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REVIEW

Polyunsaturated fatty acids and cardiovascular disease

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Abstract Replacing saturated with polyunsaturated (PUFAs) rather than monounsaturated fatty acids or carbohydrates results in cardiovascular prevention over a wide range of intakes. The mechanisms by which PUFAs reduce cardiovascular risk are manifold, and the extent and precise nature of their activities is the subject of several investigations, spanning from in vitro mechanistic studies to human intervention trials. This article reviews the most up-to-date evidence of the association between PUFA consumption and reduced cardiovascular mortality.

Keywords Fatty acids · Cardiovascular disease · Omega 3 · Omega 6 · Cholesterol · Inflammation

Introduction

Replacing saturated (SFAs) with polyunsaturated (PUFAs) rather than monounsaturated (MUFAs) fatty acids or carbohydrates is associated with cardiovascular prevention over a wide range of intakes [1]. The mechanisms by which PUFAs reduce cardiovascular (CHD) risk are manifold, and the extent and precise nature of their activities is the subject of several investigations, spanning from in vitro mechanistic studies to human intervention trials.

This article reviews the most up-to-date evidence of the association between PUFA consumption and reduced cardiovascular mortality.

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Dietary fats

Among the three major dietary components, i.e., carbohydrates, proteins, and fats, the latter—mainly composed of fatty acids esterified in triacylglycerols, although diet also contains phospholipids, glycolipids, other complex lipids, and cholesterol—are relevant determinants of health status. In fact, their intakes are quantitatively relevant in the diet, because of at least two reasons: (1) unproblematic availability due to massive cultivation of plants for oil production, and (2) current low cost of energy-rich, fatty foods. Average fat consumption has changed over time and continues to do so. These time-dependent trends differ between countries. In many developing countries, fat intake is increasing, while in developed countries, fat intake has tended to decline over the past 30 years or so. In the context of the current review, it is important to note that the type of fat has also changed over time, meaning that the fatty acid composition of the human diet has changed. As an example, data from the UK indicate that the ratio of polyunsaturated to saturated fatty acids in the diet increased from 0.17 in 1959 to 0.22 in 1979 and to 0.40 in 1990 [2]. Much of this change has been brought about by a change in consumption habits, from butter to margarine and from animal fats to vegetable oils.

In addition to providing energy, fatty acids play very relevant biological roles in our body [3]. Notably, selected types of fatty acids—such as the long chain, highly unsaturated fatty acids of the omega 6 and omega 3 series—are important modulators of cell function. The main PUFA in the diet is linoleic acid (18:2ω6, LA), followed by alpha-linolenic acid (18:3ω3, ALA). Their intakes among adults in the Western world are approximately 13.5 g and 1.7 g per day, respectively. Longer-chain PUFAs are consumed in lower amounts than LA and ALA. Arachidonic

acid (20:4ω6, ARA) intake in Western populations fluctuates between 50 and 300 mg/day.

Though slowly tapering off, the Western diet is often too rich in fat, mainly as saturated, monounsaturated, and polyunsaturated fatty acids of the omega 6 series (such as LA) present in high concentrations in most seed oils [4]. It has been suggested that this theoretical imbalance leads to a "dilution" of omega 3 fatty acids, usually scarce in most common foods [5]. In physiological terms, omega 3 PUFAs, especially those which are most relevant in biological terms, i.e., the long chain PUFAs eicosapentaenoic (EPA) and docosahexaenoic (DHA), have become analogous to micronutrients (intakes of a few hundred micrograms/day in most populations out of over 100 g/day of total fat). It is important to underline that, although several fatty acids can be synthesized in the human body, mammals are unable to synthesize PUFAs. Hence, such fatty acids need to be ingested with the diet and are, therefore, termed "essential". Essentiality might also refer to the several indispensable roles they play in cellular physiology.

