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REVIEW ARTICLE

Is There Something Fishy About Fish Oil?

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Abstract: ***Background:*** Fish is consumed as food worldwide and is considered as a rich source of essential nutrients required for a healthy life. Supplementation with fish oil has been adopted as a solution to prevent or cure many pathophysiological states and diseases by both the professionals and the civil population. The beneficial effects are, however, being questioned, as some controversial results were obtained in clinical and population studies.

Methods: Critical evaluation of studies regarding known effects of fish oil, both in favour of its consumption and related controversies.

Results: From the literature review, contradictory allegations about the positive action of the fish oil on human health emerged, so that a clear line about its beneficial effect cannot be withdrawn.

Conclusion: Scientific results on the application of fish oil should be taken with caution as there is still no standardised approach in testing its effects and there are significantly different baselines in respect to nutritional and other lifestyle habits of different populations.

Keywords: Fish oil, ω-3PUFA, CVD, diabetes, cancer, controversies.

1. INTRODUCTION

Fish is consumed as food worldwide and is considered as a rich source of the essential macro- and micro-nutrients required for a healthy life [1]. It is the richest source of vitamin D in its natural form [2] and it contains high-quality proteins and essential fatty acids (FA). Oil derived from fish is widely used for both preventive and therapeutic purposes, as it was shown that its application may ameliorate some disease states. Consumption of fish oil (FO) was, however, also associated with certain health disturbances, leading to a debate questioning its beneficial potential. The known effects of FO, both in favour of its consumption and those which alert to be more cautious are reviewed in this article.

2. FO AND ITS FATTY ACIDS

If the fat content in fish is low (1-2 %), the fish is called "white", and if it is high (5-20 %), the fish is called "oily" or "blue" [3]. Both types of fish contain the same fats that are essential for human health. FO contains ω-3 (omega-3) polyunsaturated FAs (PUFAs) [4]. The most abundant ω-3 PUFAs in fish are EPA (eicosapentaenoic acid, 20:5) and DHA (docosahexaenoic acid, 22:6), which range in FO from 0.02 to 6 % [5, 6].

The World Health Organisation (WHO) [7] recommends consumption of fish twice a week, as a preventive measure against coronary heart disease and ischaemic stroke. Food and Agriculture Organization (FAO) recommends the consumption of 250 mg/day of EPA+DHA for adult non-pregnant individuals, but there is no consensus on the minimum required dose [8]. In 2012, the European Food Safety Authority (EFSA) concluded that doses up to 5 000 mg/day do not impose a health risk for adults [9]. Accumulation of lipophilic environmental pollutants in fish may limit its intake [10]. Nevertheless, if moderately consumed, fish and FO exert more beneficial than harmful effects and are recommended for nutrition [11].

The consumption of fish depends on many factors such as the national and personal nutrition habits, geographical position of the region (*e.g.* distance from the sea), gross national product, and the price at the local market. The highest consumption of fish in the EU is in Portugal (57 kg/person/year) and the lowest in Hungary (5.2 kg/person/year) [12]. The average fish consumption in the region (Serbia, North Macedonia, and Bosnia and Herzegovina) is around 5.2 kg/person/year, which makes it an area with low usage of this food [13].

Generally, the synthesis of PUFAs involves two major pathways (Fig. **1**):

i) ω-3 pathway - the conversion of α-linolenic acid (ALA, 18:3 ω-3) to EPA, DPA, and DHA by desaturation, elongation, and oxidation reactions [14]. ALA is an essential FA for humans, as they cannot synthesise it. Although the conversion of ALA to EPA and further to DPA and DHA is possible [15], these conversions occur to a very low extent, particularly to DHA [14] and they are somewhat greater in women than in men [16].

ii) ω-6 pathway - the conversion of linoleic acid (LA, 18:2 ω-6) to arachidonic acid (AA, 20:4 ω-6) by the same reactions as mentioned for the ω-3 pathway.

Two enzymes, which are responsible for the initial metabolism of ω-6 and ω-3 FA, are Δ-5 and Δ-6 desaturase. The first reaction in this process is rate-limiting, so the FA which is in excess will react first. The optimal ratio of ω-6 to ω-3 FA should be 1-4:1 since both desaturases have a higher affinity for ω-3 FA [17]. PUFAs are sub-

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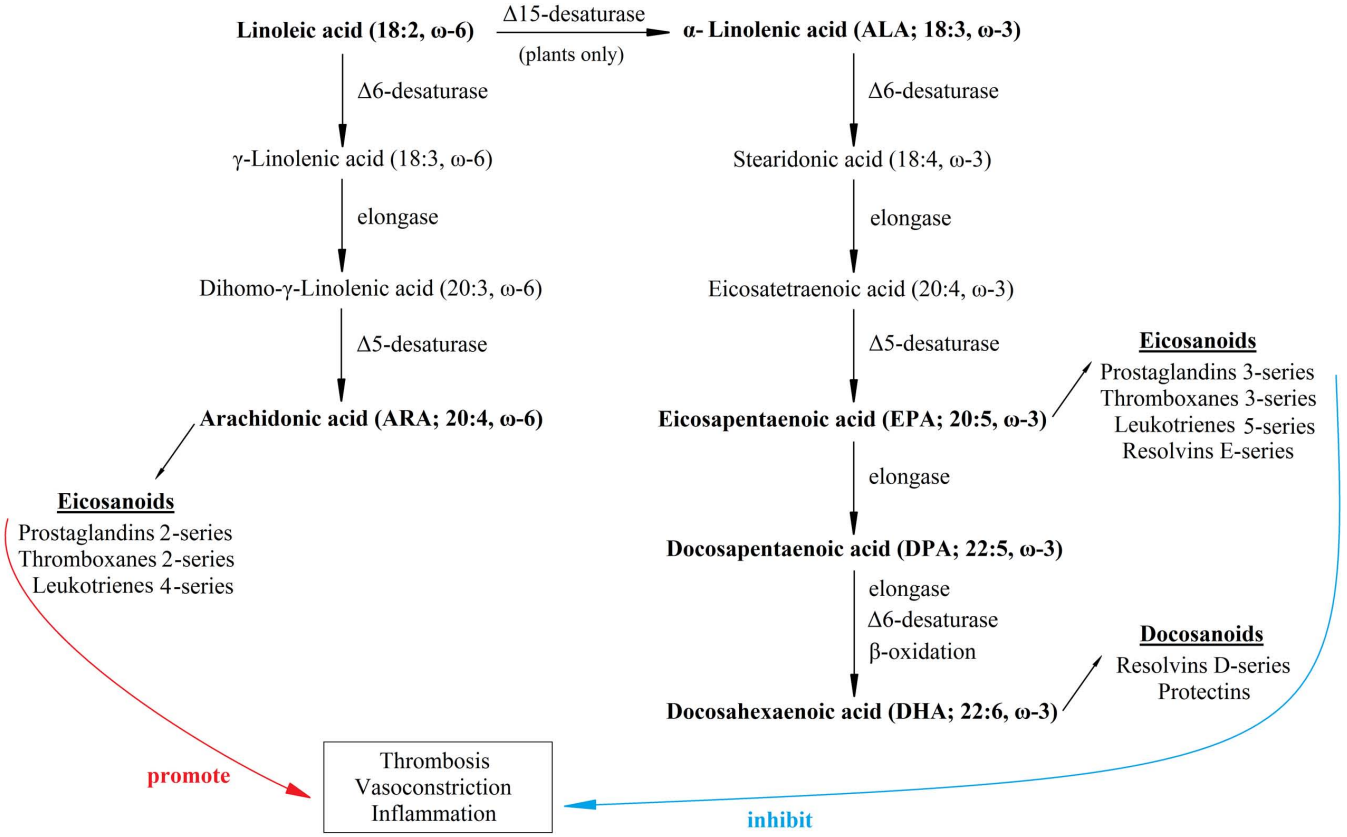

Fig. (1). The metabolism of ω-6 and ω-3 fatty acids.

strates for different enzymes: cyclooxygenases (to produce prostaglandins and thromboxanes), lipoxygenase (to produce leukotrienes), and cytochrome P450 (to produce epoxyeicosatrienoic acids) [18].

Eicosanoids (prostaglandins, prostacyclin, thromboxanes, leukotrienes) play crucial roles in the vascular physiology due to their pro/anti-inflammatory and pro/anti-(platelet) aggregatory effects. They control vasodilatation, vasoconstriction, cell growth, proliferation, and immune response [8, 19]. Specialised pro-resolving mediators (resolvins, protectins, and maresins) have been identified as new ω-3 PUFAs-derived anti-inflammatory mediators, whereas ω-6-derived products are considered as pro-inflammatory mediators [20]. Plasma and cell levels of ω-6 PUFAs in humans tend to be higher than the levels of ω-3 PUFAs due to dietary habits [8]. The concentration of ALA in plasma and tissue phospholipids is usually less than 0.5 % of the total FA [14]. Mammals lack Δ-15 desaturase, an enzyme which converts ω-6 to ω-3 PUFAs, therefore ω-3 PUFAs must be supplied in the diet. Although widely present, ω-3 PUFAs are the most abundant in neurons, retina, and myocardium [21].

Cell membrane fluidity, permeability, protein functions, and interactions are dependent on the membrane phospholipids whose important components are PUFAs. Membrane lipids form specific microdomains, the so-called "rafts", that are essential for the activity of receptors and enzymes [22]. EPA and DHA modulate the formation of "rafts", especially in the neural, immune, and cancer cells [23, 24]. PUFAs participate in and regulate cell signalling cascades and gene expression [25].

Since the effects of ω-3 and ω-6 PUFAs and their derivatives can be seen as antagonistic, their ratio was suggested to be taken into consideration when assessing the inflammatory potential of a diet [26]. In modern Western diets, the ω-6/ω-3 ratio is quite high. This ratio should not exceed 4:1, but in reality, it varies, being 5.5:1 in Denmark, 13.8:1 in France, and up to 27:1 in Spain [14]. Plasma and cell levels of ω-6 PUFAs tend to be higher than the levels of ω-3 PUFAs, due to dietary habits, which include high intake of sunflower, corn oil, and margarine, and relatively low intake of plant seeds and nuts [8]. In Serbian diet, the ω-6/ω-3 ratio is approximately 10:1. The majority of the population in Serbia (80.3 %) uses sunflower oil for daily meal preparation and 18.6 % uses olive oil. Sunflower oil contains mostly LA (62.48 %), oleic acid (26.60 %), and saturated fatty acids (10.3 %), with no ALA [27].

Encapsulated FO is the most common pharmaceutical FO product; one form is intended for the preventive application and the other for the medically prescribed. Therapeutic application of ω-3 PUFAs ameliorates severe hyperlipidaemia and atherogenic parameters, thus lowering the risk of cardiovascular disease (CVD). The American Heart Association (AHA) recommends 2-4 g/day of EPA and DHA for patients with hyperlipidaemia, but under supervision of the physician, as this supplementation may have a negative influence on the bleeding time, LDL cholesterol, and glycaemic control in non-insulin-dependent diabetics [28]. A preventive supplement usually contains 180 mg of EPA and 120 mg of DHA per 1 000 mg of FO. In a formulation intended for therapy, FAs are found in the form of ethyl esters, not triglycerides, which enables their better bioavailability, and in greater amounts (on average, three times more than in supplements). Besides, therapeutic formulations have almost no secondary substances such as pollutants, saturated FA, or cholesterol, as standardised by a Current Good Manufacturing Practices document (not applied for the production of supplements) [29].

