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MINI REVIEW

Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence

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Abstract The present review aims to illustrate current knowledge about the efficacy of omega-3 long-chain polyunsaturated fatty acids (*n*−3 LC-PUFAs) in treating/preventing several metabolic pathologies. We reviewed systematically the published evidence on the effectiveness of *n*−3 LC-PUFAs fish consumption or *n*−3 LC-PUFAs supplementation on prevention/treatment of obesity, metabolic syndrome, and cardiovascular diseases. Most of the reviewed studies were randomized-controlled interventional trials, although some relevant prospective and cross-sectional studies as well as some meta-analysis were also reviewed. Supplementation with *n*−3 LC-PUFAs might improve some obesity-associated metabolic syndrome features such as insulin resistance, hypertension and dyslipidemia by decreasing plasma triglycerides. Moreover, the blood pressure-lowering and anti-inflammatory properties of these fatty acids and their benefits in vascular function might confer cardioprotection. However, the efficacy of *n*−3 LC-PUFA on reducing myocardial infarction, arrhythmia, cardiac and sudden death, or stroke is controversial. Due to the beneficial actions of *n*−3 LC-PUFAs, several worldwide government and health organizations have established some recommendations of *n*−3 LC-PUFAs intake for groups of population. In general, the recommended levels for diseases prevention are lower than those advised for particular treatments. However, more clinical trials are necessary to recommend the most effective dosages and formulas (type of *n*−3 LC-PUFA, EPA/DHA ratio) for specific pathologies.

Keywords *n*−3 LC-PUFAs · Obesity · Insulin resistance · Dyslipidemia · Cardiovascular disease

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Introduction

Essential fatty acids: biological functions and mechanisms of action

Dietary fat constitutes an essential macronutrient in all mammals since it participates in a number of biological functions in the maintenance of homeostasis. Among those, fatty acids are one of the many representatives which have important biological activities in all cell types and tissues. Fatty acids are usually termed as short (less than six), medium (between six and 12), or long-chain (more than 12) fatty acids depending on the number of carbon atoms present in their composition. These compounds are also classified depending on the number of

double bonds present in their hydrocarbon chains as saturated or unsaturated. Saturated fatty acids (e.g., 16:0, 18:0 where first number represents number of carbons) do not have any double bonds in their chemical structure and thereby, are constituted by a straight chain of methylen-groups. On the other hand, unsaturated fatty acids contain one or more double bonds in their structure and are further classified as 'monounsaturated' (containing one double bond) or 'polyunsaturated' (PUFA, containing more than one double bond). The omega-3 (*n*−3) and omega-6 (*n*−6) fatty acids are long-chain PUFAs (LC-PUFAs) with the first of the double bonds in the *cis* configuration starting from the third and sixth carbon atom, respectively, from the methyl terminus group [100] LC-PUFAs are commonly designated as 18:3*n*−3, 18:2*n*−6, etc.

The *n*−3 LC-PUFAs and *n*−6 LC-PUFAs families are essential fatty acids in animals since they cannot be synthesized de novo and are vital for normal metabolism. They, therefore, need to be supplied in the diet [104]. One of the representatives of *n*−6 LC-PUFAs, linoleic acid (LA—C18:2*n*−6), is very abundant in the western dietary pattern, and is the precursor of arachidonic acid (AA—20:4*n*−6) [104]. On the other hand, α-linolenic fatty acid (ALA—C18:3*n*−3), which can be found in vegetable oils, is the precursor of other *n*−3 LC-PUFAs, eicosapentaenoic acid (EPA—20:5*n*−3) and docosahexaenoic acid (DHA—22:6*n*−3). EPA and DHA are found in considerable amounts in fish and fish oil and are commonly considered to be marine sourced fatty acids. Thus, regular fish intake in our diet guarantees an optimal supply of *n*−3 LC-PUFAs in our metabolism since in vivo conversion of ALA to EPA and DHA might be limited. Rich sources of ALA are flaxseed meal and oil, while rich sources of EPA and DHA are salmon, tuna, mackerel, anchovy and sardines [105]. On the other hand, vegetable oils (sunflower, corn, and soya) contain 50 % or more of *n*−6 LC-PUFAs [104].

LC-PUFAs are incorporated into cell membrane phospholipids and serve as precursors of eicosanoid synthesis. AA and EPA are converted through phospholipase A2, cyclooxygenase (COX) and lipooxygenase (LOX) to prostaglandins, thromboxanes, leukotrienes as well as various hydroxyl-fatty acids [105, 108]. Resulting metabolites from these reactions are widely known as eicosanoids, which have important biological functions in homeostasis, with a particular interest on inflammation. Although this is not true in all cases, in general, AA-derived eicosanoids are more physiologically potent and of a pro-inflammatory nature, whereas those derived from *n*−3 LC-PUFAs show anti-inflammatory activity [108].

Moreover, it has been recently discovered that EPA and DHA are precursors of important pro-resolving autacoids, resolvins (designated E and D, from EPA and DHA), protectins and maresins, which are powerful bioactive agents involved in the resolution of inflammation and also have anti-inflammatory and immune regulatory activities, as they inhibit the production of inflammatory cytokines and decrease the leukocyte recruitment and diapedesis [110] (Fig. 1).

The *n*−3 LC-PUFAs series can compete with the *n*−6 LC-PUFAs series for the same enzymes (COX and LOX) to form a different class of eicosanoids, which can counteract the effects of the *n*−6 eicosanoids [116]. In this way, the excess of a fatty acid series may lead to a decrease in the conversion of the other series [108]. Thus, an excessive intake of LA decreases the formation of other *n*−3 LC-PUFAs such as EPA and DHA. In fact, when competing with larger amounts of *n*−6 LC-PUFAs, the conversion of ALA into EPA is very low, being from 0.2 to 6 %, and the conversion into DHA is 0.05 % or less [41]. The balance of *n*−6/*n*−3 LC-PUFAs is an important determinant in homeostasis maintenance, normal development, and mental health throughout the life cycle [111, 112]. Moreover, an excessive intake of *n*−6 LC-PUFAs promotes the synthesis of pro-inflammatory eicosanoids, while a high intake of *n*−3 LC-PUFAs versus *n*−6 LC-PUFAs has anti-inflammatory effects. Inflammation is related to many chronic diseases, such as coronary heart disease, rheumatoid arthritis, obesity, diabetes, cancer, and mental illness [10]. The high *n*−6/*n*−3 LC-PUFAs ratio (15:1 to 16.7:1) that is found in today's Western diets could promote these diseases [42, 111]. Therefore, lowering the balance of *n*−6/*n*−3 LC-PUFAs can play an important role in decreasing the risk of these chronic diseases [111, 112], and an optimal balance between *n*−6 LC-PUFAs/ *n*−3 LC-PUFAs intake has been considered to be of importance when recommending LC-PUFAs supplementation. Thus, in the secondary prevention of cardiovascular disease, a ratio of 4:1 was associated with a 70 % decrease in total mortality. A ratio of 5:1 had a beneficial effect on patients with asthma and a ratio of 2–3:1 suppressed inflammation in patients with rheumatoid arthritis [111]. However, there has been much debate over the question of whether the ratio of *n*−6/*n*−3 LC-PUFAs or total amounts of dietary PUFAs is of more importance in modifying disease risk and in optimizing