Omega 3 PUFAs

Omega 3 PUFAs share a carbon chain with a methyl group at one end and a carboxyl (acid) group at the other, and have at least three double bonds (hence the term polyunsaturated fatty acids). As mentioned above, they are essential, i.e., unable to be synthesized in mammals (including humans); therefore, they need to be supplied by the diet. Furthermore, they are endowed with properties that are essential for biological systems. The detailed chemistry and the metabolism of these fatty acids are beyond the scope of this review. However, some of omega 3's key characteristics are as follows:

1. This series is termed omega 3 or n-3 because, in each component, three carbon atoms are inserted between the methyl end of the carbon chain and the first double bond. This part of the molecule cannot be modified by metabolism within the same series, i.e., elongation and desaturation of precursors to products.
2. The omega 3 fatty acids series include precursors, namely ALA, with 18 carbon atoms and three double bonds in positions Δ9, 12, and 15 from the carboxyl end. Consequently, the first double bond is located on the third position from the methyl terminal. A series of successive elongations and desaturations generates longer and more unsaturated fatty acids from ALA. It should be underlined that, even though these processes have been well documented in vitro and the associated biochemical pathways have been elucidated, the extent of ALA elongation (in particular beyond EPA) in vivo is, at best, questionable [6].
3. Desaturases are the key enzymes involved in the conversion of shorter-chain to longer-chain PUFAs, namely EPA and DHA acids. Desaturases are modulated by several factors and conditions including dietary factors (carbohydrates, insulin, and low intakes of the 18 carbon fatty acid precursors of both series) [7], agents such as peroxisome proliferators and statins [8], high fat and omega 3 fatty acids-rich diets, cholesterol [9], hormones (adrenaline, glucagone, and steroids) [10], oxysterols [11], and cigarette smoke [12].
4. The same desaturases operate in both the omega 3 and in the omega 6 fatty acid series. In the latter series, desaturases convert LA to the most abundant long-chain PUFA in biological systems, i.e., ARA. While Δ6 desaturase is ubiquitously found in nature, including plants, Δ5 desaturase is exclusively found in animals (and aquatic plants). The two, i.e., the ω3 and ω6 pathways, are completely independent, but, as the same enzymes function in both series, competitions based on the relative abundance of substrates might quantitatively affect the relative rates of conversion, at least in vitro.
5. Elongases are not rate-limiting and do not appear to play major regulatory roles in the overall metabolic conversion in the two series.
6. There are marked differences between the omega 6 and omega 3 series, in terms of their final products. In fact, while the metabolic pathways of the omega 6 series do not considerably progress beyond arachidonic acid, DHA is produced through relatively complex steps. In fact, the metabolic elongation and desaturation sequences that follow EPA synthesis involve two elongation steps, which lead to the formation of 24:5ω3 followed by a Δ6 desaturation reaction producing 24:6ω3 [13]. These steps take place in the endoplasmic reticulum and are kinetically quite efficient. However, the step yielding 22:6ω3, i.e., the retroconversion of 24:6ω3 to 22:6ω3, involves a peroxisomal β-oxidation. As a consequence, ARA is rather abundant in cells and tissues, due to high dietary intakes of both its precursor LA and its biosynthetic pathway. Conversely, omega 3 tissutal fatty acid concentrations are, in general, fairly low for the opposite reasons, i.e., lower levels in the diet and, in particular, lower efficiency of DHA synthesis from its precursors. It can be inferred that EPA and DHA are to be nearly totally derived from the diet.

Omega 3 fatty acids and cardiovascular diseases

The first epidemiological observations that populations eating high amounts of fish, namely the Greenland Eskimos, had a lower incidence of cardiovascular diseases as compared with populations following typical Western diets were published in the 1970s, thanks to the work of Bang and Dyerberg [14]. The association between fish consumption and cardiovascular protection was soon explained by the large intakes of marine fatty acids, namely those of the omega 3 series, accredited with protecting the Greenland population from ischemic processes via decreased platelet aggregability [14].

For the sake of clarity, this paragraph will use the definition "omega 3" as applied to long-chain omega 3 PUFAs, namely DHA and EPA. While the lay public often does not distinguish long- from short-chain omega 3 PUFAs, namely ALA, their proven effects on human health are very different in magnitude. While it is worth noting that ALA is only modestly converted (elongated and desaturated) into EPA and DHA [6, 15], the Lyon diet heart study, in which ALA was a component, suggested that ALA per se may confer cardiovascular benefits [16]. However, evidence in favor of a cardioprotective role of ALA is scant. Most trials are of small size and the conclusion of a recent systematic review was that while "ALA significantly decreases fibrinogen and fasting plasma glucose concentrations, no other statistically or clinically significant findings were evident in other cardiovascular risk markers" [17]. These conclusions are in agreement with the previous one by Sinclair et al. [15], who pointed to the paucity of randomized controlled trials (RCTs) and indicated that high ALA intakes might be associated with increased risk of prostate cancer. This eventuality was recently reviewed by Simon et al. [18], who emphasized the heterogeneity across studies and the likelihood of publication bias. However, the possibility that high ALA intakes or high blood and adipose tissue concentrations of ALA may be associated with a small increased risk of prostate cancer cannot, at present, be excluded.