3. BENEFICIAL EFFECTS OF FO ON HUMAN HEALTH

3.1. Cardiovascular Disease (CVD)

CVD is the most common cause of human death in the world and is associated with an increased concentration of blood lipids.

Atherosclerosis underlies CVD. It develops over time and is more likely to be prevented if the management of lipid status starts earlier [30]. The main target for this prevention is the reduction of the low-density lipoprotein particles (LDL) [31].

The first observational studies in the 1970s reported that Greenland Inuit population had a low incidence of coronary artery disease (CAD) due to its lifestyle and consumption of the marine fish and arctic mammals, both of which are rich in ω-3 PUFAs [32]. A number of meta-analyses confirmed beneficial effects of ω-3 PUFAs intake on CVD development [33], as regular consumption of ω-3 PUFAs may reduce the incidence of fatal myocardial infarction by 30 % and overall mortality rate by 20 % if the consumption of EPA and DHA were 0.3 to 6 g/day and 0.6 to 3.7 g/day, respectively [34]. In 19 studies, which included 228 864 patients all together, it was shown that fish consumption reduced the risk of fatal coronary heart disease (CHD) by 17 % [35]. In this meta-analysis of 19 studies, 8 reported that fish intake was on average 36 g/day. Meta-analysis reported by He *et al.* [36] and Xun *et al.* [37], revealed that the consumption of fish 2-4 times/week may reduce the CHD mortality rate by 23 % compared to the diet without fish or with less than one fish meal/month, whereas according to Xun *et al.* [37], the reduction in the stroke rate was 9 %, under the same nutritional regime. Two additional meta-analyses stressed a direct benefit of EPA and/or DHA supplementation on the reduction of CHD risk [38, 39]. Meta-study performed by Djoussé *et al.* [40] confirmed that consumption of ω-3 PUFAs from fish lowers the risk of heart failure and stroke, whereas a population-based cohort study of Mozaffarian *et al.* [41], showed that boiled or baked fish (but not fried) lowers the risk of the incidence of congestive heart failure by 20 % with an intake 1-2 times/week, 31 % with an intake 3-4 times/week, and 32 % with an intake greater than 5 times/week. The beneficial effect of FO on heart arrhythmia was also shown. Consumption of 3.6 g of EPA and DHA for 6 months by the implantable cardioverter defibrillator recipients led to a significant reduction of the number of ventricular tachyarrhythmic episodes [42]. EPA and DHA administration, 4.2 g/day for the first month and 0.85 g/day for the next five months, with EPA to DHA ratio of 0.9:1.5, was associated with a positive effect on the risk of arrhythmia in patients with idiopathic dilated cardiomyopathy [43]. A recent meta-analysis revealed that supplementation with PUFAs, with an intake higher than 3.5g/day, leads to a reduction in the heart rate compared to PUFAs intake lower than 3.5 g/day [44]. Antiarrhythmic effects attributed to ω-3 PUFAs include direct and indirect modulation of the properties of ion channels, membrane composition, and fluidity, as well as anti-inflammatory, anti-fibrotic effects, and modulation of sympathovagal balance [45]. Omega-3 PUFAs positively modify the blood lipid profile, thus, lowering the risk for development of CVD [46]. A study conducted by Zibaeenezhad *et al.* [47] showed that consumption of fresh fish is superior to a prescribed supplementation with ω-3 PUFAs. Participants in the study were divided into two groups: a dietary fish group received 250 g of farmed fish two times/week (they were advised not to take any ω-3 PUFAs supplements) and a supplement group received 2 g of ω-3 PUFAs every day. Participants from both groups consumed a total of 14 g of ω-3 PUFAs per week, for two months. A supplementation of ω-3 PUFAs caused a decrease in the concentration of triglycerides (TG) and total cholesterol, but there was an increase in the concentration of LDL. On the other hand, a dietary fish group exhibited improvement in all blood lipid parameters, and to a greater extent compared to the supplement group. The main mechanism involved in this effect is the enhancement of beta-oxidation of FA which reduces the amount of substrate for the formation of very low-density lipoprotein (VLDL) [48]. Different effects of DHA and EPA on LDL formation were reported [49], as only DHA treatment increased LDL synthesis. Even more, EPA treatment led to an 80 % reduction in non-HDL lipoproteins compared to a 40 % reduction due to DHA treatment. DHA was found to down-regulate the activity of the LDL receptor [50]. High doses of EPA/DHA supplementation (more than 3 g/day) were observed to protect endothelium by acting as anti-oxidant and anti-inflammatory agents [51].

Being a precursor of the family of anti-inflammatory resolvins, EPA co-administered with statin therapy induces plaque stabilisation in patients with the acute CAD [52]. Additionally, ω-3 PUFAs administration (3 g/day for 4 weeks) was shown to reduce the levels of some coagulation factors, such as factor V and thrombin, by 3.1 % and 13.9 %, respectively [53]. There is also a large body of evidence that shows a positive effect on lowering blood pressure (BP). A meta-analysis conducted by Miller *et al.* evaluated seventy randomised control trials (RCT) and concluded that supplementation of more than 2 g/day of EPA+DHA may reduce both systolic and diastolic blood pressure, with the strongest benefits being in hypertensive patients who are not receiving anti-hypertensive medications [54].

A schematic illustration of the beneficial effects of the consumption of FO is given in Fig. (**2**).

3.2. Diabetes Mellitus Type 2

Obesity, sedentary lifestyle, and high-energy diets contribute to an increase in the number of patients with diabetes mellitus type 2 (T2DM). In 2015, 415 million people were estimated to have diabetes and the projected increase is up to 642 million by the year 2040 [55, 56]. T2DM is characterised by impaired insulin secretion, insulin resistance (IR), and vascular complications, resulting in CVD, nephropathy, retinopathy, non-alcoholic fatty liver disease (NAFLD), and other disturbances [57].

Consumption of ω-3 PUFAs was recognised as a dietary and therapeutic intervention in the treatment of T2DM. Gonçalves Leão Coelho *et al.* [58] reviewed the impact of ω-3 PUFAs intake on the glycaemic control in patients with diabetes and the possible mechanisms involved in this process. They have found that supplementation of 0.4-5.2 g/day of ω-3 PUFAs for at least 8 weeks improves the glycaemic control in T2DM patients. They suggested that mechanisms responsible for the improvement of glycaemic control are interconnected with the inflammatory pathways which significantly contribute to the insulin resistance (IR). It was proposed that FA stimulate G-protein-coupled receptors (GPR), which in turn stimulate insulin secretion. GPR40 (expressed in pancreatic β-cells) and GPR120 (expressed in adipose tissue, pro-inflammatory macrophages, and gastrointestinal tract) in particular, are stimulated by ω-3 PUFAs (EPA, DHA), thus affecting insulin secretion. Furthermore, when ω-3 PUFAs bind to GPR120, they stimulate glucose uptake indirectly, by increasing the expression of the glucose transporter GLUT4 in the plasma membrane of muscle cells. Another class of receptors, toll-like receptors (TLRs) were shown to participate in the T2DM pathogenesis. Increased activation of TLR2 and TLR4 contributes to inflammation and ω-3 PUFAs supplementation was shown to suppress the TLR-induced signalling pathways and targeted gene expression, exhibiting an anti-inflammatory effect [58].

Lalia and Lanza [59] summarised the knowledge on the therapeutic value of EPA and DHA supplementation and revealed encouraging observational evidence both in T2DM patients and in non-diabetic people with IR. The strongest effect of the supplementation was seen in the Alaska Eskimo population [60]. A significant improvement in IR and reduction in the level of TNF-α was reported by Rasic-Milutinovic *et al.* [61] who conducted a study with the application of 2.4 g/day of FO for two months, and by Farsi *et al.* [62] who investigated consequences of an intake of 4 g/day of FO during 2.5 months. The research of Lepretti *et al.* [63] stressed the connection between IR, endoplasmic reticulum (ER) stress, reactive oxygen species (ROS) production, and the impairment of the mitochondrial function. They discovered that ω-3 PUFAs stimulate mitochondrial function, reduce ROS production, and attenuate ER stress, leading to the amelioration of IR. In a PREDIMED study

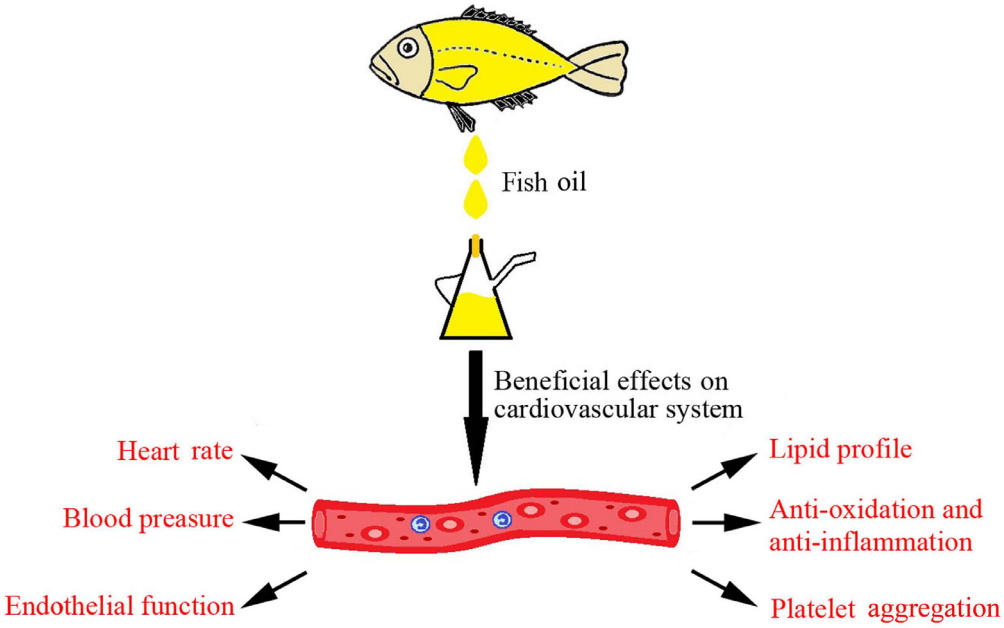

Fig. (2). Beneficial effects of the consumption of FO on the cardiovascular system.

conducted by Sala-Vila *et al.* [64], which lasted for six years, it was shown that the risk of diabetic retinopathy in T2DM patients is reduced by 48 % upon regular consumption of ω-3 PUFAs. The participants in this study were aimed at meeting a dietary recommendation of at least 0.5 g/day of long-chain ω-3 PUFAs, which equals two meals of oily fish a week.

3.3. Metabolic Syndrome and Non-Alcoholic Fatty Liver Disease (NAFLD)

Metabolic syndrome (MetS), a group of interconnected physiological, biochemical, clinical, and metabolic disturbances, is a serious risk factor for the development of CVD and T2DM [65, 66]. MetS can be also seen as a state of chronic, low-grade inflammation, accompanied by IR, visceral adiposity, atherogenic dyslipidemia, endothelial dysfunction, elevated blood pressure, hypercoagulable state - in other words, a general chronic metabolic stress [67]. The effective preventive and therapeutic approaches to deal with MetS include changes in the lifestyle: the loss of weight, diet alteration, and exercise. Application of ω-3 PUFAs from FO was reported to decrease body fat and cholesterol levels as well as to suppress appetite and reduce inflammation leading to a weight loss [68]. A meta-analysis performed by Gao *et al.* [69] investigated the relation between ω-3 PUFAs and insulin sensitivity. It included 17 individual studies with all together 672 participants and the final conclusion was that short-term supplementation with FO increased insulin sensitivity in patients with metabolic disorders. The improvement in the endothelial function, the enhancement of the fat oxidation, and increased energy expenditure were observed upon intake of ω-3 PUFAs [68]. The supplementation of ω-3 PUFAs to individuals with the obesity-associated MetS caused a decrease in their blood TG as well [70]. Beneficial effects of ω-3 PUFAs in lowering blood pressure and reducing vascular inflammatory events can be seen as cardioprotective.

