CHAPTER 13

ESSENTIAL POLYUNSATURATED FATTY ACIDS, INFLAMMATION, ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASES

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Abstract: Atherosclerosis is the primary cause of coronary and cardiovascular diseases (CVD). Epidemiological studies have revealed several important environmental (especially nutritional) factors associated with atherosclerosis. However, progress in defining the cellular and molecular interactions involved has been hindered by the etiological complexity of the disease. Nevertheless, our understanding of CVD has improved significantly over the past decade owing to the availability of new randomized trial data. In particular, the failure of antioxidant and anti-inflammatory treatments to consistently reduce the rate of CVD complications suggests that theories of atherosclerosis may have considerably exaggerated the importance of oxidized lipoprotein and vascular inflammation. In that context, one new and basic question is whether the biology of essential dietary lipids may help us understand the role of the inflammatory process in CVD. Essential dietary lipids of the omega-6 and omega-3 families are the precursors of major mediators of inflammation such as eicosanoids that regulate the production of inflammatory cytokines and the expression of some major inflammation genes. On the other hand, non-essential lipids

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(omega-9 and saturated fatty acids) interfere with biological activities of essential lipids. Finally, essential omega-3 and omega-6 fatty acids have different, often antagonistic, effects on inflammation, and their effects can vary according to the type of cells and target organs involved, as well as their respective amounts in the diet. Because of the extreme complexity in the etiology of CVD, the best strategy may be to monitor the main features of dietary patterns, such as the Mediterranean diet, that are known to be associated with a low prevalence of both CVD and chronic inflammatory diseases

1. INTRODUCTION

Coronary and cardiovascular diseases (CVD) remain the leading causes of morbidity and mortality in most developing and developed countries. Besides traditional risk factors such as hypercholesterolemia and hypertension, increased oxidant stress (or oxidized lipoproteins) and vascular inflammation have recently been considered as playing important roles in CVD [1–4]. However, in some studies, antioxidant and anti-inflammatory treatments had no protective effect against CVD [5–8]. Both nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids were ineffective. Furthermore, arthritis patients on glucocorticoids had more CVD complications (after adjustment for disease severity and other confounders) than those not exposed to glucocorticoids [9]. This is not surprising, since glucocorticoids predispose to insulin resistance and metabolic syndromes, which are major risk factors for CVD [10]. In patients with established CVD, high-dose dexamethasone-eluting stents did not reduce neointimal proliferation instead causing restenosis within the stent and creating an experimental human model of accelerated coronary atherosclerosis [11]. Finally, NSAIDs have not only failed to prevent CVD complications in some controlled trials, but rather were associated with an increased risk [7, 8]. Thus, scientific and medical knowledge about the pathogenesis of atherosclerosis and CVD complications, with primary roles attributed to high cholesterol, oxidized lipoproteins and vascular inflammation, appears to be extremely confusing at present. Even the concept of inflammation in plaque instability (the triggering event of acute CVD complications) needs to be more clearly reformulated [12]. In this context, it is certainly important to examine the role of essential polyunsaturated fatty acids (PUFAs) as mediators of inflammation in the development of CVD.

2. INFLAMMATION, ATHEROSCLEROSIS AND ESSENTIAL PUFAs

It is generally well accepted (although highly debatable) that inflammation plays a central role in atherosclerosis [1, 13]. All stages of the atherogenic process seem to be characterized by a dynamic interaction of inflammatory cells, cytokines and inflammatory eicosanoids in the arterial wall [1, 13, 14]. According to the most popular theory to date, the conventional risk factors for CVD, including oxidized low density lipoproteins (oxLDL), smoking, high blood glucose and high blood pressure, are harmful factors that initiate and promote the atherogenic process by triggering the inflammatory reaction within the arteries. The earliest detectable