In turn, the effects of ALA on human health are yet to be elucidated [15], and the general conclusion that omega 3 PUFAs are endowed with biological activities should be mostly limited to the long-chain ones.

Based on these initial observations on the inverse relationship between fish consumption and cardiovascular disease, several studies are accumulating and can be categorized as either epidemiological investigations (associations between fish consumption and cardiovascular outcomes), cohort studies, or RCTs, which assess fish or omega 3 fatty acid consumption.

While the reader is referred to the numerous specialized publications on the topic, there is general consensus on the favorable effects of adequate intakes of fish or omega 3 PUFAs supplements on cardiovascular clinical outcomes. In clinical terms, the foremost cardio-preventive effect of omega 3 fatty acid is noted on sudden death, although diminished incidence of myocardial infarction has also been associated with adequate omega 3 intakes [19]. The magnitude of the effects of omega 3 fatty acids on the cardiovascular system is, to date, not well defined. One of the major problems of omega 3 research, as applied to clinical settings, is that it is mostly based on establishing omega 3 consumption by food frequency questionnaires and composition tables.

It goes without saying that the hardest evidence should come from RCTs, where omega 3 intakes can be accurately monitored, providing more relevant results. In the realm of omega 3 research, as applied to humans, it is unfortunate that, due to practical and financial reasons, most investigators, e.g., the GISSI and the JELIS [20, 21], do not measure PUFAs levels in plasma or in red blood cells before and after intervention. This drawback makes it quite difficult to establish dose-dependent correlations between plasma PUFA variations (a reliable marker of intake) and clinical outcomes.

In most cases, e.g., in the GISSI study [20], the cardiovascular effects of omega 3 PUFAs become apparent shortly after supplementation/therapy, as discussed by Hooper et al. [22]. This phenomenon might be due to the fact that omega 3 PUFAs have minimal effects on lipid/lipoprotein profiles (as an example, they only modestly, or do not, reduce LDL cholesterol). These parameters are altered gradually and to a higher extent by other dietary manipulations such as reductions of saturated fatty acid intakes, increased consumption of monounsaturated fatty acids (MUFA), and, chiefly, increased consumption of omega 6 PUFAs, notably linoleic acid.

Cardiovascular activities of omega 3 PUFAs

Stemming from the epidemiological observations mentioned above and building on some RCTs, considerable research is being dedicated to elucidating the activities of omega 3 PUFAs on the cardiovascular system [23]. This research ranges from in vitro to animal to human studies. Evidence can be conveniently classified as follows:

Metabolic activities, namely on lipid metabolism, related to atherosclerosis

Substantial (≥2 g/day) intakes of omega 3 PUFAs efficiently reduce plasma triacylglycerol (TG) concentrations [24]. This is the most relevant effect of omega 3 PUFAs on plasma lipids and it has been explained by: (1) diminished

TG synthesis due to reduced substrate, i.e., fatty acids, availability, due to increased β-oxidation; (2) decreased delivery of free fatty acids to the liver; and (3) shift of lipid synthesis toward phospholipids rather than TG [25]. It should be noted that most of the experimental evidence in support of these hypotheses has been obtained in rodents. As pointed out by Harris and Bulchandani [25], the molecular mechanisms by which omega 3 PUFAs decrease triglyceridemia in humans remain uncertain [25]. An additional, less relevant effect of omega 3 PUFAs on lipid parameters is their hypothesized elevation of HDL cholesterolemia, though a recent review [26] did not confirm this activity. The effects on LDL cholesterol are, conversely, rather complex. In fact, plasma total and, in particular, LDL cholesterol appear to be increased by omega 3 PUFAs supplementation [26]. The LDL particles that are formed appear to be of larger volumes (with a consequently lower surface/volume ratio) [27] and in lower concentrations. These particles are considered to be less atherogenic, as arterial lesions induced by LDL are related to surface–surface interactions [28]; these processes would obviously be lessened in the case of particles with lower surface/volume ratios. Hartweg et al. [26] however, did not confirm this hypothesis. It should be underlined that near totality of data attempting to elucidate the mechanisms of action of omega 3 PUFAs on circulating lipids have been obtained in patients, e.g., dyslipidemic of diabetic subjects. The net effects of high omega 3 PUFAs intake on healthy individual—as related to cardiovascular prevention—are yet to be firmly established.