NAFLD is defined as a macrovascular accumulation of fat in more than 5 % of hepatocytes, without increased alcohol intake. It is considered as a global health problem, especially if it progresses into non-alcoholic steatohepatitis (NASH) and further into liver cirrhosis [71]. Consumption of ω-3 PUFAs in FO can reduce lipid accumulation in the liver, as well as the levels of liver enzymes, it can increase insulin sensitivity and may exert an anti-inflammatory effect. Taking into account the confirmed positive effects of FO in the treatment of CVD, its potential in the treatment of NAFLD is encouraging [17, 72].

Nobili *et al.* [17] reviewed the pathogenesis of NAFLD, together with the molecular and clinical evidence of the beneficial effects of the consumption of ω-3 PUFAs in different forms, in children, and in adults. They concluded that high doses of ω-3 PUFAs (especially DHA) may have a beneficial effect in an early stage of NAFLD, by reducing the liver fat content, but there were no convincing effects seen in the later progression of a disease, such as liver inflammation and fibrosis.

In a recently published systematic study and meta-analysis of several RCTs, ω-3 PUFAs supplementation was shown to reduce the level of alanine aminotransferase (ALT) in blood and to improve the function of liver cells in patients with NAFLD [73]. Two meta-analyses, conducted by Yan *et al.* and Musa-Veloso *et al.,* revealed that supplementation of ω-3 PUFAs leads to a reduction in liver fat, ALT, aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), TG, and improved insulin sensitivity [74, 75]. Although there is plenty of evidence that regular consumption of fatty fish benefits metabolism, the positive effect in NAFLD treatment is not straightforward and there is currently no recommended optimal dose for this purpose [17].

3.4. Cancer

Cancer is a widespread pathological state of an organism, characterised by the growth of abnormal cells beyond their usual boundaries, which can then invade adjacent parts of the body and/or spread to other organs. Globally it is considered to be the second leading cause of death, with an estimated 9.6 million deaths in 2018 [76]. Development of the cancer is under the control of various factors (age, genetics, environment, chemical substances, obesity, physical activity, lifestyle, *etc.*). One of the ways to prevent and help treat cancer is through the regulation of diet.

Application of high-energy, high-fat (Western) diet was correlated with an increased chance of developing cancer, as reviewed by Bartsch *et al.* Although the mechanism of fat-related carcinogenesis has not been fully understood, studies have associated it mainly with a high content of trans-monounsaturated fatty acids (C18:1) and a high ω-6: ω-3 ratio (>10:1) of PUFAs. Although most of the pre-clinical studies, conducted on animal or cell line

cancer models, reported beneficial effects of ω-3 PUFAs on cancer treatment, clinical data should be taken into consideration with greater importance [77].

Nutritional habits which include food rich in PUFAs (preferentially ω-3 EPA and DHA) was correlated with a lower incidence of cancer in the population consuming fish and FO more than 3 times/week [78, 79]. This protective effect of PUFAs in parental nutrition can be translated to the offspring, similarly as in the case of mammary tumors [80]. Also, it is a known fact that cancer patients have low baseline status of ω-3 PUFAs incorporated in erythrocytes membranes [81, 82]. A better outcome in patients with colorectal (CRC), prostate, gastric, lung, or breast cancer who practiced these dietary habits was also reported [83-87]. According to these data, daily supplementation of 0.2-3 g of PUFA (with the ratio EPA: DHA being 1:1) during a period of 1 to 6 weeks improves general condition, recovery and the survival rate of patients during and after the therapy.

Several RCTs have shown these beneficial effects. A study performed by Cockbain *et al.* in 2014 found that supplementation of 2 g of EPA in CRC patients undergoing liver resection surgery reduced the vascularity of CRC liver metastasis and improved overall survival compared to controls [88]. Bougnoux *et al.* showed that supplementation of 1.8 g of DHA in metastatic breast cancer patients led to greater overall survival compared to control group [89], while Murphy *et al*. found that application of 2.5 g of EPA+DHA in non-small-cell lung carcinoma patients was followed by an increased response rate and greater clinical benefit. Also, one-year survival tended to be greater in the treated group (60%) *versus* the control group (38.7%) [90]. A study of Ma *et al*. evaluated results from 11 prospective cohort RCTs, and showed that supplementation of EPA (1-6 g) and /or DHA (0.96-1 g) in inoperable pancreatic cancer patients had beneficial effect regarding an increase in body weight and lean body mass, as well as overall surviving rate [91].

While the exact mechanism of the action of these two FA remains unknown, it was proposed that they are incorporated in the membranes of cancer cells, where they participate in increased lipid peroxidation, thus targeting cancer cells for the destruction [92]. Other processes which accompany this incorporation include modulation of the pathways controlled by cyclooxygenase and lipoxygenase, impaired behavior of lipid rafts and disturbances in the signaling pathway exerted *via* GPR.

It is known that many anti-neoplastic drugs are non-selective towards cancer and healthy cells, so the supplementation of DHA and EPA as an adjuvant to standard therapies was shown to reduce negative effects of chemotherapy by modulating an immune response, manifesting anti-inflammatory effects [93], or by exerting other beneficial effects, such as those on gut microbiota in patients with colorectal carcinoma [86].

3.5. Gut Microbiota

Human gastrointestinal tract (GIT) harbors a complex and dynamic population of microorganisms, counting 10-100 trillion of symbiotic microbial cells, which makes roughly 10 times more bacterial than human cells in GIT [94]. Microbiota directly communicates with the host, as bacterial enzymes produce metabolites which are later absorbed by gut enterocytes, thus affecting the physiological processes in the host. The emerging evidence on the importance of the microbiotic flora in the gut is pointing to its vital role both in health and in disease [95]. Alteration in the composition of the microbiota is known as dysbiosis. It can be caused by the pathophysiological state of the host organism and *vice versa* [96]. Dietary habits can significantly affect the composition of the gut microbiota, thus regulating the host-microbiota relation [97]. The effect of dietary fats was not examined thoroughly, although there is growing evidence on their beneficiary impact on the gut microbiota, as reviewed by Constantini *et al.* [98].

Diet supplemented with FO has beneficial effects on the composition of the microbiota and ensures healthy ageing and wellbeing. Menni *et al.* have found a strong correlation between intake of ω-3 PUFAs, gut microbiome diversity, and composition in middle-aged and elderly women [99]. Càndido *et al*. [100] reported that consumption of FO can restore microbiotic composition by affecting short chain FA mainly produced as metabolites of the microbiota, thus influencing energy metabolism and the inflammatory response. The authors recommended avoidance of the saturated FA and recommended intake of ω-3 PUFAs in order to regulate gut microbiota, systematic low-grade inflammation, and to promote mechanisms which control body fat and weight.

Watson *et al.* [101] investigated the effect of ω-3 PUFAs on the fecal microbiome in middle-aged healthy volunteers. Two different formulations of supplements were applied: soft gel capsules and Smartfish drinks. The authors found that both formulations induced similar changes in the composition of FA in red blood cells and promoted a reversible increase in several bacterial genera. However, only drink formulation was associated with a prolonged decrease of AA levels. As can be seen from this study, there is no simple relationship between intestinal microbiome and supplementation of ω-3 PUFAs.

3.6. Asthma and Allergy

Asthma is another prevalent disease, characterised by chronic airway inflammation; it affects approximately 300 million individuals worldwide [102]. The most common form is atopic asthma which is genetically predisposed [103]. In order to reduce the incidence of asthma, researchers investigated the possible anti-inflammatory therapeutic effect of ω-3 PUFAs on the pathogenesis of this disease.

The positive effect of their consumption on asthma and other allergic diseases was demonstrated by Miyata and Arita [104]. They reviewed a large body of evidence and reported that maternal fish intake during pregnancy has beneficial effects on allergic or atopic outcomes in infants. Nutrition in pregnancy plays an important role in the development of the foetus. In the case of asthma, consumption of ω-3 PUFAs can be of particular importance. FO and ω-3 PUFAs supplementation in late pregnancy was shown to exhibit prophylactic potential in the case of asthma in newborns, as shown by Olsen *et al*. [105]. This population-based study, which recruited 533 women with normal pregnancies, supplemented with 2.7 g of ω-3 PUFAs, and 131 pregnant women who received olive oil, demonstrated that children from the ω-3 PUFAs group had a reduction in the hazard rate of asthma by 63 %, whereas the hazard rate of the allergic asthma was reduced by 87 %, after 16 years follow up. Bisgaard *et al.* [106] found that supplementation with FO in the third trimester of pregnancy reduced the risk of wheeze and asthma, as well as the incidence of lower respiratory tract infections in newborns. This clinical trial included 695 children and three years of follow up period. The risk of wheeze or asthma in children was reduced for 30.7% in the treatment group compared to the placebo. A detailed subgroup analysis revealed that the positive effect was the strongest in children whose mothers had blood levels of EPA and DHA in the lowest third of the trialled population [106], pointing out to the importance of determining the proper baseline status.

Omega -3 PUFAs from fish and FO were suggested to alter the synthesis of eicosanoid mediators produced from ω-6 PUFAs such as AA, responsible for the manifestation of the allergic disease. Increased consumption of EPA + DHA results in an elevated proportion of these FAs in the membranes of immune cells, at the expense of ω-6 PUFAs. As already said, ω-6 PUFAs act as substrates for enzymes responsible for the synthesis of prostaglandins, thromboxanes, and leukotrienes, all pro-inflammatory molecules. ω-3 PUFAs can also be substrates for the same enzymes, in which case the products of enzymatic reactions are pro-resolving mediators such as resolvins (produced from both EPA and DHA) as well as

protectins and maresins (produced from DHA). These compounds are potent anti-inflammatory mediators. An increased intake of ω-3 PUFAs acts dually, on one side the production of pro-inflammatory AA-derived mediators is decreased, and on the other, the synthesis of anti-inflammatory mediators is promoted [25].

FO supplementation in healthy babies was shown to significantly influence the composition of the erythrocyte FA by increasing ω-3 and decreasing ω-6 PUFAs, contributing to the increased production of anti-inflammatory and reduction of pro-inflammatory cytokines, and causing faster maturation of the immune system [107]. A meta-analysis conducted by Best *et al.* [108] revealed that supplementation of FO in pregnant mothers may result in a reduced risk of allergic disease in an offspring.

3.7. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is the most common autoimmune disease affecting the synovium and causing chronic inflammation of joints, destruction of the cartilage, functional disability, systemic complications, and sometimes even death [109]. The main symptoms of the disease are joint stiffness, swelling, pain, and loss of mobility [110]. RA is more common in women (18 %) than in men (9.6%) [111]. Even though it primarily affects the joints of hips, knees, and hands [112], it can also have an influence on tissues such as ligaments and subchondral bone [113]. Prolonged life expectancy is one of the main reasons which makes RA one of the major factors of disability in the elderly.

The etiology of RA lies in the infiltration of elements of the immune system in the joint tissues. Activated T cells, macrophages, antigen producing B cells and synoviocytes, the fibroblast-like synovial cells, invade the synovium, that is, the tissue lining the joints. These cells stimulate the production of pro-inflammatory molecules, such as prostaglandin E2, tumor necrosis factor (TNF), interleukin (IL)-1, and cytokines [114, 115].