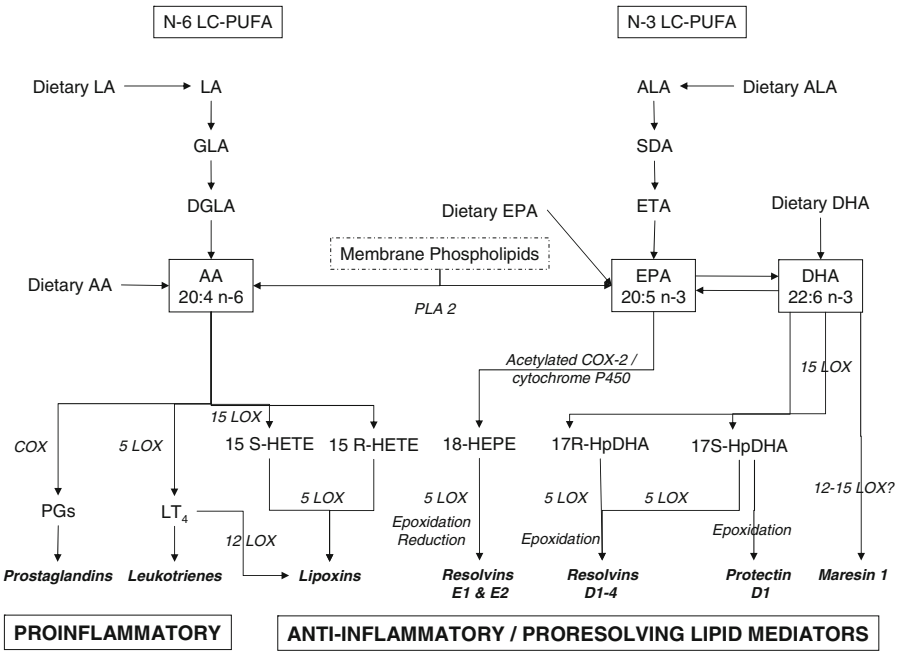

Fig. 1 *n*−6 and *n*−3 LC-PUFAs pathways leading to their derived pro-inflammatory or anti-inflammatory and pro-resolution mediators. *LA* linolenic acid, *ALA* α-linolenic acid, *GLA* γ-linoleic acid, *DGLA* dihomo-γ-linoleic acid, *SDA* stearidonic acid, *ETA* eicosatetraenoic acid, *PG* prostaglandins, *PLA2* phospholipase A2, *AA* arachidonic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *COX* cyclooxygenase, *LOX* lipoxygenase, *HEPE* hydroxyeicosapentaenoic acid, *HETE* hydroxyeicosatetraenoic acid, *HpDHA* hydroperoxydocosahexaenoic acid, *HpEPE* hydroperoxyeicosapentaenoic acid, *LOX* lipoxygenase, LT_4 leukotriene

the benefits of *n*−3 PUFAs for cardiovascular health [42, 106].

All of these recognized cellular and molecular actions of *n*−3 LC-PUFAs, and the fact that eicosanoids derived from the *n*−3 and *n*−6 PUFAs acts on selective eicosanoid receptors in nearly every tissue, including brain, retina, liver, kidney, adrenal, and gonad glands [104, 105], support the idea that they participate in the regulation of a wide range of physiological functions. The present review summarizes the main outcomes described so far in literature on the effects of *n*−3 LC-PUFAs on obesity, metabolic, and cardiovascular diseases, providing an in-depth description of most relevant studies as well as a critical discussion and brief conclusion highlighting the key facts related to each pathology.

n−3 LC-PUFAs in the prevention and treatment of obesity and metabolic syndrome

Obesity is considered to be a metabolic disorder, whose prevalence is increasing dramatically in most developed countries over the last 20 years. In fact, in 2008, the World Health Organization (WHO) estimated that at least 1.5 billion adults (20 years and older) were overweight. Of these, over 200 million men and nearly 300 million women were obese. Obesity is associated with an increased risk of developing chronic morbidities (hypertension, insulin resistance, dyslipidemia), which constitute the major components of the metabolic syndrome [56]. Low-grade inflammation has been identified as a key factor in the development of obesity-related metabolic disorders [21].

In this context, it has been proposed that an increased intake of *n*−3 LC-PUFAs, ALA, EPA, and DHA, could reduce the risk of cardiovascular disease risk, improving the metabolic syndrome features in many aspects, and may have potential anti-obesity properties [1]. However, most of the beneficial effects attributed to *n*−3 LC-PUFAs have been observed after the consumption of EPA and DHA, and most data do not support the notion that ALA will provide the same physiological effects. The initial studies suggesting the potential protective role of *n*−3 LC-PUFAs on metabolic

risk factors were based on observations from populations with a lower incidence of cardiovascular diseases, such as Eskimos and Japanese, who exhibited increased plasma levels of EPA, probably due to the high intake of fatty fish in their habitual diet [4]. After that observation, many interventional studies were performed in order to further address whether fish and fish oils may have a protective role against the development of obesity or may be useful in weight management as well as in the attenuation of insulin resistance and dyslipidemias associated to obesity (main outcomes are detailed below). Tables 1 and 2 summarize human studies that have assessed the effects of *n*−3 LC-PUFA on body weight and body fat in obesity (Table 1) and on dyslipidemia and other metabolic syndrome (Table 2)

Regulation of body weight and body composition in humans

Several studies explored the effects of oral intake of *n*−3 LC-PUFAs (either fish or fish oil) on body weight and body composition in human subjects.

Prospective studies pointed to a potential relationship between *n*−3 LC-PUFAs levels and adiposity, but lacking consistency: The study by Micallef et al. [79] showed an inverse relationship between plasma concentrations of *n*−3 PUFA and anthropometric measures of obesity including BMI, waist circumference and hip circumference. The results of the prospective Health Professional Follow-Up Study suggest that at baseline men with high fish consumption were less likely to be overweight, however no data about association of *n*−3 LC-PUFA intake and changes in BMI were provided in this 12-year follow-up cohort [49]. However, the Nurse's Health study, another prospective study, found that higher intakes of fish and *n*−3 LC-PUFAs were associated with a higher prevalence of obesity [53].

Concerning randomized controlled trials, available evidence suggests conflicting results about the effects of *n*−3 LC-PUFAs on body weight and composition. Couet et al. [18] observed in healthy adults that replacement of 6 g of visible fat in diet with 6 g of fish oil did not affect body weight loss. However, the interventional group had reduced body fat mass and increased lipid oxidation as compared to the control group. Similarly, the study performed by Kabir et al. [55] showed that *n*−3 LC-PUFAs reduced adiposity and atherogenic markers without insulin sensitivity impairment in post-menopausal women with type 2 diabetes.