Aside from the effects of omega 3 PUFAs on plasma lipids, their activities on the onset and progression of atherosclerosis are still equivocal. While some animal studies demonstrated protective activities, e.g., [29], human data are not definitive. However, Thies et al. [30] demonstrated that omega 3 PUFAs stabilize the atherosclerotic plaques of patients, possibly due to a lower infiltration with macrophages.

Modulation of cell functions

PUFAs and perhaps conjugated fatty acids uniquely alter the basic properties of cell membranes, namely by altering lipid rafts and caveolae [31]. The major activities of omega 3 PUFAs on cell function, as related to cardiovascular disease, include reduced platelets aggregability, leading to antithrombotic activity (indeed, the first evidence of cardioprotective activities by omega 3 PUFAs, as described above), and lower pro-inflammatory activity of leukocytes. These actions are largely due to lower production of bioactive metabolites (eicosanoids) generated from arachidonic acid [5]. In particular, reduced production of prothrombotic thromboxanes (TxA_2) generated by platelets and of pro-inflammatory leukotrienes (LTB_4 and LTC_4), generated by leukocytes—all derived from ARA—is noted following supplementation with omega 3 PUFAs. The lower production of bioactive mediators is associated with increased release of less active ARA metabolites, mostly derived from EPA, notably TxA_3, LTB_5, and LTC_5 [5].

More recently, the activities of anti-inflammatory protective compounds from EPA and DHA (resolvins and protectins) have also been described [32]. Resolvins (resolution-phase interaction products) are EPA- and DHA-derived mediators generated through cycloxygenase-2 (COX-2) and LOX pathways. These mediators are endowed with potent anti-inflammatory and immunoregulatory activities that play remarkable roles in the resolution of acute inflammation. Protectins are also biosynthesized from DHA and have been shown (neuroprotectin D1) to exert protective actions in neural systems and tissues [33]. It is worth noting that such pro-resolving lipid mediators have been shown to be active in vivo after administration [34, 35], though data are still scant. The discovery of resolvins and protectins (in addition to novel roles revealed for lipoxins) indicates that the resolution of inflammation is an active process and that proper dietary manipulations might accelerate it [36].

Relevant to cell function and cardiovascular disease, evidence is also accumulating of the antioxidant activities of omega 3 fatty acids [37], both in platelets [38] and in human endothelium [39]. It is noteworthy that these counterintuitive antioxidant activities have also been recorded in humans fed omega 3 PUFAs [40–43].

Direct anti-inflammatory actions

In addition to reducing the production of pro-inflammatory lipid mediators, omega 3 PUFAs reduce production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α) [44]. These activities influence the immune system, including its inflammatory components, and are the result of interactions among several cell types [45]. The fatty acid composition of immune cells can be modified by dietary interventions with omega 3 PUFAs [46], leading to altered expression of inflammatory genes, via effects on transcription factors activation [47]. Omega 3 PUFAs, namely DHA, also inhibit COX-2 in endothelial cells, resulting in lower production of prostaglandins at inflammatory sites [48]. Given the relevant role that inflammation [49] and COX-2 play in atherogenesis [50], these activities are suggestive of an athero-protective role of omega 3 PUFAs, even though, as mentioned above, this is still an unresolved issue [51].

The ability of PUFAs to decrease inflammatory gene expression in humans has also been recently investigated by Weaver et al. [52], who reported that PUFAs (both omega 3 and omega 6) may exert their clinical effects via

their capacity to regulate the expression of signal transduction genes and genes for pro-inflammatory cytokines.

Anti-arrhythmic myocardial activity

Early in vivo (dogs with myocardial infarction) experiments by the Leaf group demonstrated that omega 3 PUFAs exert antiarrhythmic activities [53]. Subsequent in vitro studies investigated the cellular mechanisms underlying such effects, which appear to be mediated by the inhibition of the fast, voltage-dependent sodium current and the L-type calcium currents, and to be responsible of the reduction of sudden death in human studies [47]. However, a recent meta-analysis of three RCTs in patients with implantable cardioverter defibrillator [54] reached the conclusion that omega 3 PUFAs do not confer protection against recurrent life-threatening ventricular arrhythmia and that more RCT are needed to prove or disprove their activities, also in light of the considerable heterogeneity between the published trials. Similar conclusions were reached by Leon et al. [55], who also included sudden cardiac death. This apparent conundrum is yet to be resolved and might be largely due to heterogeneity of the doses, of sample sizes, and of trial duration.