As already mentioned, ω-3 PUFAs possess strong anti-inflammatory action and that is why numerous RCT trials showed they could ameliorate and improve clinical parameters of RA. Rajaei *et al.* conducted a 12 week, double-blind, placebo-controlled RCT with sixty patients (49 females and 11 males) in the early stage of RA, that is the individuals which were diagnosed with the disease no more than six months ago. They adhered to earlier treatment regimens, by taking the same medication which was prescribed by the doctors. The patients consumed two ω-3 capsules per day, which contained 1.8 and 2.1 g of EPA and DHA, respectively. Every four weeks for three months, they went for a clinical and laboratory evaluation, where, according to the American College of Rheumatology (ACR), various parameters were examined. Testing was carried out with the help of the disease activity score (DAS-28) which contains variables for the number of swollen and painful joints, erythrocyte sedimentation rate (ESR), and overall assessment of the patient's general health status [116]. Forty-nine patients successfully completed the study and results showed improvement in many characteristics of patients with active rheumatoid arthritis who received diet supplements of ω - 3 along with the standard treatment. The overall effect of three months use of ω-3 capsules leads to significant reduction of pathologic parameters (mean morning stiffness decreased in the ω-3 group from 128 minutes to 40 minutes; the average number of tender joints was reduced from 21 to 5 joints; the number of swollen joints dropped from 10 to 3; the average ESR decreased from 39 to 16 mm/h and pain sensation was markedly reduced). There was a 72 % drop of analgesic use among the respondents, and of that 32 % completely discontinued the use of pain medication and 40% reduced the dose [117].

Similar, a three months study was performed by Veselinovic *et al.* with sixty female patients who were diagnosed with RA in 12-180 months time period before the beginning of the study. Except for the change in clinical parameters, the study wanted to monitor the change of plasma phospholipids, which can be considered a biomarker of FA used in nutrition. The new FA introduced, γ-linolenic acid (GLA), which, although ω-6 fatty acid, possesses an anti-inflammatory effect. The patients were assigned in three groups. The first group received 5 capsules of FO (each capsule contained 1g of FO with 300 mg of DHA, 200 mg of EPA, and 100 mg of other ω - 3 PUFAs) per day. The second group received 2 capsules of FO and 2 capsules of Evening Primrose Oil (EPO), the source of GLA (one gel capsule contains 1300 mg of evening primrose oil with 949 mg of LA and 117 mg of GLA) per day, and the third group represented controls. All patients were taking conventional doses of medication prescribed by a physician. The DAS-28 score system was used. The examination of patients' states was made two times, at the beginning of the study (baseline) and at the end.

Statistically significant reduction in DAS-28 score was observed in groups I and II. The DAS 28 score in group III trended towards a significant decrease. The pain sensation in joints was statistically reduced in both supplemented groups relative to controls. Due to a higher daily intake of FO, plasma phospholipid content in group I and II, showed significantly higher levels of EPA, DHA, ω-3 PUFA, and total PUFA, relative to baseline values. The ω-6/ω-3 ratio was significantly reduced in groups I and II, while in group III, there was no change in levels of these FAs in plasma phospholipids [118].

Increased systematic inflammation and arterial stiffness are hallmarks of the RA. The severity of the arterial stiffness is measured by an augmentation index. It was recently reported by Woodman *et al.* that patients with RA who consume more ω-3 PUFAs and FO have significantly lower augmentation index compared to the group with low ω-3 PUFAs intake. Addition of ω-3 PUFAs seems to be able to prevent arterial stiffening and possibly slow down the progression of arterial ageing [119].

In a recently published meta-analysis, Gioxari *et al.* [120] found that oral intake of ω-3 PUFAs for a minimum of 3 months leads to significant improvement in eight markers related to RA. They reported on the leukotriene B4 and TG reduction. It is important to note that this analysis was based on 20 RCTs and 1 288 patients with RA divided into two subgroups according to their daily intake of ω-3 PUFAs (<3 g/day and ≥3 g/day). The other finding was a significant amelioration in the global physical assessment, together with an increased left and right grip strength after supplementation with a high dose of ω-3 PUFAs.

Di Giuseppe *et al*. [121] performed a prospective cohort study on women who collected data for ten years *via* a self-administered questionnaire. This examination revealed that intake of dietary ω-3 PUFAs of more than 0.21 g/day resulted in 35 % decreased risk of RA, while an intake higher than 0.21 g/day during a longer period of time was associated with 52 % decreased risk. In a review paper published by Philippou and Nikiphorou [122], it is suggested that increased consumption of fatty fish contributes to lowering the risk of RA. However, they also stressed the importance of reduced consumption of sugar and the maintenance of normal body weight, in addition to fatty fish consumption.

4. CONTROVERSIES CONCERNING THE BENEFICIAL EFFECTS OF FO AND ω-3 FATTY ACIDS

Besides numerous studies which support the opinion that FO and ω-3 PUFAs are beneficial for human health, there are also others which question their effects. In addition, even some contradictory results were obtained denying the existence of firm statistical evidence of the preventative role of PUFAs.

Many of the supposed/proved benefits of FO are assigned to the effects of ω-3 PUFAs, especially EPA and DHA, but it is worth mentioning that not many trials took into account the significant amounts of liposoluble vitamins being consumed at the same time and did not review and evaluate the associated benefits and risks

involved. A recently published investigation examined the relation between intake of vitamins A and D during pregnancy, supplementation with vitamins A and D at infant age, and the risk associated with the occurrence of child asthma at 7 years of age [123]. The study used data on 61 676 school-age children, the supplement intake of their mothers during pregnancy (from various sources), as well as the information on the infant intake of supplements at 6 months, and related them to the occurrence and development of the symptoms of asthma. Excessive intake of vitamin A (≥ 2.5 times the recommended amount) during pregnancy was associated with increased risk, whereas the intake of vitamin D close to the recommended doses was associated with reduced risk of asthma. In addition, the study concluded that there were no registered antagonistic effects of vitamin A and D during high intake of both vitamins, therefore opposing the previously supposed antagonistic effects of these vitamins claimed by Bastie *et al*. in 2004 [124].

Vitamin E is also a constituent of FO and its effect on patients with CVD was also found to be controversial. Namely, in one study, DHA peroxidation and its antiarrhythmic properties in cardiomyocytes were investigated. It was shown that antioxidant properties of alpha-tocopherol oppose the antiarrhythmic effects of DHA, while in the presence of a pro-oxidant, hydrogen peroxide, the beneficial effects of DHA on the prevention of arrhythmia were potentiated [125].

In the last two decades, most investigations on FO were dedicated to confirming the potential health benefits of ω-3 PUFAs as constituents of FO. However, a number of published results revealed that there is no statistically sufficient evidence to support the preventative effect of ω-3 PUFAs, especially in CVD. For example, He *et al.* in 2002 [126] found no significant association between fish or long-chain ω-3 PUFAs intake and the risk of hemorrhagic stroke, in a large prospective study that included 43 671 men examined in 1986, 1990, and 1994. The authors concluded that eating fish once a month or more can reduce the risk of ischemic stroke, but opened the question whether high fish (ω-3 PUFAs) intake contributes to a higher risk of hemorrhagic stroke, due to the influence of EPA on platelet aggregation [126, 127].

In a cohort study on 41 836 postmenopausal women (55-69 years old), there was no firm evidence that fish and marine ω-3 PUFAs prevent CHD or stroke, or that they can be associated with a mortality rate from the stroke [128]. Similarly, Jarvinen *et al*. in 2006 followed up 2 775 men and 2 445 women (30-79 years of age) and concluded that fish consumption in the Finnish population, is not offering protection against the development of CHD in men, but it may have a protective effect in women [129]. The latest report of the Zutphen study, published by Streppel *et al*. in 2008, concluded that the association between long-term fish consumption and death from CHD decreases with increasing age. The collected data suggested a reduction in the rate of the sudden coronary death due to long-term consumption of fatty fish, but a clear dose–response relationship between EPA or DHA intake and (sudden) coronary death was not established [130]. Similar observations were published by de Goede *et al*. who followed up a group of Dutch people, 22 654 men and women (20-65 years of age) during 9-14 years. A preventative role of higher doses of EPA, DHA, and fish consumption in fatal CHD was confirmed and related to generally high intake of fish in a diet. However, they did not confirm that this diet can protect against non-fatal myocardial infarction [131].

In a recent prospective cohort study, conducted by Rhee *et al.* in 2017, a longitudinal examination of the incident major CVD in women without previous history of cardiovascular events (39 876 women over 45 years of age) in relation to the consumption of ω-3 PUFAs from different sources was performed. It was concluded that ALA and marine ω-3 PUFAs intake was not associated with major CVD or individual cardiovascular outcomes regardless of the age, BMI or the baseline history of hypertension in women, again questioning the "power" of ω-3 PUFAs [132].

The question whether FO and its constituents are beneficial to the health and in what extent, continues to raise controversies, since many of the examined and published results addressed different populations, different research hypothesis, using different statistical approaches, *etc*. This inconsistency was recognized and several authors attempted to explain the controversial results. For example, Harris [133] offered a critical review on a number of intervention studies and meta-analyses that examined the effects of ω-3 PUFAs. Harris reviewed the OMEGA trial conducted in Germany (a country with low fish intake), which encompassed 3804 CVD patients randomized to either 840mg EPA + DHA or placebo [134]. No difference between groups was observed, considering trial outcomes (sudden cardiac death within 1 year and total mortality and nonfatal cardiac events). This study was shown to be underpowered and too short, and it turned out that about 45% of study participants reported consuming fish 'several times a week'. ORIGIN trial, which addressed the efficiency of ω-3 PUFAs (study No. NCT00069784), in 12 536 patients at high risk of CVD, diagnosed with impaired glucose metabolism, was also reviewed. Supplementation of 850 mg/day of EPA + DHA was not able to reduce the rate of cardiovascular events in high-risk patients and the incidence of death due to CVD [135]. Although this study had enough power and longest trial period, the absence of positive effects can be attributed to extensive background intake of EPA + DHA.

The consequence of baseline dietary behavior in both studies (OMEGA and ORIGIN) led to an increase in baseline tissue levels of ω -3 fatty acids, where additional supplementation of 1 g or less resulted in no improvement at all, as Harris explains. Effects of FO supplementation, thus, largely, depend on the plasma/tissue status before the intervention [133].

A meta-analysis conducted by Aung *et al.* on 10 trials involving 77 917 participants [136] summarised several findings concluding that the supplementation of ω-3 PUFAs for several years (a mean of 4.4 years) had no significant benefit on the reduction of either fatal, non-fatal CHD rate or other major vascular events.

An influence of ω-3 PUFAs intake on insulin sensitivity was investigated in a meta-analysis described by Gao *et al.* which included 17 studies and 672 participants. One of the main conclusions was that short-term supplementation with FO is associated with increased insulin sensitivity in people with metabolic disorders [69]. A recent meta-analysis published by Chen *et al.* [137] stressed the population differences in respect to the development of T2DM in relation to ω-3 PUFAs supplementation (either EPA or DHA). They concluded that consumption of these FA reduced the risk of T2DM in the Asian population, whereas the risk was increased in a dose-dependent manner in the Western population.