cellular event in atherosclerosis is assumed to be the attachment of monocytes to endothelial cells. This inflammatory response is facilitated by leukocyte adhesion molecules expressed on the surface of endothelial cells under the control of proinflammatory cytokines. OxLDLs are thought to promote the formation of chemoattractant cytokines by the endothelium which stimulates the migration of monocytes into the subendothelial space where they accumulate oxLDLs as macrophages and become foam cells [13]. Foam cells are a hallmark of fatty streaks, the first (but still reversible) stage of atherosclerosis. A later stage of inflammation is characterized by fibrosis, which is also a major feature of atherosclerosis [13]. Fibrosis results from the proliferation of smooth muscle cells after their migration from the muscular layer of the artery under the control of various mitogenic and growth factors, such as the platelet derived growth factor (PGDF) [13]. Fibrosis is a reparation process that contributes to irreversible sclerosis of the lesion and arterial stenosis; nevertheless, fibrosis *per se* is a stabilizing process and does not result in acute CVD complications. In fact, it is the disruption of the atherosclerotic plaque, with the subsequent potentially total obstruction of the lumen by thrombi that leads to acute CVD complications. According to current theory, macrophages are also the primary source of plaque vulnerability because they produce matrix metalloproteinases that break up collagen (the main component of fibrosis), weaken the fibrous cap of the plaque and favor contact between blood coagulation factors and the prothrombotic components of atherosclerotic plaque. Although it is generally well accepted, the inflammatory theory of atherosclerosis [1, 13, 14] appears to be quite speculative, as illustrated by the failure of most anti-inflammatory treatments to prevent CVD complications [7, 8]. Therefore, the theory needs additional supporting evidence to become more credible.

There is increasing interest in the effects of dietary fatty acids on immune parameters and on the inflammatory process. Initially, long chain omega-6 PUFAs, linoleic acid (LA) and particularly arachidonic acid (AA), were found to inhibit lymphocyte function [15, 16]. Later studies showed that omega-3 PUFAs also inhibited lymphocyte activity [17, 18]. Other non-essential dietary fatty acids (e.g., those in the omega-9 family) also influence inflammation. Recently, PUFAs of the omega-6 and omega-3 families have been found to have antagonistic effects on inflammation and CVD; omega-6 PUFAs are now seen as pro-inflammatory and omega-3 PUFAs as anti-inflammatory [19]. Arachidonic acid (AA), the major omega-6 PUFA in inflammatory cells, is the dominant substrate for eicosanoid synthesis giving rise to major pro-inflammatory mediators potentially involved in CVD complications [13, 14]. Blocking the first step of AA metabolism in platelets, at the level of the cyclo-oxygenase (COX) enzyme system (by aspirin for instance), results in inhibition of eicosanoid synthesis and platelet aggregation. However, only very low doses of aspirin (and not large anti-inflammatory doses) were shown to effectively reduce CVD complications [20]. In fact, there is ongoing controversy about the ability of low-dose aspirin to reduce CVD complications [21, 22]. What is the optimal dose? Which patients would maximally benefit from it? Is the effect seen in secondary prevention reproducible in primary prevention? In any case,

since only very low doses were shown to be protective in certain (not all) studies [23], it is clear that the relative protection provided by low dose aspirin does not result from an anti-inflammatory effect but only from a specific anti-platelet effect. This raises some major questions about the inflammatory theory of atherosclerosis. First, as it has become clear that besides their role in hemostasis and thrombosis, platelets regulate a variety of inflammatory responses, especially through their interaction with the vascular endothelium [24], it is difficult to understand why large (anti-inflammatory) doses of aspirin were not effective in preventing CVD. Platelets represent an important link between inflammation, thrombosis and atherosclerosis, highlighting the concept of *atherothrombosis* in which thrombosis, in addition to being the consequence of plaque rupture, is also the starting point for stenosis progression through organization of residual thrombi [24]. However, apart from specific conditions such as accelerated coronary atherosclerosis after heart transplantation [25, 26], the availability of conclusive human data to support the "*atherothrombotic* theory" appears to be very limited. A second question relates to the role of inflammatory eicosanoids (from any source) in vascular inflammation and CVD. Why do substances blocking AA metabolism and the production of inflammatory eicosanoids and exhibiting potent anti-inflammatory effects (such as NSAIDs) have negative effects [7, 8] on CVD risk? A third crucial question relates to the significance of metabolic competition between the different families of PUFAs (omega-6, omega-9, and omega-3) and the vascular and anti-inflammatory effect of NSAIDs. Would COX inhibition have the same clinical effect in patients with different dietary intakes of omega-9, omega-6 and omega-3 fatty acids? In view of the complexity of these different questions, the present text aims at discussing certain aspects of the role of essential PUFAs in CVD through their pro- or anti-inflammatory properties.