In synthesis, adequate intakes of omega 3 PUFAs effectively afford cardioprotection. This activities are most noted in cardiovascular patients, where the inclusion of omega 3 PUFAs in the medication portfolio reduces mortality rates to extents comparable and often superior to those of commonly prescribed drugs [56].

Fish or drugs/supplements?

As a result of natural processes and human activity, aquatic food sources, including fish, can contain methylmercury, which has been linked to adverse health consequences [57]. Because of the presence of methylmercury in fish, the Food and Drug Administration (FDA) and the US Environmental Protection Agency issued an advisory to consumers, *What You Need to Know About Mercury in Fish and Shellfish* [58]. However, a review by Mozaffarian and Rimm [59] concluded that, although the presence of mercury and dioxins and polychlorinated biphenyls may pose health issues [60], in particular among women of childbearing age and nursing mothers, "the benefits of fish intake exceed the potential risks". A prudent advice, aimed especially at the categories at potential risk, would be to limit consumption of fish high in such pollutants [61]. In addition, fish consumption may have several advantages over formulations:

1. Fish, in addition to providing omega 3 PUFAs, is a good source of other valuable nutrients such as vitamins A, B, and D. Fish is also a valuable source of calcium and phosphorus in particular, but also of iron, copper, and selenium. Finally, saltwater fish has a high content of iodine.
2. Omega 3 PUFAs are more prone to oxidation when they are isolated from their sources and then formulated, as compared to fish (where they are protected by various stabilizing agents). This is why pharmaceutical preparations have to contain significant amounts of liposoluble vitamins, namely tocopherols.
3. It has been suggested [62, 63]—though not confirmed [64]—that the bioavailability of omega 3 PUFAs is higher when they are ingested as components of fish, or of other foods such as milk [65].
4. Some people experience gastrointestinal discomfort (diarrhea, fishy burps, halitosis) from capsules.

It should be noted, however, that selected population subgroups do not eat fish because either they do not like it, they are vegetarian, cannot find the financial resources to buy it or the time to cook it, or live in areas of limited accessibility to fish. These people should take into consideration the possibility to supplement their diet with pharmaceutical/nutraceutical preparations (some of which are suitable for vegetarians). Indeed, a recent FDA peer-reviewed draft report on the net effect of eating fish on brain development and heart health [66] concluded that every 20 g of fish consumed per day beyond the current average levels reduces the risk of CHD by 7%, on average. Based on its calculations, the report states that the current level of fish consumption is responsible for averting more than 30,000 deaths per year from CHD. Whether these health effects are due to fish in toto or to its omega 3 PUFAs is yet to be established. As a final point, it should be kept in mind that, given the serious decline in global fish stocks, dietary recommendations to increase fish consumption may not be sustainable [67].

Omega 6 PUFAs

The most abundant omega 6 PUFA in the diet is linoleic acid (LA, 18:2ω6), whose intakes are, on average, 100-fold higher than ARA [68]. Approximately 40 years ago [69], LA was shown to decrease cholesterolemia, and some dietary advice to increase its consumption was issued. As a result of this dietary advice, polyunsaturated fat (again, primarily LA) in the US increased from approximately 3% of energy in the 1950s to about 6–7% of energy [70]. As a matter of fact, the increase in LA consumption was associated with a marked (50%) reduction of cardiovascular

disease in the US [70]. This profound dietary change was confirmed by large increases in the LA acid content of adipose samples. The effects of omega 6 PUFAs on blood lipids have been computed by equations that gave almost identical results: saturated fat is positively related and polyunsaturated fat is inversely related to serum cholesterol [71, 72]. These findings were expanded by Mensink et al. [73], who also addressed the effects of dietary fats on not only total blood cholesterol but also LDL and HDL cholesterol and triglyceride levels [73]. These studies documented that, compared to carbohydrate, polyunsaturated fat (the vast majority of which is, as mentioned above, omega 6 PUFA) reduces LDL cholesterol, increases HDL cholesterol, and reduces triglycerides.