Two recent studies examined the effects of a combination of *n*−3 LC-PUFAs and a dietary energy restriction in obese volunteers. Subjects were randomized to one of four isocaloric energy-restricted diets for eight weeks: (1) control diet (without seafood), (2) diet containing lean fish, (3) diet containing oily fish rich in *n*−3 LC-PUFAs and (4) diet supplemented with six fish oil capsules per day. In this trial, Parra et al. [93] showed that satiety was increased after consumption of the *n*−3 LC-PUFAs-enriched meals. Moreover, Thorsdottir et al. [120] showed a greater weight loss and waist circumference reduction in those patients receiving fish or fish oil, although these differences were limited to men. The study by Krebs et al. [62] in 93 overweight or obese hyperinsulinemic women that followed a 5-week energy-restricted diet supplemented with fish oil (1.3 g/EPA+2.9 g/DHA) found no difference in weight loss as compared with the group that only followed the energy-restricted diet.

Another interesting aspect to consider is the effectiveness of *n*−3 LC-PUFAs intake in combination with regular exercise. Interestingly, Warner et al. [129] observed that subjects that consumed fish oils and had moderate physical activity showed a major reduction in body fat. Similar results were reported by Hill et al. [50] who also found that exercise and *n*−3 LC-PUFAs supplementation resulted in a significant reduction of body fat.

In this line, the trial performed by Kunesova et al. [66] found a higher reduction of BMI and hip circumference together with a higher increase in beta-hydroxybutyrate levels in the *n*−3 LC-PUFAs group who followed a very-low-calorie diet as compared with the placebo group, suggesting that *n*−3 LC-PUFAs combined with energy restriction specifically increases fat oxidation, leading to improvements on body composition. Moreover, a significant negative correlation between BMI change and phospholipid docosahexaenoic acid change was found, suggesting that docosahexaenoate (22:6*n*−3) seems to be the active component. The study of Vasickova et al. [126] also suggest a possible beneficial effect of DHA intake on body weight reduction in obese children that consumed an extra 300 mg DHA and 42 mg EPA daily for a period of 3 weeks.

However, Munro and Garg [87] described that dietary supplementation with *n*−3 LC-PUFAs (six capsules/day of 1 g *n*−3PUFAs containing 70 mg EPA and 270 mg

Table 1 Human studies that have assessed the effects of *n*−3 LC-PUFAs on body weight and fat mass

Reference	Study design	Diet and intervention	Outcomes (intervention group vs. control group)
Couet et al. [18]	Crossover (2×3 week), Healthy adults (*n*=6)	Diet ad libitum supplemented with 6 g/day fish oil (1.1 g/day EPA+0.7 g/day DHA)	↔ body weight loss, ↓ body fat
DeFina et al. [25]	Placebo-controlled, randomized clinical trial in overweight/ obese subjects (*n*=128)	6-month dietary intervention and exercise. 5 capsules/day of either EPA:DHA (5:1 Ratio, 5 g/day) or placebo	↔ body weight reduction, ↔ body fat loss or ↔ waist circumference
Hill et al. [50]	Randomized double-blind controlled parallel study by 12-week Overweight adults (*n*=65)	Usual diet supplemented with 6 g/day fish oil (0.36 g/day EPA+1.6 g/day) combined or not with exercise (3 times/week)	↓ body fat (independent effect of exercise),
He et al. [49]	Prospective (Health Professional Follow-up Study)	High *n*−3 LC-PUFA intake (fish consumption)	At baseline men with high fish consumption were less likely to be overweight. No data about association of *n*−3 LC-PUFA intake and changes in BMI were pro vided in this 12-years follow-up cohort.
Iso et al. [53]	Prospective (Nurse's health study)	High fish and *n*−3 LC-PUFAs intake	↑ prevalence of obesity
Kabir et al. [55]	Randomized double-blind parallel by 2-month Postmenopausal women with type 2 diabetes (*n*=26)	Intervention diet (55 % carbohydrate, 15 % protein, 30 % fat) supplemented with 3 g/day fish oil (1.08 g/day EPA+0.72 g/day DHA)	↔ weight loss ↓ fat mass ↓ adipocytes diameter of subcutaneous tissue ↔ insulin sensitivity
Krebs et al. [62]	Randomized double-blind controlled parallel study by 24-week Overweight of obese insulin-resistant women (*n*=93)	5-weeks energy-restricted diet (800–900 kcal/day), 7-week gradual increase maintenance diet and 12-week maintenance diet (~2500 kcal/day) supplemented with 1 g/day fish oil (1.3 g/EPA+ 2.9 g/DHA)	↔ body weight
Kunesova et al. [66]	Randomized controlled parallel study by 4-week, Severely obese women (*n*=20)	Very-low-calorie diet (~520 kcal/day)+ 2.8 g/day fish oil (1.9 g/day EPA+ 0.9 g/day DHA) combined with exercise (3 times/week)	↑ weight loss ↓ BMI
Munro and Garg [87]	14-weeks double-blind, randomized, controlled trial, Obese subjects aged 18–60 years	Very-low energy diet for 4 weeks followed by 10 weeks of weight maintenance supplemented with 6×1 g capsules/day of LC*n*−3PUFAs containing 70 mg EPA and 270 mg DHA	Similar weight loss reduction and metabolic profile improvements than placebo
Thorsdottir et al. [120]	Randomized controlled parallel study by 8-week Overweight and obese adults(*n*=278)	Energy-restricted diet (600 kcal/day) supplemented with lean fish (150 g cod×3 week – 0.26 g/day EPA+DHA) or fatty fish (150 g salmon×3 week – 2.1 g/day EPA+ DHA) or fish oil (6 capsules/day – 1.3 g/day EPA+DHA)	For men with fish or fish oil intake ↓ body weight ↓ waist circumference

BMI body mass index, *CRP* C-reactive protein, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *Hb* A_{1C} glycated hemoglobin, *HDL* high-density lipoprotein, *IL* interleukin, *n−3 LC-PUFAs n*−3 long-chain polyunsaturated fatty acids, *TG* triglycerides, *VLDL* very low-density lipoprotein

Table 2 Human studies reviewed that have assessed the effects of *n*−3 LC-PUFAs on dyslipidemia and other metabolic syndrome features.