3. WHAT ARE ESSENTIAL PUFAs?

Essential PUFAs are fatty acids that contain two or more double bonds. They are named by identifying the number of double bonds and the position of the first double bond counted from the methyl terminus of the acyl chain. Thus, an 18-carbon fatty acid with two double bonds in the acyl chain and with the first double bond on carbon number 6 from the methyl terminus is termed 18:2 omega-6 (or 18:2n-6). The common name of this fatty acid is linoleic acid (LA) and it is the simplest member of the omega-6 family of PUFAs. LA can be further desaturated by insertion of a double bond between carbons 3 and 4 to yield alpha-linolenic acid (ALA or 18:3 omega-3 or 18:3n-3), the simplest member of the omega-3 family of fatty acids [27]. Plants, but not mammals, have the desaturase enzymes required to synthesize LA and ALA. For this reason, LA and ALA are termed "essential fatty acids", which means that they have to be supplied through our daily diet to cover our needs (Figure 1). Plant seed oils (and margarine) from corn, sunflower and soybean are rich in LA and are the main sources of LA in the Western diet. Nuts, canola oil and green leafy vegetables are the main sources of ALA in the

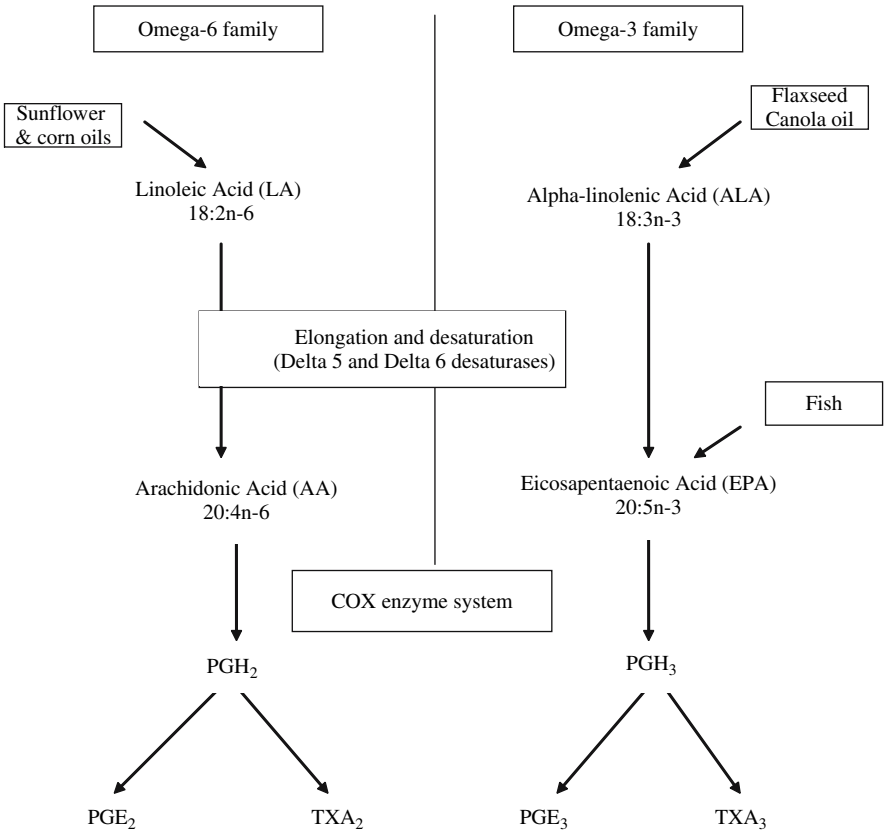

Figure 1. Schematic representation of the metabolization of 18-carbon fatty acids into longer chain fatty acids and subsequent eicosanoid metabolization under the effect of the COX system. Most AA in our body comes from LA (through endogenous biosynthesis), whereas most EPA comes from dietary intakes provided by fish. EPA can be further metabolized to produce DHA (see text). An alternative pathway for AA and EPA is the LOX system (see text). AA is arachidonic acid; EPA is eicosapentanoic acid; DHA is docosahexanoic acid; LA is linoleic acid; COX is cyclo-oxygenase and LOX is lipoxygenase