While the cholesterol-lowering activities of adequate LA intakes have never been disputed, some authors are suggesting that LA acid intake might have adverse effects on risk of heart disease. This hypothesis is largely based on the theoretical mechanism of competition discussed above. In brief, the assumption is that dietary intake of LA results in higher blood and tissue levels of ARA, in turn increasing the formation of pro-inflammatory lipid mediators. However, ARA levels are tightly regulated in the body, and increased circulating ARA levels following increases in dietary LA (within the usual dietary range) have never been clearly demonstrated, especially in humans [74, 75]. It is also worth noting that cumulative data suggest that moderately increased ARA intake is probably harmless in healthy adults [76]. In brief, it appears that significant conversion of LA to ARA happens only under essential fatty acid deficiency, namely when LA intakes fall below 2% of energy [75]. Some LA metabolites have also been suggested as direct inducers of inflammation [77, 78], though the human relevance of these findings is yet to be established. Furthermore, we have come to appreciate that dietary factors can influence risk of heart disease through many different pathways beyond cholesterol modulation. Because LA has multiple physiological influences, higher intake of this fatty acid might not necessarily translate into greater risk of CHD if other pathways favored a reduction in risk, even if there were some increase in inflammatory factors. As stated, dietary fatty acids can influence blood lipid fractions, blood pressure, thrombotic tendency, insulin resistance, oxidative stress, endothelial function, and the likelihood of ventricular arrhythmias. The net effect of specific dietary factors on risk of heart disease is the result of the balance of positive and negative influences on all of these mechanisms.

Several lines of evidence indicate that omega 6 PUFAs have anti- rather than pro-inflammatory effects, mediated by pathways that do not involve cyclooxygenase [79]. Similar to omega 3, omega 6 PUFAs inhibit the production of inflammatory factors of endothelial cells by down-regulating nuclear factor κB (NFκB), an effect that appears to be mediated by multiple mechanisms [80].

Relatively few, i.e., three, studies have actually examined the effects of omega 6 PUFAs intake on inflammatory factors. In a cross-sectional analysis among 859 men and women, Pischon et al. [81] cross-classified individuals by intakes of omega 3 and of omega 6 PUFAs and examined the relation to plasma levels of soluble tumor necrosis factor (TNF) receptors, a stable indicator of TNF activity. The lowest levels of soluble TNF receptor-2 were among individuals with highest intakes of both omega 3 and omega 6 PUFAs. Similar findings were seen in relation to soluble TNF receptor-1 levels. This study suggests that omega 6 PUFAs do not inhibit the anti-inflammatory actions of omega 3 PUFAs, but, rather, reduce inflammatory factors as well.

Data from the InCHIANTI (an epidemiological study conducted in two small towns of Tuscany, Italy), collected from 1,123 persons (aged 20–98 years) reported several significant associations between plasma concentrations of total omega 6 and omega 3 PUFAs and various pro- and anti-inflammatory markers [82]. When total omega 6 or omega 3 PUFAs were separated into quartiles (see Fig. 1 of Ferrucci et al. [82]), those subjects in the lowest quartile of plasma total omega 6 PUFAs had the highest concentrations of TNF-α and IL-6 (i.e., pro-inflammatory markers) and lowest levels of IL-10 and TGF-β (i.e., anti-inflammatory markers). Several other findings can be classified as unexpected. For example, subjects in the highest quartile of plasma ARA had the lowest IL-6 and the highest TGF-β concentrations (see Table 2 of Ferrucci et al. [82]). In turn, these data suggest—counterintuitively—that people with higher circulating ARA have lower inflammatory reactivity. Also, plasma LA by itself was neither positively nor negatively associated with any of the inflammatory markers measured in this study. It is worth mentioning that omega 3 PUFAs were also inversely associated with concentrations of inflammatory markers, as expected. The conclusion that can be drawn from this study is that increased intakes of both omega 3 and omega 6 PUFAs decrease the concentrations of circulating markers of inflammation.

More recently, Liou et al. [83] reported their findings from a randomized cross-over designed study in which LA intake was purposely reduced to explore the possible impact on circulating IL-6 and C-reactive protein, two markers of inflammation. In this study, the diet of 22 individuals was modified such that LA intake was reduced from 12% of total calories down to 4%, while maintaining a constant intake of n-3 PUFA (i.e., 1% of total energy as ALA), for 4 weeks. The results show that decreased intakes of LA do not significantly modify either the plasma concentrations of ARA or those of inflammatory markers. This

lack of effect on inflammation was noted despite significant decreases in the EPA:ARA ratio in the plasma, which was due to diminished EPA concentrations rather than changes in ARA.