Reference	Study design	Diet and intervention	Outcomes (intervention group vs. control group)
Chan et al. [14]	6-week randomized, placebo-controlled, 2×2 factorial intervention study, 48 obese men with dyslipidemia	Fish oils (4 g/day) and atorvastatin (40 mg/day)	↓ TG ↑ HDL cholesterol ↓ fractional catabolic rate of HDL apo A-I and HDL apo A-II
Davidson et al. [22]	8-week, randomized, double-blind, placebo-controlled study in hypertriglyceridemic patients (*n*=254 patients, of whom 57.5 % were male)	Addition of prescription *n*−3 LC-PUFAs (4 g/day) to simvastatin (40 mg/day) treatment	↓ TG ↓ non HDL-cholesterol ↑ HDL-cholesterol ↓ VLDL
DeFina et al. [25]	Placebo-controlled, randomized clinical trial in overweight/obese subjects (*n*=128)	6-month dietary intervention and exercise. 5 capsules/day of either EPA:DHA (5:1 Ratio, 5 g/day) or placebo	No differences in lipid and glucose profiles and in blood pressure
Kabir et al. [55]	Randomized double-blind parallel by 2-month Postmenopausal women with type 2 diabetes (*n*=26)	Intervention diet (55 % carbohydrate, 15 % protein, 30 % fat) supplemented with 3 g/day fish oil (1.08 g/day EPA+0.72 g/day DHA)	↓ TG ↓ ratio of triacylglycerol to HDL cholesterol (atherogenic index) ↓ plasminogen activator inhibitor-1
He et al. [49]	Prospective (Health Professional Follow-Up Study), men 40–75 years old (*n*=43671)	High *n*−3 LC-PUFAs intake (fish consumption)	↓ relative risk of ischemic stroke
Hill et al. [50]	Randomized double-blind controlled parallel study by 12-week Overweight adults (*n*=65)	Usual diet supplemented with 6 g/day fish oil (0.36 g/day EPA+1.6 g/day) combined or not with exercise (3 times/week)	↑ HDL cholesterol ↓ TG
Kelley et al. [57]	double-blind, randomized, placebo-controlled parallel study, 34 hypertriglyceridemic men	7.5 g DHA oil/day (3 g DHA/day) or olive oil (placebo) for 90 days	↓ TG, large VLDL and intermediate-density lipoproteins ↑ LDL cholesterol and small VLDL particles, and large LDL particles
Krebs et al. [62]	Randomized double-blind controlled parallel study by 24-week, Overweight of obese insulin-resistant women (*n*=93)	5-weeks energy-restricted diet (800–900 kcal/day), 7-week gradual increase maintenance diet and 12-week maintenance diet (~2500 kcal/day) supplemented with 1 g/day fish oil (1.3 g/EPA+2.9 g/DHA)	↓ TG ↑ adiponectin
Lopez-Alvarenga et al. [70]	Cross-sectional study 212 men and 240 women from Alaska	*n*−3 LC-PUFAs	Associated with lower TG levels, even in the presence of high levels of saturated fat
Lovegrove et al. [74]	12 weeks-randomized, double-blind, placebo-controlled, parallel, fish oil intervention study, 44 Europeans and 40 Indo-Asian Sikhs	4.0 g fish oil (1.5 g EPA and 1.0 g DHA) or 4.0 g olive oil (control)	↓ TG ↓ apolipoprotein B-48, and platelet phospholipid arachidonic acid ↑ HDL cholesterol and platelet phospholipid EPA and DHA
Micallef et al. [78]	3 week randomized, double-blind, placebo-controlled, 2×2 factorial trial in 4 parallel groups of 60 hyperlipidemic individuals	sunola oil or 1.4 g/day *n*−3 LC-PUFAs capsules with or without 2 g phytosterols per day	*n*−3 LC-PUFA+phytosterols: ↓ total and LDL-cholesterol *n*−3 LC-PUFA alone or with phytosterols: ↓ TG, ↓ total cholesterol, ↑ HDL cholesterol

Table 2 (continued)

Reference	Study design	Diet and intervention	Outcomes (intervention group vs. control group)
Mori et al. [81]	Randomized controlled trial by 16-week Overweight and obese adults (n=63)	Energy-restricted diet (~500–1,550 kcal/day) supplemented with fish (3.65 g/day $n-3$ LC-PUFAs)	↔ HDL cholesterol ↔ LDL cholesterol ↑ HDL_2 cholesterol ↓ TG ↓ insulin resistance
Nestel et al. [89]	Seven weeks double-blind trial, 38 dyslipidemic subjects	3 g EPA/day (n=12), 3 g DHA/day (n =12), or a placebo (n=14)	↑ systemic arterial compliance (36 % with EPA and 27 % with DHA) ↓ total and VLDL triacylglycerol
Popp-Snijders et al. [97]	Intervention diet by 8-weeks, Non-insulin-dependent diabetic subjects (n=6)	Usual diet supplemented with 3 g/day EPA+DHA	↑ insulin sensitivity, ↓ triacylglycerol
Rivellese et al. [102]	Randomized double-blind placebo-controlled parallel study by 6-months Non-insulin-dependent diabetic subjects (n=16)	Usual diet supplemented with 2.7 g/day EPA+DHA for 2-month, then 1.7 g/day for 4-month	↔ insulin-mediated glucose uptake ↓ TG ↓ VLDL-TG ↓ Fasting non-sterified fatty acids
Stene & Joner [114]	Case–control study, 545 cases of childhood-onset type 1 diabetes and 1668 population control subjects	Use of cod liver oil in the first year of life	↓ risk of type 1 diabetes
Tsitouras et al. [123]	Intervention diet by 8-weeks, Elderly subjects>60 year (n=12)	6-week control diet+8-week experimental diet (720 g/week fatty fish+15 ml/day sardine oil)	↑ insulin sensitivity ↓ hs-CRP
Warner et al. [129]	Randomized controlled parallel study by 12-week, Hyperlipidemic adults (n=34)	Usual diet supplemented with 50 mL/day fish oil (14 g/day EPA+10 g/day DHA) combined with exercise (3 times/week)	↑ HDL cholesterol ↓ TG ↓ Cholesterol ↓ Serum apo-B ↓ LDL cholesterol ↓ LDL protein
Woodman et al. [131]	Double-blind placebo-controlled parallel study by 6-week, Obese type 2 diabetes mellitus subjects 40–75 year old with treated hypertension (n=51)	Usual diet supplemented with 4 g/day EPA+DHA	↔ Hb A_{1C} ↔ LDL cholesterol ↔ HDL cholesterol ↓ TG ↑ HDL_2 cholesterol ↑ fasting glucose

DHA docosahexaenoic acid, *EPA* eicosapentaenoic acid, *Hb A_{1C}* glycated hemoglobin, *HDL* high-density lipoprotein, *IL* interleukin, *n −3 LC-PUFAs* $n-3$ long-chain polyunsaturated fatty acids, *TG* triglycerides, *VLDL* very low-density lipoprotein

DHA for 14 weeks) did not promote additional weight loss when combined with a very-low-energy diet for 4 weeks, followed by 10 weeks of weight maintenance. Similarly, DeFina et al. [25] described that $n-3$ LC-PUFAs supplementation (3 g EPA plus DHA at a 5:1 ratio for 24 weeks) in combination with diet and exercise

did not significantly modify weight loss and body composition in overweight/obese subjects in comparison to a placebo supplemented group. The most probable cause for the disparities in the outcomes obtained in these studies may be related to phenotypical characteristics of the subjects included in the study. In previously published reports, subjects recruited for nutritional interventions with fish oils or *n*−3 PUFAs supplements were overweight with additional metabolic disorders (glucose intolerance, dislipidemia, etc.) [50, 129], while in the study carried out by DeFina et al. [25], subjects were overweight but otherwise healthy from a metabolic point of view. Therefore, it can be suggested that *n*−3 LC-PUFAs supplementation could be effective in improving metabolic syndrome symptoms rather than ameliorating overweight/obesity per se. Indeed, mechanisms underlying this differential response in body weight in obese humans remain a challenging point still to be addressed in future.