Western and Mediterranean diets [27]. LA is by far the main essential PUFA in the Western diet (average intake is between 12 and 20 grams per day), with an LA to ALA ratio of 20 or 25 according to recent studies. It has been clearly shown that the preferred LA to ALA ratio for the prevention of CVD should be 4 or lower, with a minimum ALA intake of about 2 grams per day [27]. In many countries, however, the ALA intake is lower than 1 gram per day. LA and ALA are the main PUFAs in the Western and Mediterranean diets and longer chain PUFAs (with 20 carbons or more) are consumed in small amounts: from 50 mg (often) to 500 mg (rarely) per day for AA (20:4n-6) and for the long chain omega-3 PUFAs mostly found in fish, eicosapentanoic acid (EPA, 20:5n-3) and docosahexanoic acid (DHA,

22:6n-3). Mammals are in theory able to synthesize EPA and DHA from ALA [19, 27]. In patients at high risk for CVD complications, high ALA intake resulted in a significant increase in blood and tissue EPA levels, whereas the increase in DHA was low and non-significant [27, 28]. Thus, DHA is often considered an "essential fatty acid" like LA and ALA, and it is prudent to provide for minimum amounts of it in our daily diet (at least 200 to 500 mg DHA per day depending on the associated amounts of ALA and EPA).

Unlike ALA (the precursor of EPA), oleic acid (18:1n-9) is consumed in substantial amounts in the typical Western diet and is not an essential fatty acid. Oleic acid is the precursor of eicosatreinoic acid (ETA, 20:3n-9), the main omega-9

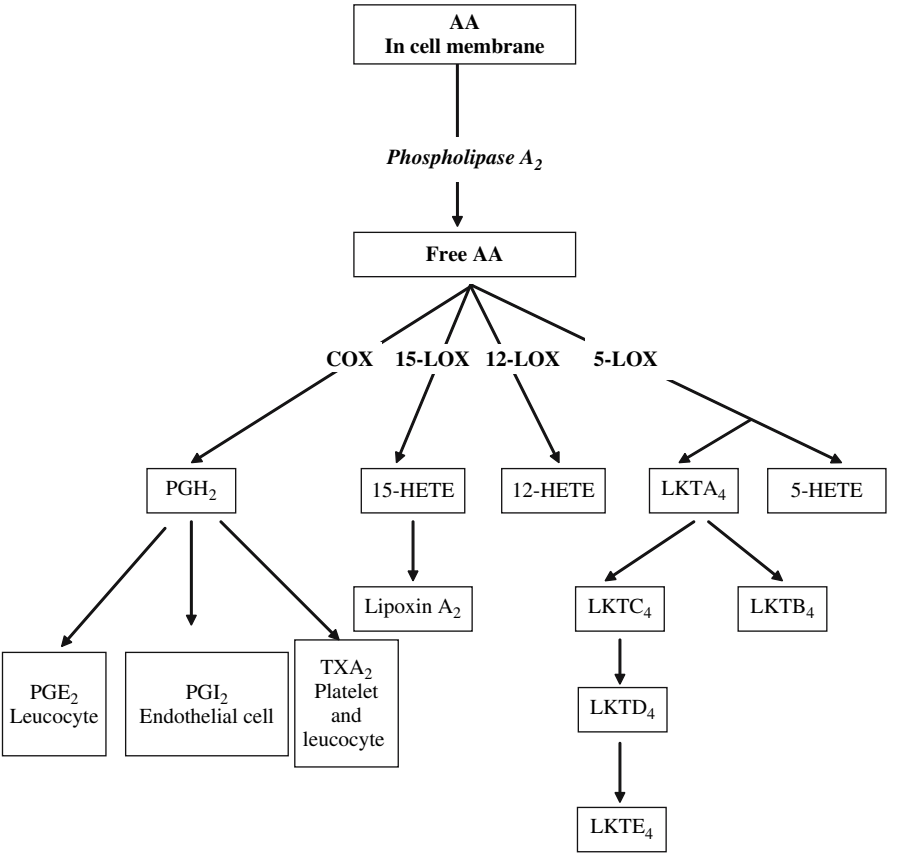

Figure 2. Metabolism of AA (arachidonic acid) in various cells. EPA can substitute for AA as a substrate for COX and LOX systems. This may result in the release of compounds that are generally less active (TXA_3 and $LKTB_5$ instead of TXA_2 and $LKTB_4$) than those produced from AA. There is one exception with PGI_3, which is as active as PGI_2 as an anti-platelet and vasodilating substance. TX is thromboxane, PGI_2 is prostacyclin and LKT is leukotriene