There is another controversy in the application of ω-3 PUFAs for therapeutic purposes, as not all ω-3 PUFAs are equally applicable or recommended. Deanen *et al*. [138] conducted a clinical study examining the effects of the application of different FA, which can be found in the commercial FO formulations, during cancer treatment. They found that one of these FA, 16:4 (ω-3) (hexadeca-4,7,10,13-tetraenoic acid) ω-3 FA, present in several commercial preparations, hampered tumor-directed cytotoxicity of platinum compounds at concentrations which are commonly reached in the blood of patients who take FO supplements.

Although dietary ω-3 PUFAs were suggested as potential supplements in the prevention and suppression of colorectal carcinoma (CRC), Weylandt *et al.* [139] reported no anti-proliferative, no pro-apoptotic and no anti-inflammatory effect of these PUFAs in CRC. Even more, the same authors suggested the potential causal connection between FO intake and the incidence of CRC in the high-risk groups.

Studies performed by Chua *et al.* and Haas-Haseman *et al.* reported no or even negative correlation between the use of PUFAs and the incidence of cancer in a certain population. According to

data from the WHO and the FAO, predicted incidence of cancer for the population of island Maldives, with the highest fish consumption per capita in the world (185 kg in 2013) or Iceland (92 kg) is equal or even higher compared to the similar island population of Madagascar, with low consumption of fish (just 4.6 kg) [140, 141]. This data imply that investigations need to be more population-adjusted, in order to see more clearly the effect of PUFAs on the development and treatment of cancer.

Stark *et al*. [142] performed a global survey of the blood concentrations of DHA and EPA in healthy adults and presented a geographical distribution of their levels. As expected, the levels differ in different populations as a result of different diets. Inhabitants of Japan, Scandina*via*, and from areas where fish is often consumed had the highest concentrations of EPA and DHA. On the other hand, very low levels of these acids (≤ 4 %) were measured in the populations from North, Central, and South America, Europe, the Middle East, Southeast Asia, and Africa. Indeed, Nakamura *et al*. investigated the hypothesis that a high fish intake contributes to longevity in Japanese people. In a 19-year lasting follow up of 3 945 men and 4 934 women, they did not find enough statistical evidence to support this hypothesis and reasoned that the absence of the effect was due to the generally high consummation of fish in the regular Japanese diet [143].

As already mentioned, different baseline levels of ω-3 PUFAs are variables which need to be taken into consideration when discussing the effects of supplementation.

In a recent review, Roy *et al.* [125] suggested raising an international initiative to standardize the approach in conducting trials related to EPA and DHA health benefits. They proposed to take into account the dose, the type of FA supplemented and to include a "PUFA index" in evaluation, referring to the index proposed by von Schacky in 2010, which expresses the percentage of EPA+DHA in total lipids in red blood cells [144]. These authors also stressed the importance of defining a baseline standard for FA concentrations in order to overcome multi-factorial impacts, such as different eating habits of different populations.

Moreover, the effects of ω-3 PUFAs depend on its source; whether it is fish, FO, or a pharmaceutical supplement. For instance, the supplements mostly contain no vitamins, only negligible concentrations of vitamin E (added as an antioxidant). When consuming fish, not only FO is ingested, but also proteins and trace minerals, lipophilic polyphenols which are lost during the FO extraction, and encapsulation. These elements could be the very thing that contributes to the health benefits of fish as food. They protect the ω-3 PUFAs from oxidation and possess health benefits by themselves [145]. FO is considered a supplement (not counting the prescription form) and as such does not comply with the strict pharmaceutical regulations. There are a number of cases where the real content of ω-3 PUFAs in capsules was lower than it was written on the label, and the amount of oxidized forms were above recommended limits [146]. On the other hand, capsules are more convenient to consume and devoided of mercury or chemical contaminants found in fish [147].

CONCLUSION

In conclusion, it is known that long-chain ω-3 PUFAs as constituents of FO can contribute more to prevent a disease than to manifest a therapeutic outcome. A deficiency arising from long-chain ω-3 PUFAs is not registered in a regular diet, so the real effect remains practically unknown so far. Furthermore, increased knowledge of the beneficial effects of ω-3 PUFAs has inevitably led to an increase in the consummation of food and supplements that contain them.

Although the effects of FO are being investigated for many decades and many results exist, this article gives a brief summary of the examined effects of the constituents of FO, especially in CVD, T2DM, cancer, asthma and allergy, NAFLD, RA, and on the gut microbiota. In addition, we tried to summarize the reported controversies regarding some claimed health benefits.

It is to be expected that controversial reports will continue to emerge, since many of the examined and published results so far addressed different populations and different research hypothesis, using different statistical approaches, and standardization may reduce the influence of specific variables to some extent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Weichselbaum E, Coe S, Buttriss J, Stanner S. Fish in the diet: A review. Nutr Bull 2013; 38: 128-77. [http://dx.doi.org/10.1111/nbu.12021]

[2] Lamberg-Allardt C. Vitamin D in foods and as supplements. Prog Biophys Mol Biol 2006; 92(1): 33-8. [http://dx.doi.org/10.1016/j.pbiomolbio.2006.02.017] [PMID: 16618499]

[3] SACN (Scientific Advisory Committee on Nutrition). 2004. Advice on fish consumption: Benefits & Risks. Office, T.S.: London.

[4] Shahidi F. Functional seafood lipids and proteins.Functional Foods: Biochemical and Processing Aspects. Lancaster, UK: Technomic Publ. 1998; pp. 381-401.

[5] Shahidi F, Ambigaipalan P. Omega-3 polyunsaturated fatty acids and their health benefits. Annu Rev Food Sci Technol 2018; 9: 345-81. [http://dx.doi.org/10.1146/annurev-food-111317-095850] [PMID: 29350557]

[6] Strobel C, Jahreis G, Kuhnt K. Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products. Lipids Health Dis 2012; 11: 144. [http://dx.doi.org/10.1186/1476-511X-11-144] [PMID: 23110317]

[7] WHO (World Health Organisation), Population nutrient intake goals for preventing diet-related chronic diseases. [updated 2018; cited 2018 Oct 28]. Available from: (www.who.int/nutrition/ topics/5_population_nutrient/en/index13.html)

[8] Fats and fatty acids in human nutrition Report of an expert consultation. Rome: WHO 2010.

[9] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion related to the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA J 2012; 10(7): 2815. [48 pp.] doi:10.2903/j.efsa.2012.2815.

[10] Fernandes A, Smith F, Petch R, *et al.* Investigation into the Levels of Environmental Contaminants in Scottish Marine and Freshwater Fin Fish and Shellfish. York: Food and Environment Research Agency 2009.www.food.gov.uk/sites/default/files/media/document/572-1-1005_S14040-S14041__Environmental_Contaminants_in_Scottish_Marine_and_ _Freshwater_Fish.pdf [updated 2018; cited 2018 Dec 1]

[11] FAO/WHO. 2011. Report of the Joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish Consumption. Rome and Geneva.

[12] EUMOFA 2018. The EU Fish Market. HIGHLGHTS. THE EU IN THE WORLD. EU MARKET SUPPLY. CONSUMPTION.

TRADE. EU LANDINGS. AQUACULTURE PRODUCTION. [updated 2019; cited 2019 Apr 26]. Available from: (http://www.eumofa.eu/)

[13] FAOSTAT, FAO. Food Balance Sheets: Fish, Seafood - Food supply quantity (kg/capita/yr) [updated 2019; cited 2019 Apr 26]. Available from: (www.fao.org/faostat/en/?#data/)

[14] Burdge GC, Calder PC. Dietary alpha-linolenic acid and health-related outcomes: A metabolic perspective. Nutr Res Rev 2006; 19(1): 26-52. [http://dx.doi.org/10.1079/NRR2005113] [PMID: 19079874]

[15] Calder PC. Very long chain omega-3 (n-3) fatty acids and human health. Eur J Lipid Sci Technol 2014; 116(10): 1280-300. [http://dx.doi.org/10.1002/ejlt.201400025]

[16] Welch AA, Shakya-Shrestha S, Lentjes MA, Wareham NJ, Khaw KT. Dietary intake and status of n-3 polyunsaturated fatty acids in a population of fish-eating and non-fish-eating meat-eaters, vegetarians, and vegans and the product-precursor ratio [corrected] of α-linolenic acid to long-chain n-3 polyunsaturated fatty acids: Results from the EPIC-Norfolk cohort. Am J Clin Nutr 2010; 92(5): 1040-51. [http://dx.doi.org/10.3945/ajcn.2010.29457] [PMID: 20861171]

[17] Nobili V, Alisi A, Musso G, Scorletti E, Calder PC, Byrne CD. Omega-3 fatty acids: Mechanisms of benefit and therapeutic effects in pediatric and adult NAFLD. Crit Rev Clin Lab Sci 2016; 53(2): 106-20. [http://dx.doi.org/10.3109/10408363.2015.1092106] [PMID: 26463349]

[18] Scaioli E, Liverani E, Belluzzi A. The imbalance between n-6/n-3 polyunsaturated fatty acids and inflammatory bowel disease: A comprehensive review and future therapeutic perspectives. Int J Mol Sci 2017; 18(12): E2619. [http://dx.doi.org/10.3390/ijms18122619] [PMID: 29206211]

[19] Shahidi F, Miraliakbari H. Omega-3 (n-3) fatty acids in health and disease: Part 1--cardiovascular disease and cancer. J Med Food 2004; 7(4): 387-401. [http://dx.doi.org/10.1089/jmf.2004.7.387] [PMID: 15671680]

[20] Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014; 510: 92-101.

[21] Schuchardt JP, Hahn A. Bioavailability of long-chain omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids 2013; 89: 1-8.

[22] Pike LJ. Lipid rafts: Bringing order to chaos. J Lipid Res 2003; 44(4): 655-67. [http://dx.doi.org/10.1194/jlr.R200021-JLR200] [PMID: 12562849]

[23] Yaqoob P. The nutritional significance of lipid rafts. Annu Rev Nutr 2009; 29: 257-82. [http://dx.doi.org/10.1146/annurev-nutr-080508-141205] [PMID: 19400697]

[24] Hou TY, McMurray DN, Chapkin RS. Omega-3 fatty acids, lipid rafts, and T cell signaling. Eur J Pharmacol 2016; 785: 2-9. [http://dx.doi.org/10.1016/j.ejphar.2015.03.091] [PMID: 26001374]

[25] Miles EA, Calder PC. Can early omega-3 fatty acid exposure reduce risk of childhood allergic disease? Nutrients 2017; 9(7): 784. [http://dx.doi.org/10.3390/nu9070784] [PMID: 28754005]

[26] Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta 2015; 1851(4): 469-84. [http://dx.doi.org/10.1016/j.bbalip.2014.08.010] [PMID: 25149823]

[27] Vučić V. The Role of dietary polyunsaturated fatty acids in inflammation. Serb J Exp Clin Res 2013; 14: 93-9. [http://dx.doi.org/10.5937/sjecr14-4774]

[28] U. S. Food and Drug Administration. Center for Food Safety and Applied Nutrition. Office of Nutritional Products, Labeling, and Dietary Supplements. 2000. Letter Regarding Dietary Supplement Health Claim for Omega-3 Fatty Acids and Coronary Heart Disease.

