

## REVIEW ARTICLE

## DRUG THERAPY

## n-3 Fatty Acids in Cardiovascular Disease

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CARDIOVASCULAR DISEASE IS THE LEADING CAUSE OF DEATH WORLDWIDE, and preventive approaches, particularly achievable dietary changes, have major public health implications. An increased dietary intake of n-3 (polyunsaturated) fatty acids is one such dietary approach. This review discusses advances since the topic was last reviewed in the *Journal*<sup>1</sup> and highlights current gaps in knowledge.

## HISTORICAL PERSPECTIVE

In response to anecdotal reports of a low prevalence of coronary heart disease among Greenland Eskimos (Inuits), Bang and Dyerberg undertook six expeditions to Greenland starting in the late 1960s. They confirmed a very low incidence of myocardial infarction and reported an antiatherogenic blood lipid pattern, as well as markedly reduced platelet reactivity, in this population as compared with Danish controls.<sup>2,3</sup> These findings were attributed to the Inuit diet, which was composed mainly of seal and whale and was extremely rich in marine n-3 fatty acids. The prevalence of inflammatory and immune diseases among the Inuits was also reported to be very low.<sup>4</sup> In a seminal article in 1978, Dyerberg and colleagues presented the hypothesis that marine n-3 fatty acids might provide protection against atherosclerosis and thrombosis,<sup>5</sup> and they began research on the potential effects of n-3 fatty acids in the prevention and treatment of vascular disease.

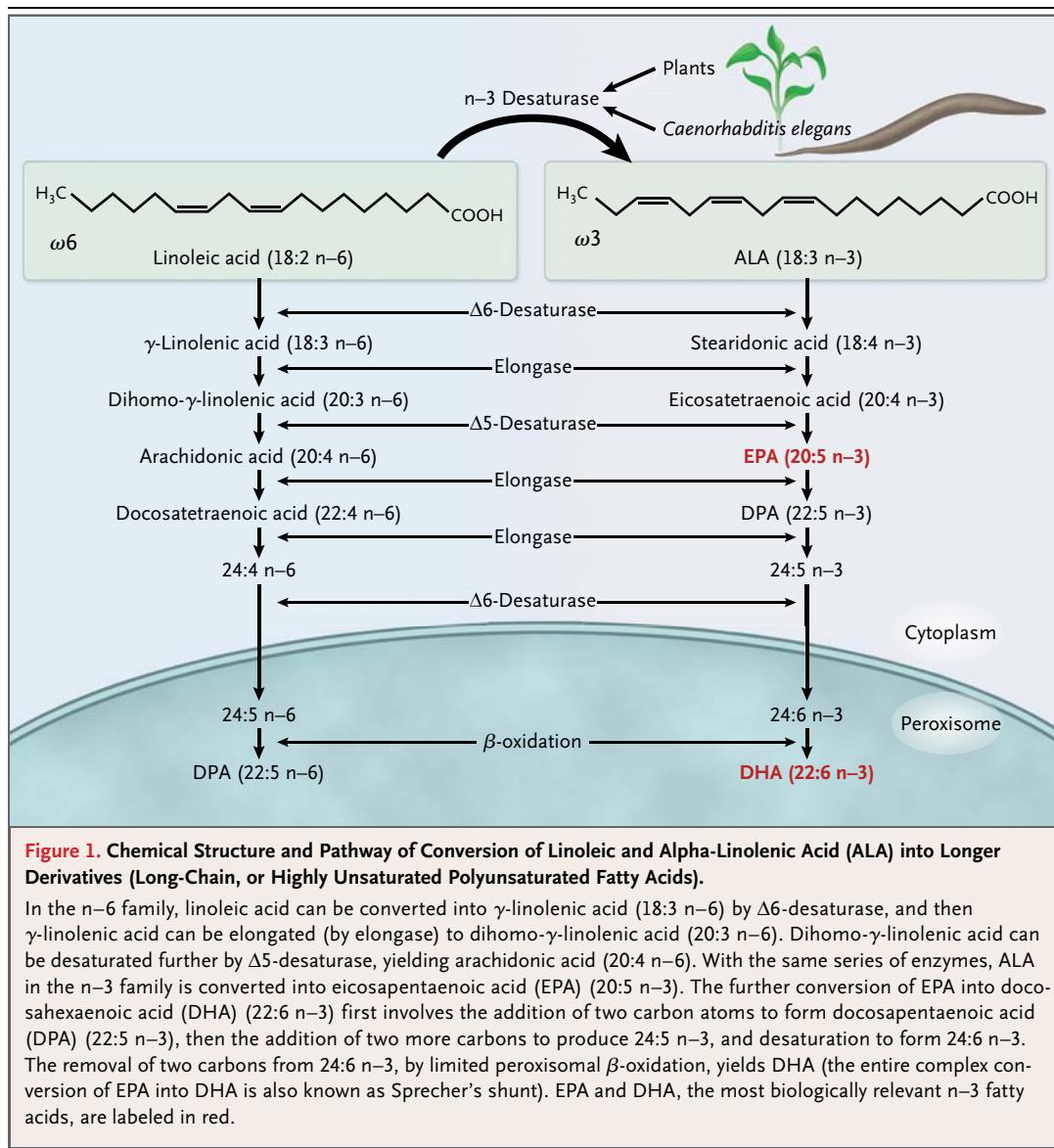
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## BIOSYNTHESIS OF ESSENTIAL POLYUNSATURATED FATTY ACIDS