PUFA potentially involved in inflammation by competing with AA (and EPA) as substrates in the COX and LOX (lipoxygenase) enzyme systems. However, there is little ETA in cell membranes, probably because of the overwhelming competition from dietary LA and ALA for the relevant desaturase and elongase enzymes [29]. ETA is nevertheless assumed to decrease synthesis of leukotriene (LKT) B_4, a major inflammatory mediator, partly through a direct effect on $LKTA_4$ hydrolase (Figure 2). ETA is also a substrate for 5-LOX and may compete with AA for the formation of $LKTA_4$, especially in case of severe LA restriction leading to elevated ETA concentrations [29]. It is noteworthy that the Mediterranean diet is poor in LA and rich in oleic acid, which is another circumstance where ETA concentrations are relatively high compared with the LA-rich Western diet. Thus, whatever the nutritional context (severe LA restriction or Mediterranean diet), and in partial analogy to the situation with EPA, elevated ETA concentrations can alter the balance of eicosanoids produced by leukocytes toward a potentially less inflammatory mixture [29]. The effect of ETA on COX is less clear than on 5-LOX although inhibition of endothelial PGI_2 production has been ascribed to ETA [30]. This could, at least theoretically, increase the risk of thrombosis. Thus, a traditional Mediterranean diet with high intake of oleic acid and omega-3 PUFAs from both vegetable and marine sources and low intake of saturated fatty acids and LA may be the best compromise to reduce the risk of both inflammation and thrombosis. This has been confirmed in clinical trials [28, 31–33]. In any case, as emphasized by several major investigators in the field, the background omega-6 PUFA content of the diet is a key issue when fortifying diets with either omega-9 and/or omega-3 fatty acids for therapeutic or health-enhancing purpose [19, 27, 29].

4. INFLAMMATORY EICOSANOID SYNTHESIS FROM ESSENTIAL PUFAs

A key link between PUFAs and inflammation is that the family of inflammatory mediators termed eicosanoids is generated from 20-carbon PUFAs released by cell-membrane phospholipids (Figure 2). Inflammatory cells typically contain a high proportion of omega-6 AA and low proportions of omega-3 EPA. In fact, the AA to EPA ratio is extremely dependent on the dietary habits of the populations examined [28, 29, 34]. In persons following a typical Western diet (with a high AA to EPA ratio), AA is the dominant substrate for eicosanoid synthesis. In contrast, in persons following a Mediterranean diet poor in omega-6 PUFAs (but rich in omega-9 oleic acid and omega-3 PUFAs), the relevance of AA and AA-derived eicosanoids is reduced. Eicosanoids include prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LKTs), and many other less studied substances (Figure 2). AA is mobilized from cell membranes by phospholipases and subsequently acts as a substrate for the enzymes that synthesize eicosanoids. The metabolism of AA by COX gives rise to the 2-series PGs and TXs. However, when EPA is the substrate for COX instead of AA, the eicosanoids that are produced belong to 3-series PGs, the properties of which are very different (less inflammatory, less vasoconstrictive,

less prothrombotic) from those of 2-series PGs [19]. Substances derived from ETA are less well-characterized and their physiological roles are not clearly determined.

There are two isoforms of COX: COX-1 is a constitutive enzyme and COX-2 is induced in inflammatory cells as a result of stimulation (for instance by cytokines produced by activated leukocytes) and accounts for the marked increase in eicosanoid production that occurs in activated cells. It is very important to understand that PGs are formed in a cell-specific manner (Figure 2). For instance, monocytes (and macrophages) produce large amounts of PGE_2 and PGF_2, neutrophils produce moderate amounts of PGE_2 and mast cells produce PGD_2. AA metabolism through the 5-lipoxygenase (5-LOX) pathway gives rise to hydroxyl and hydroperoxyl derivatives and to the 4-series LKTs. EPA metabolism by the 5-LOX pathway gives rise to 5-series LKTs, which have a considerably lower inflammatory effect than 4-series LKTs.