Regulation of insulin sensitivity

There is some controversy concerning the effects of *n*−3 LC-PUFAs on insulin resistance and type 2 diabetes. Mori et al. [81] found a beneficial effect of fish oil on insulin sensitivity in obese women. In addition, a study conducted by Tsitouras et al. [123] found that insulin sensitivity increased significantly after 8 weeks on the *n*−3 LC-PUFAs diet, which was also accompanied by a significant reduction in the serum C-reactive protein. In this context, a 2-year nutritional intervention carried out by Esposito et al. [29], showed that a Mediterranean-style diet with a higher intake of *n*−3 LC-PUFAs in subjects with metabolic syndrome significantly improved inflammation biomarkers and insulin sensitivity, reducing the prevalence of metabolic syndrome compared to a control group [29]. Likewise, Ramel et al. [99] showed that *n*−3 LC-PUFAs consumption during energy reduction exerts positive effects on insulin resistance in young overweight individuals, independently of changes in body weight, triglycerides, erythrocyte membrane or adiponectin. Additionally, a recent study also demonstrated an improvement in insulin sensitivity in young iron-deficient women after 8 weeks with a high intake of oily fish (four portions/week) [88]. In this context, Feskens et al. [30] suggested that oily fish intake delays the development of glucose intolerance in humans. Furthermore, Popp-Snijders et al. [97] observed improved insulin sensitivity upon supplementation with dietary *n*−3 LC-PUFAs (3 g of EPA+DHA) in non-insulin-dependent diabetic subjects. In contrast, Rivellese et al. [102] found no changes in insulin sensitivity in type 2 diabetic patients with hypertriglyceridemia after moderate dietary supplementation with *n*−3 LC-PUFAs (fish oil, 2.7 g EPA+DHA). Other studies also suggest that *n*−3 LC-PUFAs do not influence insulin sensitivity in obese and type 2 diabetic women [42, 55]. On the other hand, the double-blind, placebo-controlled study by Woodman et al. [131] involving type 2 diabetic subjects who were randomly assigned to consume 4 g of EPA or DHA showed that both fatty acids had similar benefits on lipids, but had adverse effects on short-term glycemic control as compared to the placebo group that received olive oil, suggesting that fish oil may worsen glycemic control.

Regulation of dyslipidemia

Regarding the effects of *n*−3 LC-PUFAs on dyslipidemia, several studies demonstrated that fish oil supplements and diets containing fish and fish oils are useful to reduce plasma triglycerides and VLDL lipoprotein concentrations [70, 89] contributing to improve the symptoms of metabolic syndrome. Moreover, other studies also reported increases in plasma HDL-cholesterol concentrations after *n*−3 LC-PUFA supplementation (1.4–4 g/day), either as oily fish [78] or as fish oil (EPA+DHA) [55, 78, 81], contributing to decreased risk of atherosclerosis.

The meta-analysis conducted by Harris et al. [46] in placebo-controlled studies showed that oral supplementation with fish oils for 7 to 10 weeks (<7 g/day) decreases fasting plasma triglyceride concentrations by approximately 25 % in normolipidemic subjects and up to 34 % in hyperlipidemic subjects. In the same way, another meta-analysis showed that 0.1–5.4 g/day of EPA and DHA was able to reduce fasting triacylglycerol (TG) concentrations in healthy subjects as well as in diabetic, hypertensive, dyslipidemic patients and in subjects that had been diagnosed with cardiovascular disease [34].

On the other hand, other authors have reported that fish oil supplementation has no significant effects on HDL-cholesterol or total cholesterol concentrations [48,

57] and that *n*−3 LC-PUFAs have only a small HDL-raising effect in hypertriglyceridemic subjects [54].

By way of conclusion, published evidence suggests that *n*−3 LC-PUFAs supplementation might have a preventive role against weight gain by modulating fat oxidation and satiety, although conflicting results have been found in body weight reduction and body composition. It should be stated that the effects of *n*−3 LC-PUFAs on fat mass and weight regulation might be difficult to address due to important differences in how the studies were designed/conducted (for instance, whether diet was combined with exercise or not) as well as the inclusion criteria and source for *n*−3 LC-PUFAs supplementation. Therefore, effectiveness of the *n*−3 LC PUFA supplementation might be related to dietary and exercise patterns and gender aspects might also be relevant. In addition, oral supplementation with *n*−3 LC-PUFAs seems to be an interesting tool to reduce plasma triglycerides and increase HDL-cholesterol, helping to improve metabolic syndrome and reduce risk of mortality and morbidity.

However, the effects of these fatty acids on insulin sensitivity remain controversial since one of the main limitations while examining the effects on insulin sensitivity is the fact that insulin resistance is usually linked to other pathological conditions such as hypertrigliceridemia, overweight and cardiovascular diseases and might be difficult to study on itself. Thus, further studies are needed to evaluate this aspect of *n*−3 LC-PUFAs in insulinemia management.

n−3 LC-PUFA and Cardiovascular Diseases

Cardiovascular diseases are the leading cause of death in many countries. It is widely known that a high intake of saturated fat increase the risk of cardiovascular diseases, while intakes enriched in *n*−3 LC-PUFAs seem to lower this risk [84].

Initial studies reporting cardiovascular beneficial effects of *n*−3 LC-PUFAs were performed in the late 1970s when Bang and Dyerberg observed a lower incidence of cardiovascular disease in Eskimos in spite of the fact that they had a high fat intake from marine animals [4]. Several years afterwards, the low incidence of atherosclerotic heart disease among the Eskimos was attributed to the diet rich in fish oil from marine vertebrates. Later on, several controlled trials provided evidence that the consumption of *n*−3 LC-PUFAs was responsible for the protective effects of fish oils against cardiovascular diseases [5, 27].

Prevention of myocardial infarction

Several investigators have addressed the benefits of fish oils in the secondary prevention of myocardial infarction. The Diet and Reinfarction trial (DART) observed that a moderate intake of fatty fish (two or three portions per week) may reduce mortality (29 %) in men who have recovered from myocardial infarction. Moreover, the subgroup consuming a fish oil capsule containing 450 mg of EPA plus DHA daily showed a reduction of 56 % in the mortality rate [9].