Polyunsaturated fatty acids, organic acids that naturally contain more than one double bond in the aliphatic chain, are named according to the number (>1), position, and configuration of such double bonds, which also largely determine their physical and biologic properties. Biologically relevant families of polyunsaturated fatty acids are the n-6 and the n-3 fatty acids (Fig. 1). Mammals lack enzymes to insert the double bond in the n-6 or n-3 position; therefore, linoleic acid and alpha-linolenic acid (ALA), as well as some of their elongation products, are essential nutrients for mammals; the lack of these nutrients leads to a syndrome of deficiency of essential fatty acids.<sup>6,7</sup> In humans, this deficiency is usually characterized by desquamative rashes and hyperkeratotic dermatoses.<sup>8</sup> Current estimates of the minimum requirements for n-6 and n-3 fatty acids in adults are 1.0% and 0.2% of daily energy intake, respectively,<sup>9</sup> with acceptable (but not necessarily ideal) macronutrient distribution ranges for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of 0.25 to 2.0 g per day.<sup>10</sup>

Plant tissues and oils are good sources of linoleic acid and ALA. Photosynthesizing plants are especially rich in ALA, accounting for up to 55% of the fatty acids present in green vegetables. However, green vegetables generally contribute little to the optimal intake of ALA in humans, as compared with certain plant oils such as



soybean, flaxseed, linseed, and rapeseed (canola) oils, as well as walnuts. Mammalian cells cannot synthesize linoleic acid and ALA, though limited (1 to 5%)<sup>11</sup> conversion to longer-chain polyunsaturated fatty acids occurs through further desaturation and elongation. Linoleic acid can ultimately be converted to arachidonic acid (20:4 n-6) and, through the same series of enzymes, ALA can be converted to EPA (20:5 n-3). The further conversion of EPA to DHA (22:6 n-3) involves a complex series of additional reactions,<sup>12</sup> but the conversion is very limited<sup>11</sup> (Fig. 1). DHA accumulates in all tissues, including the heart and the vessel wall,

but it particularly accumulates in the nervous system and the retina, where it serves important physiological functions.