One of the major inflammatory AA-derived 2-series PGs is PGE_2. Its pro-inflammatory effects include fever, increased vascular permeability and vasodilatation, as well as increased pain and edema. PGE_2 induces COX-2, up-regulates its own production by leukocytes, and induces the production of inflammatory cytokines (TNF, interleukins), which are other major mediators of inflammation that are able to recruit new leukocytes and again induce COX-2. However, PGE_2 also inhibits 5-LOX, decreasing the production of the 4-series LKTs, and induces 15-LOX, promoting the formation of lipoxins [35]. The latter mediators have potent anti-inflammatory effects [36, 37] indicating that the same compound, namely PGE_2, possesses both pro- and anti-inflammatory actions, whereas PGE_3 derived from EPA apparently is less active than PGE_2 [17–19]. This may explain some puzzling data showing benefits from PGE_2 in some inflammatory compartments, especially those where 4-series LKTs exert damaging effects [38]. In fact, $LKTB_4$, one of the major inflammatory AA-derived eicosanoids of the 4-series LKTs which increases vascular permeability is a potent chemotactic agent for leukocytes and increases the generation of reactive oxygen species and production of inflammatory cytokines. $LKTB_4$ was recently shown to play an important role in the atherosclerotic process (using the intima-media thickness as a surrogate marker of atherosclerosis) in certain patients with a specific polymorphism (variant 5-LOX genotypes) [39]. Interestingly, a protective effect of omega-3 PUFAs (and a deleterious effect of omega-6 PUFAs) was shown in that study, suggesting that long-chain omega-3 PUFAs may also be able to slow down the progression of the atherosclerotic process contrary to the results of large randomized trials where the protective effect of EPA+DHA appeared to be confined to myocardial anti-arrhythmic effects [40–42]. In addition, a recent study demonstrated that incorporation of EPA and DHA in the plaque might have a stabilizing (anti-inflammatory) effect thereby preventing acute ischemic events [43]. This suggests that EPA+DHA may inhibit the generation of metalloproteinases [44, 45], compounds that are potentially involved in plaque vulnerability and ulceration and subsequent thrombotic complications. Further studies are obviously needed to support this assertion.

The molecular biology of PGE_2 and 4-series LKTs illustrates the complexity of the health effects of eicosanoids and the necessity to be careful when using potent

pharmacological agents to manage them. As shown with the anti-COX-2 (coxib) agents, the ultimate outcome may be less appealing than previously expected, e.g., an increased risk of CVD complications [7, 8].

The EPA-derived 3-series PGs and 5-series LKTs are considerably less inflammatory than those derived from AA [17–19]. Increased consumption of omega-3 PUFAs results in increased proportions of omega-3 PUFAs (especially EPA) in inflammatory cell phospholipids, at the expense of AA. This was shown to result in decreased production of PGE_2, TXB_2 and $LKTB_4$ by inflammatory cells and, at the same time, increased production of PGE_3, TXB_3 and $LKTB_5$. The functional significance is that the mediators derived from EPA are less potent than those derived from AA. It may be exaggerated, however, to say that EPA-derived eicosanoids are anti-inflammatory. Let it simply be said that they are less pro-inflammatory than the AA-derived eicosanoids.

Finally, recent studies have identified novel groups of mediators, termed E-series resolvins (for "*resolution phase interaction products*") when derived from EPA by COX-2, and D-series resolvins (or docosatrienes and neuroprotectins) when derived from DHA by COX-2, which appear to have anti-inflammatory properties, especially during the resolution phase of the inflammatory process [46]. The relevance of this specific anti-inflammatory activity for vascular inflammation associated with atherosclerosis remains to be elucidated.

Thus, the key "anti-inflammatory effect" of omega-3 PUFAs appears to be antagonism of AA, the major inflammatory PUFA (Table 1). But another major question is whether omega-3 PUFAs have anti-inflammatory effects that occur downstream of altered eicosanoid production.

5. OTHER ANTI-INFLAMMATORY EFFECTS OF OMEGA-3 PUFAs

Proposed mechanisms by which omega-3 PUFAs may have anti-inflammatory effects are shown in Table 1. In addition to competing with omega-6 PUFAs at various levels of PUFA metabolism, EPA and DHA have been shown to inhibit the production of cytokines by leukocytes and other inflammatory cells *in vitro* and *ex vivo* [47]. In clinical studies, EPA+DHA-rich fish oil supplementation resulted in decreased production of TNF and interleukins by leukocytes [48]. Also, diets enriched in ALA have been associated with reduced vascular inflammation and endothelial activation [49]. Which bioactive components (ALA itself or its metabolite, EPA,or both) inhibit endothelial activation is not clear. In fact, De Caterina et al. [50] showed that DHA and EPA significantly decrease cytokine-induced expression of adhesion molecules by endothelial cells. This has the functional effect of decreasing the binding of leukocytes, a crucial step of vascular inflammation and atherosclerosis [1, 13]. Interestingly, oleic acid (the precursor of the omega-9 PUFA ETA) was also shown to inhibit endothelial activation [51] and olive oil itself (the oil typically used around the Mediterranean Sea) had similar effects in middle-aged men [52].