The direct impact of dietary ARA on inflammation has also been investigated in two studies. Kelley et al. [84] reported that the addition of 1,200 mg ARA per day to the diet of healthy individuals for up to 7 weeks had no significant effect on the production of TNF-α, IL-1β, or IL-6 by peripheral blood mononuclear cells, stimulated ex vivo by bacterial endotoxin. Likewise, Thies et al. [85] reported that the addition of 700 mg of ARA per day to the habitual diet of healthy subjects for 12 weeks does not significantly increase ex vivo production of these same pro-inflammatory cytokines, possibly because of the concomitant synthesis of several other ARA-derived products.

Effects on insulin resistance and risk of type 2 diabetes

Controlled feeding studies also show that increasing polyunsaturated fat, namely LA intake, has a beneficial effect on insulin resistance [86, 87]. The risk of type 2 diabetes has also been inversely related to LA intake in large prospective studies [88]. Within the Nurses' Health Study cohort, Salmeron et al. [89]. reported that women with the highest intake of LA had about a 20% lower risk of type 2 diabetes compared to those with the lowest intake. Mechanistically, these findings might be explained by the observation that insulin resistance increases many inflammatory factors via TOLL-like receptors [90]. Hence, the beneficial effect of LA in reducing insulin resistance could translate into lower production of pro-inflammatory factors, in turn mitigating the clinical sequelae of type 2 diabetes through pathways other than the mere modulation of plasma lipids.

It is also worth mentioning that Mostad et al. [91]. reported that high doses of omega 3 PUFAs moderately increase blood glucose and decrease insulin sensitivity in persons with type 2 diabetes without hypertriacylglycerolemia and alters carbohydrate and fat utilization in a time-dependent manner. These data, of course, need to be confirmed.

Risk of coronary heart disease

Even though a direct, causal correlation cannot be proven, Dwyer and Hetzel [92] performed a time-trend analysis which formulated the hypothesis that increased intake of polyunsaturated fats, namely LA, was probably the primary factor underlying these large decreases in cardiovascular mortality.

Some intervention trials have also been performed, in the mid-1960s, with high intakes of polyunsaturated fat for the prevention of cardiovascular disease [93–96]. In terms of PUFA consumption, these trials used either corn oil, which contains large amounts of LA and minimal amounts of ALA, or soybean oil, which is also very high in LA. The results showed a consistent decrease in serum total cholesterol of 12–15% and reduced incidence of coronary heart disease by 10–45%. Notably, Dayton et al. [94], who most clearly documented a benefit, used corn oil to increase intake of LA to nearly 20% of calories.

Other studies examined the relation between total PUFA intake, namely linoleic acid intake, and risk of coronary heart disease. Significant inverse associations were seen in 5 out of 13 studies, and in no study was a positive association between LA and CHD risk observed. The most detailed analysis is that of the Nurses' Health Study cohort [97], where nearly 90,000 women were followed for 14 years. During this period, nearly 1,000 women died of CHD or were hospitalized for acute myocardial infarction. Dietary fat intake was quali/quantitatively assessed by repeated, validated food frequency questionnaires. Any increment of 5% of PUFAs equicalorically replacing carbohydrates was associated with nearly 40% lower risk of coronary heart disease. This association was stronger than that obtained by simply adding the effects on LDL and HDL, thus strongly supporting the importance of other metabolic pathways. In a sub-analysis of the same study, Hu et al. [98] singled out ALA and the ALA/LA ratio in relation to risk of fatal CHD. ALA intake was inversely related to risk of fatal CHD to a similar degree as LA; hence, the ratio between these two PUFAs (the former belonging to the ω3 and the latter to the ω6 series) was not influential on cardiovascular risk. However, caution must be used when interpreting these results, as the necessary calculations are difficult to perform based on the manuscript.

Recently, Jakobsen et al. [1] analyzed 11 high-quality American and European prospective cohort studies on CHD endpoints and intake of carbohydrates, saturated, monounsaturated, and polyunsaturated (PUFA, pooling together ω3 and ω6 fatty acids) fats. The conclusion was that replacing SFA intake with PUFA intake rather than MUFA or carbohydrate intake prevents CHD over a wide range of intakes and among both middle-aged and older men and women.

The activities of omega 6 PUFAs on cardiovascular risk were summarized in a recent AHA advisory, which concluded that consumption of at least 5–10% of energy from omega 6 PUFAs, in the context of other appropriate lifestyle and dietary behavior, reduces the risk of CHD relative to low intakes [99].