In the Indian Experiment of Infarct Survival Trial, patients suspected of suffering from myocardial infarction received fish oil capsules (1.8 g/day, EPA+DHA), mustard oil (2.9 g of ALA) or placebo. After 1 year follow-up, cardiac events were significantly lower in the fish oil and mustard oil groups as compared to the placebo group, and total cardiac deaths were significantly lower in the fish oil as compared with the control (placebo) group [113]. The findings of this study suggest that fish oil and mustard oil, possibly due to the presence of *n*−3 LC-PUFAs, may provide rapid protective effects in patients with acute myocardial infarction. However, this study is now being challenged, since a larger trial is necessary to confirm these outcomes.

The GISSI-Prevention trial is considered the largest randomized controlled trial which examined the role of *n*−3 LC-PUFAs in the prevention of further cardiovascular events. This study enrolled around 11,000 subjects with recent myocardial infarction who were randomized to receive a gelatine capsule of *n*−3 LC-PUFAs (equivalent to 850 mg/day as EPA+DHA ethyl-esters), vitamin E, or the combination of both or any of these supplements. After 3.5 years' follow-up, subjects receiving *n*−3 LC-PUFAs had a decreased risk ratio of all-cause death plus nonfatal myocardial infarction and stroke [43].

The Japan EPA Lipid Intervention Study (JELIS) is an important trial that analyzed the effects of the addition of 1.8 g/day of highly purified EPA to statin therapy in Japanese patients with hypercholesterolemia [132] and found that the incidence of major coronary events,

which included nonfatal myocardial infarction, coronary artery disease death and unstable angina, was reduced by 19 % in the group that consumed EPA.

Moreover the meta-analysis performed by Wang et al. [127] showed that *n*−3 LC-PUFAs from fish or fish oil supplementation significantly reduced all-cause mortality, myocardial infarction, cardiac and sudden death or stroke [127]. Similarly, the recent analysis of Delgado-Lista et al. [26] also suggests that marine *n*−3 LC-PUFAs are effective in preventing cardiovascular events, cardiac death and coronary events, especially in persons with high cardiovascular risk. In contrast, other meta-analysis have suggested that *n*−3 LC-PUFAs supplementation was not associated with a lower risk of all-cause mortality, cardiac death, sudden death, myocardial infarction, or stroke based on relative and absolute measures of association in a population at increased risk of cardiovascular risk [103]. This study included all available published randomized evidence regardless of methodological study characteristics, prevention setting or supplementation mode. Therefore, the relevance of this meta-analysis on the efficacy of omega-3 PUFAs on cardiovascular events is considerable since included more than 68,000 patients from 20 randomized controlled trials, including DART, GISSI, JELIS among others. However, as randomized evidence accumulates, further meta-analysis will clarify the efficacy of omega-3 on major cardiovascular endpoints.

Other studies also found no beneficial effects of *n*−3 LC-PUFAs on the prevention of myocardial infarction. The recent Alpha Omega trial [65] examined whether margarine supplemented with EPA–DHA (400 mg/day) and/or ALA (2 g/day) could prevent major fatal and nonfatal cardiovascular events and cardiac interventions in 60–80-year-old patients with previous history of myocardial infarction. Neither EPA–DHA nor ALA reduced the primary endpoint in this study and the effect of EPA–DHA supplementation is difficult to address since supplementation dose was small and might have been masked by the large differences in ALA doses.

The study conducted by Burr et al. [8] recruited male patients with angina pectoris from general practice, who were assigned to one of four groups. The first group was advised to consume two portions of oily fish each week or three fish oil capsules daily. The second group was advised to consume more fruits, vegetables, and oats. The third group received both recommendations, and the fourth group received no specific dietary advice. The results showed that after 3 to 9 years, there was no reduction in mortality in the group of patients that received dietary advice on increasing fish consumption. Nor were other benefits attributed to the group advised to consume more fruit. However, it was not possible to check long-term adherence to the assigned treatments or changes in lifestyle and medication during this time.

Arrhythmia and prevention of arrhythmia in high-risk patients

Several studies, such as the Fish oil Anti-arrhythmia study, reported different effects of these *n*−3 LC-PUFAs on arrhythmia and prevention of ventricular arrhythmias in high-risk patients [67]. In this study, daily ingestion of 2.6 g of EPA plus DHA for at least 11 months significantly reduced the risk of potentially fatal ventricular arrhythmias, compared to an olive oil placebo consumption [67]. Moreover, tuna or other broiled or baked fish consumption was associated with a 28 % reduced incidence of atrial fibrillation over a 12-year follow-up [86]. In the trial performed by Calo et al. [12], 160 patients were assigned to receive 2 g of EPA+DHA per day or placebo before elective coronary artery bypass surgery. The *n*−3 LC-PUFAs consumption was associated with a lower incidence of atrial fibrillation and with a shorter hospital stay.

However, in another randomized study with patients receiving either 1.5 g of EPA plus DHA per day or placebo, there was no significant decrease in the number of premature ventricular contractions, although there was a trend to decrease the heart rate [35].

In any case, not only positive results were found concerning this issue: while in patients experiencing non-ischemic ventricular arrhythmia, *n*−3 LC-PUFAs may be proarrhythmic, other studies evidenced a neutral effect [15]. Indeed, Saravanan et al. [107], suggested that the role of *n*−3 LC-PUFAs in primary prevention of coronary artery disease and in atrial fibrillation is far from clear since the results discussed are secondary prevention studies, and when the groups with and without previous coronary artery disease were individually analyzed, there was no benefit in the primary prevention group. In fact, a recent meta-analysis does not suggest a major effect of fish/fish oil or *n*−3 fatty acids on the risk of atrial fibrillation [58]. Other meta-analysis analyzing effects of *n*−3 LC-

PUFAs in the prevention of atrial fibrillation recurrences after cardioversion concludes that the heterogeneity among the studies was significant and that subgroup analysis showed that by administering *n*−3 LC-PUFAs at least 4 weeks prior to cardioversion and continuing thereafter, the recurrence rate of AF is significantly low [13].

Therefore, nowadays, there are no conclusive results and further investigations are needed to demonstrate the potential functionality of *n*−3 LC-PUFAs as antiarrhythmic drugs.

Blood pressure

Another important aspect of *n*−3 LC-PUFA's role on cardiovascular disease is the potential ability to decrease blood pressure. In fact, *n*−3 LC-PUFAs have been shown to decrease both systolic and diastolic blood pressure and people who eat *n*−3 LC-PUFAs regularly are less likely to develop hypertension [20]. The potential blood pressure-lowering effect of *n*−3 LC-PUFAs has been well established both in normotensive and hypertensive subjects [63]. In this regard, the International Study of Macro- and Micro-nutrients and Blood Pressure showed an inverse relationship between the total *n*−3 LC-PUFAs intake and systolic and diastolic blood pressures [124]. Several meta-analyses [36, 47] showed that *n*−3 LC-PUFAs supplementation (3–7.7 g/day EPA and DHA) lowered both systolic and diastolic blood pressure in hypertensive patients. Moreover, these hypotensive effects for *n*−3 LC-PUFAs seem to be mediated through the ability of *n*−3 LC-PUFAs, especially DHA, to decrease vascular wall thickness in the coronary arteries and aorta as well as blunting the renin–angiotensin–aldosterone system and modulation of calcium release in smooth muscle cells [28].