Some of the anti-inflammatory effects of omega-3 PUFAs may also be exerted at the level of gene expression. Although the extent of these effects in humans *in vivo* is

Table 1. A summary of the main potential anti-inflammatory effects of omega-3 PUFAs

1.	The 18-carbon omega-3 ALA (18:3n-3) decreases the synthesis of pro-inflammatory AA from the omega-6 LA (18:2n-6) through competition at the level of their common elongation and desaturation pathways (Figure 1).
2.	The 20-carbon omega-3 PUFA EPA (20:5n-3) decreases the levels of AA in inflammatory cells. EPA replaces AA in membrane phospholipids (Figure 2).
3.	EPA decreases the production of AA-derived inflammatory eicosanoids by decreasing the release of AA from cell membranes and competing at the levels of the COX and LOX enzyme systems.
4.	EPA gives rise to a family of eicosanoid mediators that are analogs of those produced from AA (Figure 2) but are often less potent (less inflammatory).
5.	Omega-3 PUFAs reduce the production of inflammatory cytokines (including TNF and interleukins) by leukocytes and other cells involved in the inflammation process through decreased production of TXA_2 and $LKTB_4$ (see text).
6.	Omega-3 PUFAs induce production of the anti-inflammatory E-resolvins from EPA and D-resolvins from DHA (see text).
7.	The omega-3 PUFAs EPA and DHA alter the expression of inflammatory genes via inhibition of the non-specific transcription factor NF-?B (see text).

not yet clear, animal studies indicate potentially significant effects on the expression of a range of inflammatory genes. For instance, omega-3 PUFAs were shown to decrease the cytokine-mediated induction of expression of COX-2, TNFα and various interleukins in cultured chondrocytes and human cartilage explants [19]. Similar data were reported with DHA and vascular endothelial cells [50]. This effect on gene expression was independent of the effect on eicosanoid production; thus, omega-3 PUFAs may directly modulate the intracellular signaling pathway that leads to activation of one or more transcription factors such as nuclear factor κB (NF-κB) [19]. For instance, omega-3 PUFAs were shown to prevent NF-κB activation by TNFα and to decrease endotoxin-induced activation of NF-κB by leukocytes [51]. Thus, in addition to directly decreasing the production of inflammatory eicosanoids and leukocyte cytokines, omega-3 PUFAs act by altering the expression of inflammatory genes.

6. ESSENTIAL PUFAs, COX, ASPIRIN AND COXIBS

Once mobilized from cell membrane phospholipids, 20-carbon PUFAs (either AA or EPA) are oxygenated into eicosanoids along various pathways including COX, LOX, P450 epoxygenase and (nonenzymatic) isoprostane synthesis. In addition, free PUFAs are available to exert direct effects on membrane receptors and ion channels, e.g. to deploy anti-arrhythmic effects in the ischemic myocardium [54].

As indicated above, the fate and distribution of AA or EPA metabolites depend on the cell type where they are formed. For example, leukocytes, endothelial cells, smooth muscle cells in the arterial wall, as well as platelets express PGE synthase and are thus all capable of producing proinflammatory PGE. Platelets also express TXA synthase and elaborate the prothrombotic and vasoconstrictive TXA_2. Endothelial cells express prostacyclin synthase and synthesize the antithrombotic and vasodilating PGI_2. In addition to cell-specific synthesis, the biological effects of eicosanoids are governed by cell-specific receptor-dependent signaling pathways that define biological responses. Pharmacological inhibition of eicosanoid synthesis has been the focus of intensive drug development, from aspirin to NSAIDs and specific coxibs. NSAIDs provide antipyretic, analgesic and anti-inflammatory properties but the relative degree of these effects varies markedly from one compound to another. NSAIDs also share the common side-effects of gastro-intestinal ulceration and renal function impairment.