The omega 6/omega 3 ratio

An ongoing debate concerns the usefulness of calculating the dietary omega 6-to-omega 3 ratio. The discussion has been mostly triggered by observations by Simopoulos [100], who noted how an absolute and relative change of omega 6/omega 3 in the food supply of Western societies has occurred over the last 100 years. A balance existed between omega 6 and omega 3 for millions of years during the long evolutionary history of the genus *Homo*, and genetic changes occurred partly in response to these dietary influences [100]. Theoretically, i.e., based on in vitro competition mechanisms, reverting the omega 6/omega 3 ratio in our diet back to the original $\sim$1 rather than the current 15/1 to 16.7/1 should lessen the incidence of degenerative diseases, including those with a strong inflammatory component. While this hypothesis has strong biochemical bases [101], its validity in vivo has been questioned [102], in particular after the OPTILIP study [103]. Data from the literature and this article demonstrate that omega 3 and omega 6 PUFAs are both essential, and that intakes of both are related to lower risk of CHD. For this reason, the ratio of omega 6 to omega 3 PUFAs might not a useful concept from a nutritional viewpoint. This concept is reinforced by considering that, at any ratio, both omega 6 and omega 3 PUFA intakes could be deficient. In absolute terms, any equal amount of omega 6 and omega 3 (even 1 g) can provide a presumably optimal ratio while clearly being overall insufficient. Experimental evidence also indicates that reduced tissue/blood levels of omega 3 PUFAs provide a better indication of increased cardiovascular risk than the omega 6:omega 3 ratio [104]. In synthesis, it might be better to considered PUFAs individually rather than as a ratio (which, incidentally, does not distinguish between ALA and EPA + DHA) and to provide evidence-based dietary advice to increase consumption of omega 3 PUFAs rather than just focusing on decreasing their omega 6 counterparts [105].

"Minor" PUFAs

One potentially nutritionally important PUFA is gamma-linolenic acid (18:3ω6; GLA). GLA can be synthesized by humans from LA, via Δ6 desaturase, though the quantitative extent and in vivo relevance of this desaturation is yet to be determined.

Gamma-linolenic acid is rapidly converted to dihomo-γ-linolenic acid (20:3ω6; DGLA), which is the precursor of prostaglandin 1, an inhibitor of platelet aggregation with anti-inflammatory properties. As a matter of fact, the putative health effects of GLA might be due to its derivatives [106].

Major sources of GLA are evening primrose, black currant seeds, and borage oils. The popularity of GLA as a supplement stems from the works of Horrobin [107], who proposed supplementation of 500-2,000 mg/day of GLA to provide pharmacological benefits. Though some of these activities are based on hypotheses, there is substantial evidence of the benefits of GLA supplementation is a series of inflammation-based pathologies, including skin (atopic dermatitis [108]), joint (rheumatoid arthritis [109]), and ocular (dry eye [110]) disorders.

Stearidonic acid (18:4ω3, SDA) is a metabolic intermediate in the conversion of ALA to EPA. SDA is a minor (0.5–2%) component of fish fat, but is abundant in echium and borage. The cardiovascular benefits of SDA per se have never been explored in humans, as recently reviewed by Whelan [111]. However, SDA is quite efficiently converted to EPA [112, 113]. Hence, its putative cardioprotective effects might be due to EPA [21] and to the fact that SDA increases the Omega 3 index [19]. In brief, while SDA can be considered a pro-EPA PUFA [111], there are, currently, insufficient human data to suggest direct health benefits.

Conclusions

Fat is indispensible for energy, delivery of lipid-soluble vitamins, and physiology. While an overall reduction of fat intake is advisable in Western countries, more emphasis should be placed on the relative proportion of individual fatty acids. Based on available data—both ecological and experimental—shifting the fraction of saturated fat toward PUFAs rather than MUFAs appears to confer the foremost benefits [1]. In quantitative terms, several guidelines have been issued by scientific bodies. As an example, the International Society for the Study of Fatty Acids and Lipids (ISSFAL), the foremost International scientific society dealing exclusively with the health impact of dietary lipids, recommends an adequate LA intake of 2% of energy, a healthy intake of ALA of 0.7% of energy, and a minimum intake of EPA and DHA combined, of 500 mg/day, for cardiovascular health [114]. It should also be mentioned that the diverse biological roles played by PUFAs (either ω6 or ω3) extend beyond the cardiovascular system.

In conclusion, basic and applied research will continue exploring the cardioprotective properties of PUFAs, and more accurate evidence-based recommendations will be issued in the near future.

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