[29] Brunton S, Collins N. Differentiating prescription omega-3-acid ethyl esters (P-OM3) from dietary-supplement omega-3 fatty acids. Curr Med Res Opin 2007; 23(5): 1139-45. [http://dx.doi.org/10.1185/030079907X188017] [PMID: 17519081]

[30] Rosin S, Ojansivu I, Kopu A, Keto-Tokoi M, Gylling H. Optimal use of plant stanol ester in the management of hypercholesterolemia. Cholesterol 2015; 2015: 706970. [http://dx.doi.org/10.1155/2015/706970] [PMID: 26543642]

[31] Baigent C, Blackwell L, Emberson J, *et al.* Efficacy and safety of more intensive lowering of LDL cholesterol: A meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet 2010; 376(9753): 1670-81. [http://dx.doi.org/10.1016/S0140-6736(10)61350-5] [PMID: 21067804]

[32] Bang HO, Dyerberg J, Nielsen AB. Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. Lancet 1971; 1(7710): 1143-5. [http://dx.doi.org/10.1016/S0140-6736(71)91658-8] [PMID: 4102857]

[33] Mori TA. Marine OMEGA-3 fatty acids in the prevention of cardiovascular disease. Fitoterapia 2017; 123: 51-8. [http://dx.doi.org/10.1016/j.fitote.2017.09.015] [PMID: 28964873]

[34] Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: A meta-analysis of randomized controlled trials. Am J Med 2002; 112(4): 298-304. [http://dx.doi.org/10.1016/S0002-9343(01)01114-7] [PMID: 11893369]

[35] Whelton SP, He J, Whelton PK, Muntner P. Meta-analysis of observational studies on fish intake and coronary heart disease. Am J Cardiol 2004; 93(9): 1119-23. [http://dx.doi.org/10.1016/j.amjcard.2004.01.038] [PMID: 15110203]

[36] He K, Song Y, Daviglus ML, *et al.* Accumulated evidence on fish consumption and coronary heart disease mortality: A meta-analysis of cohort studies. Circulation 2004; 109(22): 2705-11. [http://dx.doi.org/10.1161/01.CIR.0000132503.19410.6B] [PMID: 15184295]

[37] Xun P, Qin B, Song Y, *et al.* Fish consumption and risk of stroke and its subtypes: Accumulative evidence from a meta-analysis of prospective cohort studies. Eur J Clin Nutr 2012; 66(11): 1199-207. [http://dx.doi.org/10.1038/ejcn.2012.133] [PMID: 23031847]

[38] Alexander DD, Miller PE, Van Elswyk ME, Kuratko CN, Bylsma LC. Eicosapentaenoic and docosahexaenoic long-chain omega-3 fatty acids and coronary heart disease risk. Mayo Clin Proc 2017; 92(1): 15-29. [http://dx.doi.org/10.1016/j.mayocp.2016.10.018] [PMID: 28062061]

[39] Maki KC, Palacios OM, Bell M, Toth PP. Use of supplemental long-chain omega-3 fatty acids and risk for cardiac death: An updated meta-analysis and review of research gaps. J Clin Lipidol 2017; 11(5): 1152-1160.e2. [http://dx.doi.org/10.1016/j.jacl.2017.07.010] [PMID: 28818347]

[40] Djoussé L, Akinkuolie AO, Wu JH, Ding EL, Gaziano JM. Fish consumption, omega-3 fatty acids and risk of heart failure: A meta-analysis. Clin Nutr 2012; 31(6): 846-53. [http://dx.doi.org/10.1016/j.clnu.2012.05.010] [PMID: 22682084]

[41] Mozaffarian D, Bryson CL, Lemaitre RN, Burke GL, Siscovick DS. Fish intake and risk of incident heart failure. J Am Coll Cardiol 2005; 45(12): 2015-21. [http://dx.doi.org/10.1016/j.jacc.2005.03.038] [PMID: 15963403]

[42] Weisman D, Beinart R, Erez A, *et al.* Effect of supplemented intake of omega-3 fatty acids on arrhythmias in patients with ICD: Fish oil therapy may reduce ventricular arrhythmia. J Interv Card Electrophysiol 2017; 49(3): 255-61. [http://dx.doi.org/10.1007/s10840-017-0267-1] [PMID: 28664342]

[43] Nodari S, Metra M, Milesi G, *et al.* The role of n-3 PUFAs in preventing the arrhythmic risk in patients with idiopathic dilated cardiomyopathy. Cardiovasc Drugs Ther 2009; 23(1): 5-15. [http://dx.doi.org/10.1007/s10557-008-6142-7] [PMID: 18982439]

[44] Hidayat K, Yang J, Zhang Z, *et al.* Effect of omega-3 long-chain polyunsaturated fatty acid supplementation on heart rate: A meta-analysis of randomized controlled trials. Eur J Clin Nutr 2018; 72(6): 805-17. [http://dx.doi.org/10.1038/s41430-017-0052-3] [PMID: 29284786]

[45] Tribulova N, Szeiffova Bacova B, Egan Benova T, Knezl V, Barancik M, Slezak J. Omega-3 index and anti-arrhythmic potential of omega-3 PUFAs. Nutrients 2017; 9(11): 1191.

[http://dx.doi.org/10.3390/nu9111191] [PMID: 29084142]
[46] Burke MF, Burke FM, Soffer DE. Review of cardiometabolic effects of prescription omega-3 fatty acids. Curr Atheroscler Rep 2017; 19(12): 60. [http://dx.doi.org/10.1007/s11883-017-0700-z] [PMID: 29116404]
[47] Zibaeenezhad MJ, Ghavipisheh M, Attar A, Aslani A. Comparison of the effect of omega-3 supplements and fresh fish on lipid profile: A randomized, open-labeled trial. Nutr Diabetes 2017; 7(12): 1. [http://dx.doi.org/10.1038/s41387-017-0007-8] [PMID: 29259181]
[48] Backes J, Anzalone D, Hilleman D, Catini J. The clinical relevance of omega-3 fatty acids in the management of hypertriglyceridemia. Lipids Health Dis 2016; 15(1): 118. [http://dx.doi.org/10.1186/s12944-016-0286-4] [PMID: 27444154]
[49] Jacobson TA, Glickstein SB, Rowe JD, Soni PN. Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: A review. J Clin Lipidol 2012; 6(1): 5-18. [http://dx.doi.org/10.1016/j.jacl.2011.10.018] [PMID: 22264569]
[50] Fialkow J. Omega-3 fatty acid formulations in cardiovascular disease: Dietary supplements are not substitutes for prescription products. Am J Cardiovasc Drugs 2016; 16(4): 229-39. [http://dx.doi.org/10.1007/s40256-016-0170-7] [PMID: 27138439]
[51] Nastel P, Shige H, Pomeroy S, Cehun M, Abbey M, Raederstorff D. The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans. Am J Clin Nutr 2002; 76: 326-30.
[52] Yamano T, Kubo T, Shiono Y, *et al.* Impact of eicosapentaenoic acid treatment on the fibrous cap thickness in patients with coronary atherosclerotic plaque: An optical coherence tomography study. J Atheroscler Thromb 2015; 22(1): 52-61. [http://dx.doi.org/10.5551/jat.25593] [PMID: 25132379]
[53] Vanschoonbeek K, Feijge MAH, Paquay M, *et al.* Variable hypocoagulant effect of fish oil intake in humans: Modulation of fibrinogen level and thrombin generation. Arterioscler Thromb Vasc Biol 2004; 24(9): 1734-40. [http://dx.doi.org/10.1161/01.ATV.0000137119.28893.0b] [PMID: 15217806]
[54] Miller PE, Van Elswyk M, Alexander DD. Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: A meta-analysis of randomized controlled trials. Am J Hypertens 2014; 27(7): 885-96. [http://dx.doi.org/10.1093/ajh/hpu024] [PMID: 24610882]
[55] Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet 2017; 389(10085): 2239-51. [http://dx.doi.org/10.1016/S0140-6736(17)30058-2] [PMID: 28190580]
[56] De Fronzo RA, Ferrannini E, Groop L, *et al*. Type 2 diabetes mellitus. Nat Rev/ Dis Primers 2015; 1: 15019.
[57] Itsiopoulos C, Marx W, Mayr HL, *et al.* The role of omega-3 polyunsaturated fatty acid supplementation in the management of type 2 diabetes mellitus: A narrative review. J Nutr Intermed Metab 2018; 14: 42-51. [http://dx.doi.org/10.1016/j.jnim.2018.02.002]
[58] Coelho OGL, da Silva BP, Rocha DMUP, Lopes LL, Alfenas RCG. Polyunsaturated fatty acids and type 2 diabetes: Impact on the glycemic control mechanism. Crit Rev Food Sci Nutr 2017; 57(17): 3614-9. [http://dx.doi.org/10.1080/10408398.2015.1130016] [PMID: 26853892]
[59] Lalia AZ, Lanza IR. Insulin-Sensitizing Effects of Omega-3 Fatty Acids: Lost in Translation? Nutrients 2016; 8(6): 329-53. [http://dx.doi.org/10.3390/nu8060329] [PMID: 27258299]
[60] Ebbesson SO, Risica PM, Ebbesson LO, Kennish JM, Tejero ME. Omega-3 fatty acids improve glucose tolerance and components of the metabolic syndrome in Alaskan Eskimos: The Alaska Siberia project. Int J Circumpolar Health 2005; 64(4): 396-408. [http://dx.doi.org/10.3402/ijch.v64i4.18016] [PMID: 16277123]
[61] Rasic-Milutinovic Z, Perunicic G, Pljesa S, *et al.* Effects of N-3 PUFAs supplementation on insulin resistance and inflammatory biomarkers in hemodialysis patients. Ren Fail 2007; 29(3): 321-9. [http://dx.doi.org/10.1080/08860220601184092] [PMID: 17497447]
[62] Farsi PF, Djazayery A, Eshraghian MR, *et al.* Effects of supplementation with omega-3 on insulin sensitivity and non-esterified free fatty acid (NEFA) in type 2 diabetic patients. Arq Bras Endocrinol Metabol 2014; 58(4): 335-40. [http://dx.doi.org/10.1590/0004-2730000002861] [PMID: 24936727]
[63] Lepretti M, Martucciello S, Burgos Aceves MA, Putti R, Lionetti L. Omega-3 Fatty Acids and Insulin Resistance: Focus on the Regulation of Mitochondria and Endoplasmic Reticulum Stress. Nutrients 2018; 10(3): 349-59. [http://dx.doi.org/10.3390/nu10030350] [PMID: 29538286]
[64] Sala-Vila A, Díaz-López A, Valls-Pedret C, *et al.* Dietary Marine ω-3 Fatty Acids and Incident Sight-Threatening Retinopathy in Middle-Aged and Older Individuals With Type 2 Diabetes: Prospective Investigation From the PREDIMED Trial. JAMA Ophthalmol 2016; 134(10): 1142-9. [http://dx.doi.org/10.1001/jamaophthalmol.2016.2906] [PMID: 27541690]
[65] Grundy SM, Cleeman JI, Daniels SR, *et al.* Diagnosis andmanagement of the metabolic syndrome: An American Heart Association/NationalHeart, Lung, and Blood Institute scientific statement. Circulation 2005; 112(17): 2735-52. [http://dx.doi.org/10.1161/CIRCULATIONAHA.105.169404] [PMID: 16157765]
[66] Wilson PWF, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation 2005; 112(20): 3066-72. [http://dx.doi.org/10.1161/CIRCULATIONAHA.105.539528] [PMID: 16275870]
[67] Kaur J. A comprehensive review on metabolic syndrome. Cardiol Res Pract 2014; 2014: 943162. [http://dx.doi.org/10.1155/2014/943162] [PMID: 24711954]
[68] Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. A natural solution for obesity: Bioactives for the prevention and treatment of weight gain. A review. Nutr Neurosci 2015; 18(2): 49-65. [http://dx.doi.org/10.1179/1476830513Y.0000000099] [PMID: 24621068]
[69] Gao H, Geng T, Huang T, Zhao Q. Fish oil supplementation and insulin sensitivity: A systematic review and meta-analysis. Lipids Health Dis 2017; 16(1): 131. [http://dx.doi.org/10.1186/s12944-017-0528-0] [PMID: 28673352]
[70] Lorente-Cebrián S, Costa AGV, Navas-Carretero S, Zabala M, Martínez JA, Moreno-Aliaga MJ. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: A review of the evidence. J Physiol Biochem 2013; 69(3): 633-51. [http://dx.doi.org/10.1007/s13105-013-0265-4] [PMID: 23794360]
[71] Torres DM, Williams CD, Harrison SA. Features, diagnosis, and treatment of nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2012; 10(8): 837-58. [http://dx.doi.org/10.1016/j.cgh.2012.03.011] [PMID: 22446927]
[72] Gupta V, Mah XJ, Garcia MC, Antonypillai C, van der Poorten D. Oily fish, coffee and walnuts: Dietary treatment for nonalcoholic fatty liver disease. World J Gastroenterol 2015; 21(37): 10621-35. [http://dx.doi.org/10.3748/wjg.v21.i37.10621] [PMID: 26457022]
[73] Yu L, Yuan M, Wang L. The effect of omega-3 unsaturated fatty acids on non-alcoholic fatty liver disease: A systematic review and meta-analysis of RCTs. Pak J Med Sci 2017; 33(4): 1022-8. [http://dx.doi.org/10.12669/pjms.334.12315] [PMID: 29067086]
[74] Yan J-H, Guan B-J, Gao H-Y, Peng XE. Omega-3 polyunsaturated fatty acid supplementation and non-alcoholic fatty liver disease: A meta-analysis of randomized controlled trials. Medicine (Baltimore) 2018; 97(37): E12271. [http://dx.doi.org/10.1097/MD.0000000000012271] [PMID: 30212963]
[75] Musa-Veloso K, Venditti C, Lee HY, *et al.* Systematic review and meta-analysis of controlled intervention studies on the effectiveness of long-chain omega-3 fatty acids in patients with nonalcoholic fatty liver disease. Nutr Rev 2018; 76(8): 581-602. [http://dx.doi.org/10.1093/nutrit/nuy022] [PMID: 29917092]
[76] World Health Organization. [updated 2018; cited 2018 Dec 6]. Availbale from: (www.who.int/cancer/en/).