EPA and DHA enter the food chain through marine phytoplankton, and they pass through fish and marine mammals — seals, walrus, and whales — which are the main components of the Eskimo diet. In Western diets, the main source of EPA and DHA intake is fish, especially oily fish (e.g., mackerel, trout, salmon, herring, and sardines). Fish-oil preparations contain variable amounts of n-3 fatty acids in the form of triglycerides, ethyl esters, or free fatty acids.

Transgenic animals that express the *fat-1* gene from the worm *Caenorhabditis elegans* have been raised. This gene encodes an n-3 fatty acyl desaturase catalyzing the conversion of 18- and 20-carbon n-6 substrates into n-3 fatty acids. A supplementation approach with the use of meat from these transgenic animals might permit a marked enrichment of mammalian cells with n-3 fatty acids beyond that which is possible through dietary approaches, with obvious clinical implications.<sup>13,14</sup>

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METABOLISM AND MECHANISMS  
OF ACTION

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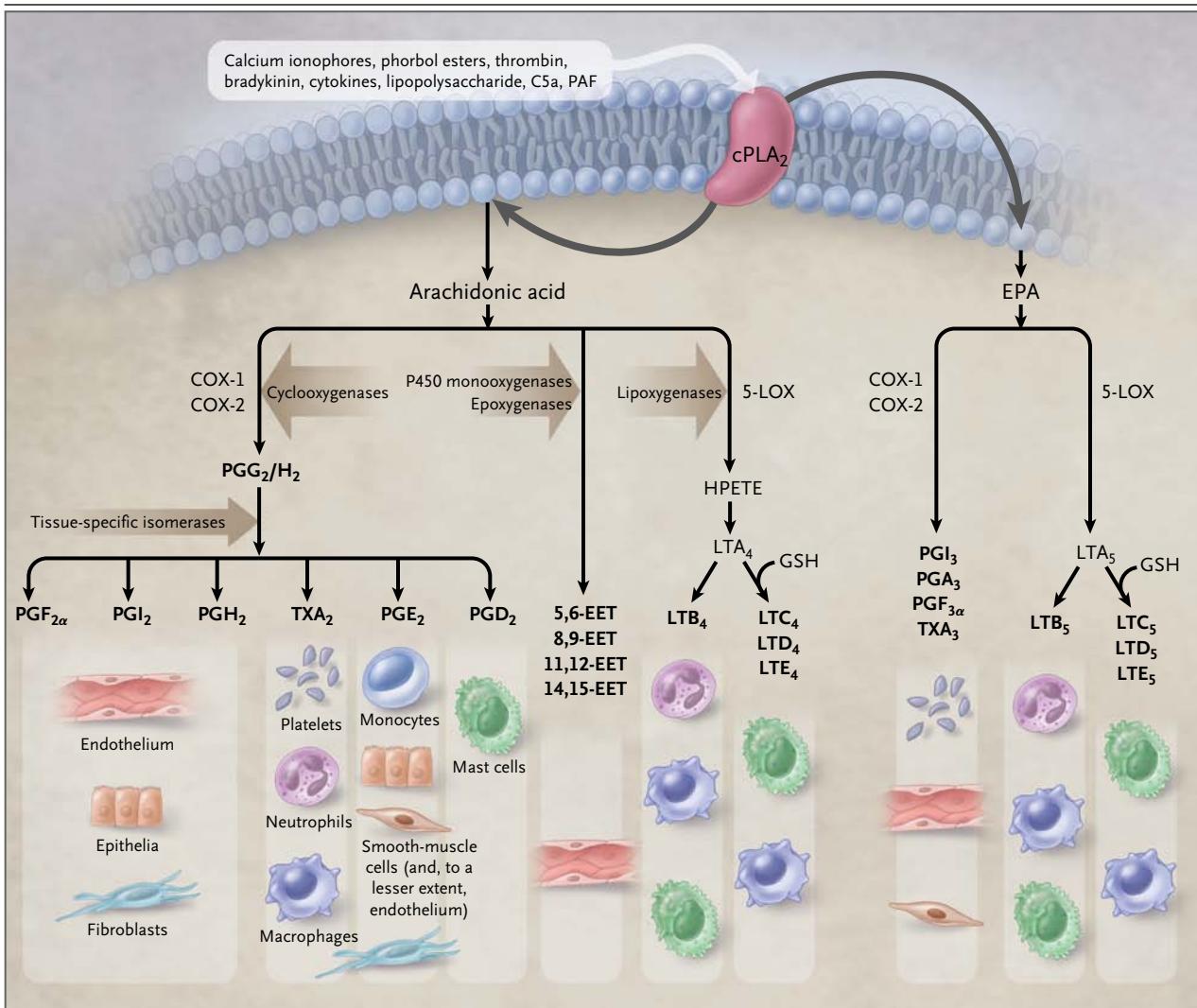
Polyunsaturated fatty acids modulate local signaling and structure, primarily after esterification (prevalently in the sn-2 position) in glycerophospholipids and incorporation into cell membranes (see the Supplementary Appendix, available with the full text of this article at NEJM.org). There are three broad categories of biologic effects: those mediated by the release of bioactive mediators, direct effects on ion channels that modulate a number of events (e.g., arrhythmogenesis), and other direct effects on membranes that require incorporation into cell phospholipids.