Although other studies have not observed a direct decrease in blood pressure after treatment with *n*−3 LC-PUFAs, it has been stated that these fatty acids improve other related parameters leading to a final positive effect on hypertension [128]. In this context, Nestel et al. [89], observed that the consumption of 3 g/day of either EPA or DHA increased systemic arterial compliance, and consequently decreased systolic and pulse pressure, although these outcomes did not reach significance. The absence of the expected improvement in blood pressure in this study may have been related to the limited number of volunteers enrolled (*n*=12 for each group).

Vascular function

Considerable evidence concerning the effects of *n*−3 LC-PUFAs suggests that these fatty acids may have a protective role against the development of coronary heart disease and cardiovascular events.

Goodfellow and colleagues [39] showed that both EPA and DHA improved endothelium vasodilation in hypercholesterolemic subjects. In agreement with these findings, a trial performed in patients with coronary heart disease showed that long-term treatment with EPA increased both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilatation [117].

Consumption of *n*−3 LC-PUFAs may also reduce the risk of thrombosis by affecting platelet aggregation and hemostasis [60]. *n*−3 LC-PUFAs also prolong the template bleeding time and they may also exert positive effects on erythrocyte flexibility although it seems they do not lower fibrinogen or interact with the fibrinolytic system directly [60].

Inflammation is considered as a key process in the development of cardiovascular diseases. Thus, the anti-inflammatory effects of *n*−3 LC-PUFAs [10, 11] could also partially contribute to the beneficial effects of these fatty acids on the improvements of cardiovascular function. It has been observed that *n*−3 LC-PUFAs reduce adhesion and migration of monocytes and decrease the production of pro-inflammatory mediators such as leukotrienes, which promote infiltration of macrophages to the vessels contributing to the development of the atherome plaque [68]. Thies et al. [119] reported in a randomized controlled trial that consumption of *n*−3 LC-PUFAs contributed to the stability of atherosclerotic plaques and the vessels of these patients were also less infiltrated by macrophages than those of the control group. Similarly, Baumann et al. [7] showed in a randomized trial that supplementation with *n*−3 LC-PUFAs for 4 weeks decreased the transcription of pro-inflammatory molecules such as MCP-1 (monocyte chemoattractant protein-1), which contributes to improve vascular reactivity and endothelial function.

As concluding remarks, a growing body of evidence supports that *n*−3 LC-PUFAs supplementation could have a protective role against the development of cardiovascular diseases. These positive effects seem to be related to a hypotensive action of *n*−3 LC-PUFAs as well as an improvement in vascular function

as these fatty acids stimulate vasodilatation and decrease risk of thrombosis. Available data strongly support that, due to their anti-inflammatory properties, *n*−3 LC-PUFAs could be considered as potential therapeutic agents for the prevention of cardiovascular diseases. However, some studies have questioned the beneficial role of these fatty acids in the prevention of myocardial infarction, arrhythmia, coronary heart disease and sudden cardiac death. In this context, the limitations of the studies should be considered (interventional period, amount and source of *n*−3 LC-PUFAs, number of participants among others) since the outcomes of the studies could be misinterpreted. Therefore, more trials are necessary to clarify their role in other cardiovascular diseases and to further explore their mechanisms of action.

Recommendations

Due to the potential beneficial actions of *n*−3 LC-PUFAs on metabolic diseases, several worldwide government and health organizations have recommended some patterns for *n*−3 LC-PUFAs intake. Some institutions recommend two to three servings of fish per week, whereas others recommend a quantity of nutrients intake per day. Moreover, other organizations encourage the consumption of fish oil and fish rich in *n*−3 LC-PUFAs, but do not establish consumption levels [23, 98]. Table 3 summarizes the recommendations for consumption of *n*−3 LC-PUFAs from several Boards and Associations. For primary prevention of cardiovascular disease in adult general population a consumption of two to three servings per week of fish rich in *n*−3 LC-PUFAs is suggested since it should provide about 500 mg/day of EPA and DHA [3, 6, 33, 52, 64, 77, 115, 118, 125]. However, it should be noted that some reports consider that the evidences suggesting a direct association between the reduced risk of mortality from cardiovascular disease and the consumption of *n*−3 LC-PUFAs in fish are still limited [2].

The American Heart Association distinguishes between the previous recommendation for people without diseases and those for people with specific diseases, to whom a higher consumption of EPA and DHA is recommended. Thus, patients with documented coronary heart disease should consume approximately 1 g EPA+DHA/day from oily fish or fish oil capsules for secondary prevention [69]. For patients with severe hypertriglyceridemia (>500 mg of triglycerides per deciliter), the effective doses of EPA–DHA are higher (2 to 4 g of EPA–DHA per day to lower triglyceride levels by 20 to 40 %). [24, 69, 109]. However, for other pathologies (obesity, type 2 diabetes) the wide range of dosages and formulations tested and substantial variation between results from different trials makes it difficult to recommend dosages for specific treatment goals [31].

An important issue to be solved is the potential differences in DHA and EPA effectiveness. Indeed, several studies have suggested that EPA and DHA have different effects on cell function [40, 91, 121]. Regarding the differential effects observed after supplementation with purified EPA or DHA, while both have shown to be able to decrease TG levels, only DHA appears to increase HDL and LDL particle size [82]. Moreover, it was found that DHA, but not EPA, enhances vasodilator mechanisms and attenuates constrictor responses in the forearm microcirculation [83]. Evidence to date also suggests that DHA is more efficient in decreasing blood pressure, heart rate and platelet aggregation compared to EPA [17].

There is a conflicting point regarding the health benefits of selected species of fish and concerns about contaminants present in these species. Fish such as shark, swordfish, golden bass, or albacore tuna are at the top of the food chain and are more likely to bioaccumulate heavy metals, and it is not easy to control the contaminants in seafood. The presence of toxic contaminants such as mercury, arsenic and lead in fish is of importance for dietary recommendations at the general population level [85]. Since it was suggested that long-term exposure to mercury might increase cardiovascular risk [61], the dietary recommendations regarding fish consumption should be an optimal balance between relative harm and benefits. Moreover, this fact is of importance especially in pregnant women who should avoid some specific fish species that may contain relatively high levels of mercury and other contaminants. However, epidemiological data published so far suggest that the benefits of fish consumption outweigh the potential harm [24], and it is possible to choose fish species that are both high in *n*−3 LC-PUFAs and low in MeHg [76]. Another issue of importance when considering fish oil supplementation of diet is the stability to oxidation of these products, as *n*−3 LC-PUFAs are easily oxidized, which may lead to an increase in potentially hazardous

Table 3 Recommended daily fish and/or *n*−3 LC-PUFAs (ALA, EPA and DHA) intakes from government and health organizations and scientific experts to particular diseases/collectives