[77] Bartsch H, Nair J, Owen RW. Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: Emerging evidence for their role as risk modifiers. Carcinogenesis 1999; 20(12): 2209-18. [http://dx.doi.org/10.1093/carcin/20.12.2209] [PMID: 10590211]

[78] Abel S, Riedel S, Gelderblom WCA. Dietary PUFA and cancer. Proc Nutr Soc 2014; 73(3): 361-7. [http://dx.doi.org/10.1017/S0029665114000585] [PMID: 24850051]

[79] Zhang Y, Zhuang P, He W, *et al.* Association of fish and long-chain omega-3 fatty acids intakes with total and cause-specific mortality: Prospective analysis of 421 309 individuals. J Intern Med 2018; 284(4): 399-417. [http://dx.doi.org/10.1111/joim.12786] [PMID: 30019399]

[80] Li J, Li K, Gao J, *et al.* Maternal exposure to an n-3 polyunsaturated fatty acid diet decreases mammary cancer risk of female offspring in adulthood. Food Funct 2018; 9(11): 5768-77. [http://dx.doi.org/10.1039/C8FO01006D] [PMID: 30327803]

[81] Mikirova N, Riordan HD, Jackson JA, Wong K, Miranda-Massari JR, Gonzalez MJ. Erythrocyte membrane fatty acid composition in cancer patients. P R Health Sci J 2004; 23(2): 107-13. [PMID: 15377058]

[82] Coviello G, Tutino V, Notarnicola M, Caruso MG. Erythrocyte membrane fatty acids profile in colorectal cancer patients: A preliminary study. Anticancer Res 2014; 34(9): 4775-9. [PMID: 25202057]

[83] Camargo CQ, Mocellin MC, Brunetta HS, *et al.* Fish oil decreases the severity of treatment-related adverse events in gastrointestinal cancer patients undergoing chemotherapy: A randomized, placebo-controlled, triple-blind clinical trial. Clin Nutr ESPEN 2019; 31: 61-70. [http://dx.doi.org/10.1016/j.clnesp.2019.02.015] [PMID: 31060836]

[84] Lovegrove C, Ahmed K, Challacombe B, Khan MS, Popert R, Dasgupta P. Systematic review of prostate cancer risk and association with consumption of fish and fish-oils: Analysis of 495,321 participants. Int J Clin Pract 2015; 69(1): 87-105. [http://dx.doi.org/10.1111/ijcp.12514] [PMID: 25495842]

[85] Luu HN, Cai H, Murff HJ, *et al.* A prospective study of dietary polyunsaturated fatty acids intake and lung cancer risk. Int J Cancer 2018; 143(9): 2225-37. [http://dx.doi.org/10.1002/ijc.31608] [PMID: 29905376]

[86] Volpato M, Hull MA. Omega-3 polyunsaturated fatty acids as adjuvant therapy of colorectal cancer. Cancer Metastasis Rev 2018; 37(2-3): 545-55. [http://dx.doi.org/10.1007/s10555-018-9744-y] [PMID: 29971573]

[87] Song M, Zhang X, Meyerhardt JA, *et al.* Marine ω-3 polyunsaturated fatty acid intake and survival after colorectal cancer diagnosis. Gut 2017; 66(10): 1790-6. [http://dx.doi.org/10.1136/gutjnl-2016-311990] [PMID: 27436272]

[88] Cockbain AJ, Toogood GJ, Hull MA. ω-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. Gut 2012; 61(1): 135-49. [http://dx.doi.org/10.1136/gut.2010.233718] [PMID: 21490374]

[89] Bougnoux P, Hajjaji N, Ferrasson MN, Giraudeau B, Couet C, Le Floch O. Improving outcome of chemotherapy of metastatic breast cancer by docosahexaenoic acid: A phase II trial. Br J Cancer 2009; 101(12): 1978-85. [http://dx.doi.org/10.1038/sj.bjc.6605441] [PMID: 19920822]

[90] Murphy RA, Mourtzakis M, Chu QS, Baracos VE, Reiman T, Mazurak VC. Supplementation with fish oil increases first-line chemotherapy efficacy in patients with advanced nonsmall cell lung cancer. Cancer 2011; 117(16): 3774-80. [http://dx.doi.org/10.1002/cncr.25933] [PMID: 21328326]

[91] Ma YJ, Yu J, Xiao J, Cao BW. The consumption of ω-3 polyunsaturated fatty acids improves clinical outcomes and prognosis in pancreatic cancer patients: A systematic evaluation. Nutr Cancer 2015; 67(1): 112-8. [http://dx.doi.org/10.1080/01635581.2015.976315] [PMID: 25425246]

[92] Fuentes NR, Kim E, Fan YY, Chapkin RS. Omega-3 fatty acids, membrane remodeling and cancer prevention. Mol Aspects Med 2018; 64: 79-91. [http://dx.doi.org/10.1016/j.mam.2018.04.001] [PMID: 29627343]

[93] Miccadei S, Masella R, Mileo AM, Gessani S. ω3 Polyunsaturated Fatty Acids as Immunomodulators in Colorectal Cancer: New Potential Role in Adjuvant Therapies. Front Immunol 2016; 7: 486. [http://dx.doi.org/10.3389/fimmu.2016.00486] [PMID: 27895640]

[94] Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J 2017; 474(11): 1823-36. [http://dx.doi.org/10.1042/BCJ20160510] [PMID: 28512250]

[95] Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. Nat Med 2018; 24(4): 392-400. [http://dx.doi.org/10.1038/nm.4517] [PMID: 29634682]

[96] Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 2015; 26: 26191. [PMID: 25651997]

[97] Flint HJ, Duncan SH, Louis P. The impact of nutrition on intestinal bacterial communities. Curr Opin Microbiol 2017; 38: 59-65. [http://dx.doi.org/10.1016/j.mib.2017.04.005] [PMID: 28486162]

[98] Costantini L, Molinari R, Farinon B, Merendino N. Impact of Omega-3 Fatty Acids on the Gut Microbiota. Int J Mol Sci 2017; 18(12): 2645-63. [http://dx.doi.org/10.3390/ijms18122645] [PMID: 29215589]

[99] Menni C, Zierer J, Pallister T, *et al.* Omega-3 fatty acids correlate with gut microbiome diversity and production of N-carbamylglutamate in middle aged and elderly women. Sci Rep 2017; 7(1): 11079. [http://dx.doi.org/10.1038/s41598-017-10382-2] [PMID: 28894110]

[100] Cândido FG, Valente FX, Grześkowiak LM, Moreira APB, Rocha DMUP, Alfenas RCG. Impact of dietary fat on gut microbiota and low-grade systemic inflammation: Mechanisms and clinical implications on obesity. Int J Food Sci Nutr 2018; 69(2): 125-43. [http://dx.doi.org/10.1080/09637486.2017.1343286] [PMID: 28675945]

[101] Watson H, Mitra S, Croden FC, *et al.* A randomised trial of the effect of omega-3 polyunsaturated fatty acid supplements on the human intestinal microbiota. Gut 2018; 67(11): 1974-83. [http://dx.doi.org/10.1136/gutjnl-2017-314968] [PMID: 28951525]

[102] Global Iniative For Asthma (GINA). Pocket Guide for Astma Management and Prevention (for adults and Children Older than 5 Years). A Pocket Guide for Health Professionals. [updated 2018; cited 2018 Dec 1]. Available from: Https://ginasthma.org/2018-pocket-guide-for-asthma-management-and-prevention/.

[103] Hall S, Agrawal DK. Key mediators in the immunopathogenesis of allergic asthma. Int Immunopharmacol 2014; 23(1): 316-29. [http://dx.doi.org/10.1016/j.intimp.2014.05.034] [PMID: 24933589]

[104] Miyata J, Arita M. Role of omega-3 fatty acids and their metabolites in asthma and allergic diseases. Allergol Int 2015; 64(1): 27-34. [http://dx.doi.org/10.1016/j.alit.2014.08.003] [PMID: 25572556]

[105] Olsen SF, Østerdal ML, Salvig JD, *et al.* Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. Am J Clin Nutr 2008; 88(1): 167-75. [http://dx.doi.org/10.1093/ajcn/88.1.167] [PMID: 18614738]

[106] Bisgaard H, Stokholm J, Chawes BL, *et al.* Fish oil-derived fatty acids in pregnancy and wheeze and asthma in offspring. N Engl J Med 2016; 375(26): 2530-9. [http://dx.doi.org/10.1056/NEJMoa1503734] [PMID: 28029926]

[107] Damsgaard CT, Lauritzen L, Kjaer TM, *et al.* Fish oil supplementation modulates immune function in healthy infants. J Nutr 2007; 137(4): 1031-6. [http://dx.doi.org/10.1093/jn/137.4.1031] [PMID: 17374672]

[108] Best KP, Gold M, Kennedy D, Martin J, Makrides M. Omega-3 long-chain PUFA intake during pregnancy and allergic disease outcomes in the offspring: A systematic review and meta-analysis of observational studies and randomized controlled trials. Am J Clin Nutr 2016; 103(1): 128-43. [http://dx.doi.org/10.3945/ajcn.115.111104] [PMID: 26675770]

[109] Abdulrazaq M, Innes JK, Calder PC. Effect of ω-3 polyunsaturated fatty acids on arthritic pain: A systematic review. Nutrition 2017; 39-40: 57-66.