With the recognition that aspirin inhibits platelet function via inhibition of thromboxane formation, the anti-thrombotic effects of NSAIDs gained unique therapeutic emphasis. Because endothelial PGI_2 also has an anti-platelet action, nonselective inhibition of COX attenuates the anti-platelet effect of aspirin. Thus, in view of the irreversible inhibition of thromboxane formation in platelets by aspirin and the differences in half-lives of platelet and endothelial COX, very low dose aspirin was found to provide optimal antithrombotic activity for prevention of thrombotic CVD complications [22, 23]. Finally, the recognition that there are two different COXs led to the straightforward view that COX-2 is specifically responsible for the adverse proinflammatory effects of eicosanoids and that selective COX-2 inhibitors (coxibs) would provide adequate analgesia and anti-inflammatory effects without the gastrointestinal side effects due to COX-1 inhibition and without platelet and endothelial cell effects [23]. Unfortunately, this clean mechanistic distinction between the COXs is an oversimplification [7]. In fact, inhibition of COX-2 appears to be associated with suppression of prostacyclin (PGI_3 from EPA and PGI_2 from AA) synthesis [55]. The complexity of the interactions between the different factors in arterial physiology is illustrated by the fact that suppression of COX-2 results in an increasing flux of AA towards the different LO pathways, with potential additional inflammatory effects. This may be especially important in the setting of inflammation in atherosclerotic plaque, as suggested by the study of Dwyer et al. [39] on the role of LKTs in plaque progression.

The consequences of COX-2 inhibition by coxibs recorded in some of the observational studies and randomized trials were therefore—perhaps not surprisingly—an increased risk of CVD complications [7, 8, 56, 57]. It is important to note that increased risks have been observed in populations at low risk for CVD complications [56, 57] and have also been found in some studies with nonselective NSAIDs [58]. This suggests that the increased risk of CVD complications associated with coxibs and other anti-inflammatory drugs may not be an effect of a specific class of drugs (such as the coxibs) but rather may be related to the indiscriminant inhibition of the inflammatory process itself.

Beyond the practical problems regarding the treatment of painful inflammatory chronic diseases such as arthritis, the controversy surrounding effects of coxibs on

CVD raises several major questions regarding the inflammatory theory of atherosclerosis. The main one is that it is difficult to accept that vascular inflammation is a prominent feature of CVD development if anti-inflammatory treatments (whatever the class of drugs) tested in randomized trials are not able to reduce the risk of CVD complications. In fact, a major question is whether we should not reconsider our conception of atherosclerosis and CVD complications. For instance, if arterial lesion fibrosis is a key factor in lesion stabilization, altering the process by any anti-inflammatory treatment may increase the risk of plaque ulceration and CVD complications.

The potential role of omega-3 PUFAs in vascular inflammation and CVD may help open new areas of investigation. From a biological point of view omega-3 PUFAs appear to have anti-inflammatory properties that make them good candidates to reduce vascular inflammation and prevent atherosclerosis (see Table 1). However, evidence from randomized trials supports the complex nature of PUFAs in modulating vascular inflammation and atherosclerosis. In clinical trials of CVD outcomes, omega-3-PUFAs administered at low dosages (less than 1 gram per day) reduced the risk of sudden cardiac death and ventricular arrhythmias [40, 41], and a combination of increased intake of omega-9 and omega-3 fatty acids and decreased intake of omega-6 PUFAs in a Mediterranean diet produced significant reductions in fatal and non-fatal CVD complications including ventricular arrhythmias [28, 31]. In mechanistic studies, the traditional Mediterranean diet produced significant anti-inflammatory effects associated with less endothelial dysfunction and lower vascular endothelial growth factor [32, 33]. The exact mechanisms of these effects remain to be elucidated, and the anti-inflammatory effects of low dosages of omega-3-PUFAs are not clear. Whether anti-inflammatory effects can be adequately balanced to prevent vascular inflammation without altering the reparation-fibrosis process that stabilizes atherosclerotic plaques is unknown, but these observed benefits should be used to establish a working hypothesis.

Taken together, the human data indicate that vascular inflammation is a complex multi-step process and atherosclerosis is a multifactorial disease. Considering only dietary lipids, it is clear that essential PUFAs of the omega-6 and omega-3 families, saturated fatty acids, as well as omega-9 fatty acids are collectively involved. Thus, to be effective and safe, any anti-inflammatory approach to prevent atherosclerosis and CVD should be prudent, preferably non-pharmacological, multifactorial, and primarily dietary. This is compatible with the well-accepted concept that CVD is a lifestyle disease that will require lifestyle (especially dietary) changes for prevention.

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