Organization or reference	Focus	Recommendation	
		High *n*−3 fish (weekly)[a]	*n*−3 LC-PUFAs (daily)
American Heart Association [69]	Primary prevention CHD For patients with CVD	2 servings	1 g/day
American Diabetes Association [6]	Primary prevention CHD	2 or more servings	
World Health Organization [33]	Primary prevention CHD	2 servings	200–500 mg of EPA+DHA
The American Dietetic Association [64]	Primary prevention CHD	2 servings	500 mg of EPA+DHA
Dutch Health Council [118]	Primary prevention CHD	2 servings	450 mg
United Kingdom Scientific Advisory Committee on Nutrition [125]	Primary prevention CHD	2 servings	450 mg of EPA+DHA
Australia and New Zealand National Health and Medical Research Council [3]	Primary prevention CHD (19 to 70 years of age)		610 mg (men) or 430 mg (women) of EPA+DHA
French organizations [77]	Primary prevention CHD	500 mg of EPA+DHA	
Superior Health Council of Belgium [115]	Primary prevention CHD		0.3 % energy (~670 mg) of EPA+DHA
International Society for the Study of Fatty Acids and Lipids [52]	Primary prevention CHD		500 mg of EPA+DHA
European Food Safety Authority [109]	Maintenance of normal blood pressure.		3.0 g EPA+DHA
	Maintenance of normal blood triglycerides		2.0–4.0 g EPA+DHA

[a] 1 serving: ~112 g of fish

CHD Chronic Heart Disease, *CVD* Cardiovascular Disease

organic compounds. Thus, the contents of hydroperoxides and alkenals in omega-3 supplements are higher than in vegetable oils [45]. Therefore reduction of contaminants and oxidation products is also an industrial focus in fish oil elaboration, and industrial processes have been developed to reach this objective, so that alternative processes like carbon absorption, steam deodorization, and short-path distillation [92] are now used.

According to safety standards for human consumption, the Council for Responsible Nutrition voluntary monograph on *n*−3 LC-PUFA products states the relevance of determining polychlorinated biphenyls, heavy metals (lead, cadmium, mercury, and arsenic) and oxidation levels as well as purity and total amount of EPA and DHA as standards for quality in *n*−3 EPA and DHA supplements [19]. Moreover, these supplements must also fit WHO standards for dioxin and furan levels which will guarantee a high-quality product.

On the other hand, a question has been raised about the recommendation to eat more fish or take fish oil supplements as a source of *n*−3 LC-PUFAs with respect to disease prevention. In this context, several aspects should be taken into consideration. Some authors have questioned whether or not the *n*−3 LC-PUFAs in fish explain all the potential benefits. Indeed, fish are an important source of vitamin D and trace minerals, such as iodine and selenium, and increased intake of them associated with fish consumption may also contribute to the health benefits ascribed to *n*−3 LC-PUFAs. It is important, therefore, to consider that interactions between *n*−3 LC-PUFAs and other constituents of fish providing synergistic beneficial effects cannot be ruled out [31]. Another issue to be considered is that the health benefits of fish consumption may be dramatically altered by the cooking method. Because fish oil supplements are claimed to be contaminant-free and provide a better standardized dosage, some authors consider that the intake of *n*−3

LC-PUFAs as capsules may be a better choice [130], especially in some specific situations such as increased needs of *n*−3 LC-PUFA supply or when fish and seafood consumption is not recommended due to food allergies or intolerance.

Regarding the potential adverse effect of *n*−3 LC-PUFAs fish oil supplements, the most commonly reported ones are fishy aftertaste and gastrointestinal upset, which appears to be dose-dependent. The US Food and Drug Administration has ruled that up to three g of fish oils daily is generally recognized as safe for inclusion in the diet. At higher doses, potential adverse effects, such as increase bleeding time, and drug interactions, could be of concern; however, this effect is generally not considered to be clinically significant. Currently, there are many *n*−3 LC-PUFAs supplements available. When choosing an *n*−3 LC-PUFAs supplement, several factors should be taken into account, especially the quality and purity of the product [31].

Conclusions

Chronic metabolic diseases such as cardiovascular disease, obesity, and diabetes have an intimate relationship with an increased production of pro-inflammatory cytokines. Due to its anti-inflammatory properties an elevated consumption of *n*−3 LC-PUFAs, especially EPA and DHA may help to prevent or improve these conditions. Therefore, several organizations advise to increase the consumption of *n*−3 LC-PUFAs (at least two servings of fish per week) for primary prevention of coronary disease in general population. However, there is still limited evidence about the association of *n*−3 LC-PUFAs intake and prevention of mortality from cardiovascular disease. For people with specific diseases such hypertriglyceridemia and hypertension the effective doses of EPA–DHA required are much higher. Since many chronic diseases begin in utero or early infancy, dietary consumption of EPA and DHA even prior to pregnancy may be important. Similarly, the regular dietary consumption of fish rich in *n*−3 LC-PUFAs may improve the quality of life of elderly persons. Further larger clinical trials are needed in order to be able to recommend the most effective dosages and formulas (type of *n*−3 LC-PUFA, EPA/DHA ratio) for prevention/treatment of specific pathologies.

Even though the extraordinary amount of studies about the possible health benefits of EPA and DHA, the molecular and cellular mechanisms for their action are still insufficiently deciphered. In this context, *n*−3 LC-PUFAs have been shown to modulate adipogenesis [59], to suppress lipogenesis [95], and to induce fatty acid oxidation [32, 44] in key metabolic organs such as liver, skeletal muscle and white adipose tissue. These actions are in part mediated by the regulation of key transcription factors such as the peroxisome proliferator-activated receptors and sterol regulatory element binding protein [37, 51]. Moreover, *n*−3 LC-PUFAs actions have been also related to its ability to regulate AMPK activity [71], mitochondrial biogenesis [32], glucose transporters expression and insulin signaling pathway [90], as well as the secretion of bioactive adipokines, such as leptin, adiponectin, and visfatin, involved in the control of body weight, nutrient metabolism, and insulin sensitivity [72, 73, 80, 96]. In addition, *n*−3 LC-PUFA have been shown to prevent and/or ameliorate inflammation associated to obesity and metabolic syndrome [94, 122] by inhibiting the formation of *n*−6 fatty acids-derived pro-inflammatory eicosanoids, and by promoting the formation of endogenous anti-inflammatory and pro-resolving lipid mediators such as resolvins and protectins [16, 38, 101]. Evolution of "-omics"—epigenomics, transcriptomics, and lipidomics—is contributing greatly to the deciphering of the molecular networks regulated by *n*−3 LC-PUFAs. Moreover, the development of nutrigenetics will also allow us to identify different genotype-dependent responses to *n*−3 LC-PUFAs supplementation [75]. The major challenge lies in translating these findings into dietary guidelines and personalized recommendations.

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entirely free to express whatsoever views they thought appropriate.

Statement of authorship All authors have made substantial contributions to the writing and/or review of the manuscript. All authors have approved the final manuscript.

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