[http://dx.doi.org/10.1016/j.nut.2016.12.003] [PMID: 28606571]
[110] Musumeci G. The effect of mechanical loading on articular cartilage. J Funct Morphol Kinesiol 2016; 1: 154-61. [http://dx.doi.org/10.3390/jfmk1020154]
[111] Kaneko Y, Takeuchi T. A paradigm shift in rheumatoid arthritis over the past decade. Intern Med 2014; 53(17): 1895-903. [http://dx.doi.org/10.2169/internalmedicine.53.2834] [PMID: 25175120]
[112] Reynard LN, Loughlin J. Genetics and epigenetics of osteoarthritis. Maturitas 2012; 71(3): 200-4. [http://dx.doi.org/10.1016/j.maturitas.2011.12.001] [PMID: 22209350]
[113] Brandt KD, Dieppe P, Radin EL. Etiopathogenesis of osteoarthritis. Rheum Dis Clin North Am 2008; 34(3): 531-59. [http://dx.doi.org/10.1016/j.rdc.2008.05.011] [PMID: 18687271]
[114] Woo SJ, Lim K, Park SY, *et al.* Endogenous conversion of n-6 to n-3 polyunsaturated fatty acids attenuates K/BxN serum-transfer arthritis in fat-1 mice. J Nutr Biochem 2015; 26(7): 713-20. [http://dx.doi.org/10.1016/j.jnutbio.2015.01.011] [PMID: 25910895]
[115] Bannenberg G, Arita M, Serhan CN. Endogenous receptor agonists: Resolving inflammation. ScientificWorldJournal 2007; 7: 1440-62. [http://dx.doi.org/10.1100/tsw.2007.188] [PMID: 17767360]
[116] Landewé R, van der Heijde D, van der Linden S, Boers M. Twenty-eight-joint counts invalidate the DAS28 remission definition owing to the omission of the lower extremity joints: A comparison with the original DAS remission. Ann Rheum Dis 2006; 65(5): 637-41. [http://dx.doi.org/10.1136/ard.2005.039859] [PMID: 16219709]
[117] Rajaei E, Mowla K, Ghorbani A, Bahadoram S, Bahadoram M, Dargahi-Malamir M. The Effect of Omega-3 Fatty Acids in Patients With Active Rheumatoid Arthritis Receiving DMARDs Therapy: Double-Blind Randomized Controlled Trial. Glob J Health Sci 2015; 8(7): 18-25. [http://dx.doi.org/10.5539/gjhs.v8n7p18] [PMID: 26925896]
[118] Veselinovic M, Vasiljevic D, Vucic V, *et al*. Clinical Benefits of n-3 PUFA and ɤ-Linolenic Acid in Patients with Rheumatoid Arthritis. Nutrients 2017; 25: 9(4).
[119] Woodman RJ, Baghdadi LR, Shanahan EM, de Silva I, Hodgson JM, Mangoni AA. Diets high in n-3 fatty acids are associated with lower arterial stiffness in patients with rheumatoid arthritis: A latent profile analysis. Br J Nutr 2019; 121(2): 182-94. [http://dx.doi.org/10.1017/S0007114518003100] [PMID: 30430957]
[120] Gioxari A, Kaliora AC, Marantidou F, Panagiotakos DP. Intake of ω-3 polyunsaturated fatty acids in patients with rheumatoid arthritis: A systematic review and meta-analysis. Nutrition 2018; 45: 114-124.e4. [http://dx.doi.org/10.1016/j.nut.2017.06.023] [PMID: 28965775]
[121] Di Giuseppe D, Wallin A, Bottai M, Askling J, Wolk A. Long-term intake of dietary long-chain n-3 polyunsaturated fatty acids and risk of rheumatoid arthritis: A prospective cohort study of women. Ann Rheum Dis 2014; 73(11): 1949-53. [http://dx.doi.org/10.1136/annrheumdis-2013-203338] [PMID: 23940215]
[122] Philippou E, Nikiphorou E. Are we really what we eat? Nutrition and its role in the onset of rheumatoid arthritis. Autoimmun Rev 2018; 17(11): 1074-7. [http://dx.doi.org/10.1016/j.autrev.2018.05.009] [PMID: 30213695]
[123] Parr CL, Magnus MC, Karlstad Ø, *et al.* Vitamin A and D intake in pregnancy, infant supplementation, and asthma development: The Norwegian Mother and Child Cohort, Am J Clin Nutrn, 107 (5); 789-98.
[124] Bastie JN, Balitrand N, Guidez F, *et al.* 1 alpha,25-dihydroxyvitamin D3 transrepresses retinoic acid transcriptional activity *via* vitamin D receptor in myeloid cells. Mol Endocrinol 2004; 18(11): 2685-99. [http://dx.doi.org/10.1210/me.2003-0412] [PMID: 15284334]
[125] Roy J, Oger C, Thireau J, *et al.* Nonenzymatic lipid mediators, neuroprostanes, exert the antiarrhythmic properties of docosahexaenoic acid. Free Radic Biol Med 2015; 86: 269-78. [http://dx.doi.org/10.1016/j.freeradbiomed.2015.04.014] [PMID: 25911196]
[126] He K, Rimm EB, Merchant A, *et al.* Fish consumption and risk of stroke in men. JAMA 2002; 288(24): 3130-6. [http://dx.doi.org/10.1001/jama.288.24.3130] [PMID: 12495393]
[127] Driss F, Vericel E, Lagarde M, Dechavanne M, Darcet P. Inhibition of platelet aggregation and thromboxane synthesis after intake of small amount of icosapentaenoic acid. Thromb Res 1984; 36(5): 389-96. [http://dx.doi.org/10.1016/0049-3848(84)90295-0] [PMID: 6098051]
[128] Folsom AR, Demissie Z. Fish intake, marine omega-3 fatty acids, and mortality in a cohort of postmenopausal women. Am J Epidemiol 2004; 160(10): 1005-10. [http://dx.doi.org/10.1093/aje/kwh307] [PMID: 15522857]
[129] Järvinen R, Knekt P, Rissanen H, Reunanen A. Intake of fish and long-chain n-3 fatty acids and the risk of coronary heart mortality in men and women. Br J Nutr 2006; 95(4): 824-9. [http://dx.doi.org/10.1079/BJN20051687] [PMID: 16571163]
[130] Streppel MT, Ocké MC, Boshuizen HC, Kok FJ, Kromhout D. Long-term fish consumption and n-3 fatty acid intake in relation to (sudden) coronary heart disease death: The Zutphen study. Eur Heart J 2008; 29(16): 2024-30. [http://dx.doi.org/10.1093/eurheartj/ehn294] [PMID: 18641046]
[131] de Goede J, Geleijnse JM, Boer JM, Kromhout D, Verschuren WM. Marine (n-3) fatty acids, fish consumption, and the 10-year risk of fatal and nonfatal coronary heart disease in a large population of Dutch adults with low fish intake. J Nutr 2010; 140(5): 1023-8. [http://dx.doi.org/10.3945/jn.109.119271] [PMID: 20335635]
[132] Rhee JJ, Kim E, Buring JE, Kurth T. Fish consumption, omega-3 fatty acids, and risk of cardiovascular disease. Am J Prev Med 2017; 52(1): 10-9. [http://dx.doi.org/10.1016/j.amepre.2016.07.020] [PMID: 27646568]
[133] Harris WS. Are n-3 fatty acids still cardioprotective? Curr Opin Clin Nutr Metab Care 2013; 16(2): 141-9. [http://dx.doi.org/10.1097/MCO.0b013e32835bf380] [PMID: 23196817]
[134] Rauch B, Schiele R, Schneider S, *et al.* OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. Circulation 2010; 122(21): 2152-9. [http://dx.doi.org/10.1161/CIRCULATIONAHA.110.948562] [PMID: 21060071]
[135] Bosch J, Gerstein HC, Dagenais GR, *et al.* n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia. N Engl J Med 2012; 367(4): 309-18. [http://dx.doi.org/10.1056/NEJMoa1203859] [PMID: 22686415]
[136] Aung T, Halsey J, Kromhout D, *et al.* Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease Risks: Meta-analysis of 10 Trials Involving 77 917 Individuals. JAMA Cardiol 2018; 3(3): 225-34. [http://dx.doi.org/10.1001/jamacardio.2017.5205] [PMID: 29387889]
[137] Chen C, Yang Y, Yu X, Hu S, Shao S. Association between omega-3 fatty acids consumption and the risk of type 2 diabetes: A meta-analysis of cohort studies. J Diabetes Investig 2017; 8(4): 480-8. [http://dx.doi.org/10.1111/jdi.12614] [PMID: 28032469]
[138] Daenen LG, Cirkel GA, Houthuijzen JM, *et al.* Increased plasma levels of chemoresistance-inducing fatty acid 16:4(n-3) after consumption of fish and fish oil. JAMA Oncol 2015; 1(3): 350-8. [http://dx.doi.org/10.1001/jamaoncol.2015.0388] [PMID: 26181186]
[139] Weylandt KH, Serini S, Chen YQ, *et al.* Omega-3 Polyunsaturated Fatty Acids: The Way Forward in Times of Mixed Evidence. Biomed Res Int 2015; 2015: 143109. [http://dx.doi.org/10.1155/2015/143109] [PMID: 26301240]
[140] Chua ME, Sio MC, Sorongon MC, Dy JS. Relationship of dietary intake of omega-3 and omega-6 Fatty acids with risk of prostate cancer development: A meta-analysis of prospective studies and review of literature. Prostate Cancer 2012; 2012: 826254.

[http://dx.doi.org/10.1155/2012/826254] [PMID: 23193480]

[141] Haas-Haseman M. Weighing the Benefits of Fish Oil for Patients With Prostate Cancer: A Subcohort Review From the SELECT Trial. J Adv Pract Oncol 2015; 6(4): 376-8. [PMID: 26705498]

[142] Stark KD, Van Elswyk ME, Higgins MR, Weatherford CA, Salem N Jr. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. Prog Lipid Res 2016; 63: 132-52. [http://dx.doi.org/10.1016/j.plipres.2016.05.001] [PMID: 27216485]

[143] Nakamura Y, Ueshima H, Okamura T, *et al.* Association between fish consumption and all-cause and cause-specific mortality in Japan: NIPPON DATA80, 1980-99. Am J Med 2005; 118(3): 239-45. [http://dx.doi.org/10.1016/j.amjmed.2004.12.016] [PMID: 15745721]

[144] von Schacky C. Omega-3 Index and sudden cardiac death. Nutrients 2010; 2(3): 375-88. [http://dx.doi.org/10.3390/nu2030375] [PMID: 22254028]

[145] Elvevoll EO, Osterud B. Impact of processing on nutritional quality of marine food items. Forum Nutr 2003; 56: 337-40. [PMID: 15806925]

[146] Clayton PR, Ladi S. From alga to omega; have we reached peak (fish) oil? J R Soc Med 2015; 108(9): 351-7. [http://dx.doi.org/10.1177/0141076815599673] [PMID: 26359136]

[147] Harris WS, Pottala JV, Sands SA, Jones PG. Comparison of the effects of fish and fish-oil capsules on the n 3 fatty acid content of blood cells and plasma phospholipids. Am J Clin Nutr 2007; 86(6): 1621-5. [http://dx.doi.org/10.1093/ajcn/86.6.1621] [PMID: 18065578]