, myelination and neurotransmission. The ω-3 and 6 fatty acids regulate cholesterol metabolism, blood clotting and control inflammation. They are important for brain activity, structure and function, form nerve cell membranes, and insulate neurons. An ALA-enriched diet cannot reproduce the predictable changes in plasma lipids and the LDL pattern that is produced with fish oil. Marine omega-3 FA cause a significant increase in HDL2, LDL particle size and shift LDL particle distribution from atherogenic small, dense LDL particles (pattern B) to large, buoyant particles (pattern A). These benefits are likely to occur only when TG levels are significantly low and one has pattern B LDL to begin with. Anti-atherosclerotic effects of EPA include antiplatelet aggregation, vasodilation, anti-inflammation and maintaining low plasma TG levels. DHA functions through alterations in membrane structure and function, in membrane protein function, cellular signalling and lipid mediator production. Low DHA levels have been linked to poor cognitive development and visual function. Decosapentaenoic acid reduces platelet aggregation, improves lipid metabolism, endothelial cell migration, and resolution of chronic inflammation. While a low ratio of ω-6 to ω-3 seems to alleviate most disease conditions, the beneficial ratio of ω-6 to ω-3 PUFA seems to differ with different diseases. Clarification on whether the nutritional and biological functions of DPA are unique or overlap with those of EPA and DHA needs further study. The roles of ApoB particles in relation to other lipids remain unclear. Meta-analyses on major depression suggested a small, but non-significant benefit, implying that the preventive role of ω-3 PUFA may depend on other not yet understood factors. The roles of marine ω-3 FA on brain health are being established, but mechanisms of action are not yet clear, especially with depression.

Notes

ApoB-48: Is the primary apolipoprotein of CM, VLDL, Lp(a), IDL (intermediate density lipoprotein), and LDL (commonly known as the "bad cholesterol"), which is responsible for carrying fat molecules (lipids) including cholesterol around the body to all cells within all tissues. While all the functional roles of ApoB within the LDL (and all larger) particles remain unclear, it is the primary organizing protein (of the entire complex shell enclosing/carrying fat molecules within) of the particles and is required for the formation of these particles.

CM (chylomicrons): Are the largest lipoproteins, with diameters of 75–600 nm. They have the lowest protein-to-lipid ratio (being about 90% lipid) and therefore the lowest density. CM are synthesized by the absorptive cells of the intestinal lining and are secreted by these cells into the lymphatic system which joins the blood circulation at the subclavian vein. They transport lipids from the intestinal tract to body cells for further metabolism.

***De novo* synthesis**: Refers to the synthesis of complex molecules from simple molecules such as sugars or amino acids, as opposed to recycling after partial degradation. For example, nucleotides are not needed in the diet as they can be constructed from small precursor molecules such as formate and aspartate. Methionine, on the other hand, is needed in the diet because while it can be degraded to and then regenerated from homocysteine, it cannot be synthesized *de novo*.

Myelin: Is an insulating layer, or sheath, that forms around nerves, including those in the brain and spinal cord. It is made up of protein and fatty substances. The myelin sheath allows electrical impulses to transmit quickly and efficiently along the nerve cells. If myelin is damaged, the impulses slow down.

A neurite: Refers to any projection from the cell body of a neuron. This projection can be either an axon or a dendrite.

Oligodendrocytes: Are a type of large glial cell found in the central nervous system. Oligodendrocytes produce the myelin sheath that insulates neuronal axons (analogous to Schwann cells in the peripheral nervous system), although some oligodendrocytes (called satellite oligodendrocytes) are not involved in myelination.

Small, dense LDL (**sdLDL**): Are a sub-class or fraction of LDL that seems to be more atherogenic than the larger LDL sub-fractions. sdLDL is characterized by an enhanced ability to penetrate the arterial wall which makes it a potent source of cholesterol for the development of atherosclerotic plaque.

Comment [I19]: ???????? UNUSUAL????

REFERENCES

Comment [I20]: REVIEW AND CORRECT ERRORS

Reference style

References must be listed at the end of the manuscript and numbered in the order that they appear in the text. Every reference referred in the text must also present in the reference list and vice versa. In the text, citations should be indicated by the reference number in brackets [3].

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Note: Authors are also encouraged to add other database's unique identifier (like PUBMED ID).

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