The n-3 fatty acids can be released through the actions of phospholipase A<sub>2</sub>, induced by a variety of stimuli, and are converted through both orthodox and novel pathways to signaling molecules. The orthodox pathways involve reactions catalyzed by cyclooxygenases and lipoxygenases to biologically active eicosanoids, including prostaglandins, thromboxanes, and leukotrienes (Fig. 2). Thromboxanes and leukotrienes derived from n-3 fatty acids are in general much less potent local mediators than the corresponding n-6 fatty acid derivatives in orthodox pathways. The n-3 fatty acids are also converted to biologically active epoxides, alcohols, diols, and ketones through recently discovered new pathways (Fig. 3). A number of local mediators, discovered as a result of elucidating such new pathways, are potent (in the nanomolar range) and stereoselective, with anti-inflammatory actions and actions prompting the resolution of inflammation, and they are produced during this resolution phase of inflammation through transcellular biosynthetic routes (i.e., the product of one cell is transformed by neighboring cells). Thus, these compounds are termed “res-

olution-phase interaction products” (resolvins)<sup>16,17</sup> (Fig. 3). In addition, unsaturated fatty acids, through nitration, can generate nitro-fatty acids that globally suppress inflammation at micromolar concentrations<sup>18</sup> (Fig. 3).

In low micromolar concentrations, n-3 fatty acids have direct effects without undergoing metabolism. Some direct effects, such as antiarrhythmic effects, occur rapidly,<sup>19</sup> without incorporation of n-3 fatty acids into the cell membranes. These free fatty acids cause steric interference with sodium, potassium, and calcium channels; this blocking mechanism is distinct from that of other known classes of antiarrhythmic agents.<sup>20</sup> Conversely, incorporation into cell membranes is required for slow-onset and long-acting direct anti-inflammatory, antiatherogenic effects, modulating the expression of endothelial proinflammatory, proatherogenic genes (genomic effects).<sup>21,22</sup> The incorporation of fatty acids appears to alter the properties of lipid rafts and caveolae, contributing to membrane fluidity. Thus, hormone-receptor binding and the function of membrane-associated proteins are affected.<sup>23,24</sup> In turn, this incorporation has been associated with decreased generation of intracellular reactive oxygen species and a consequent diminished activation of redox-sensitive transcription factors, such as the nuclear factor- $\kappa$ B system, modifying the expression of proinflammatory, proatherogenic genes.<sup>22,25</sup> There is evidence that n-3 fatty acids signal through G-protein-coupled receptor 120, modulating inflammatory and insulin-sensitizing effects that occur in monocytes and macrophages.<sup>26</sup>

Through one or more of the above mechanisms, n-3 fatty acids may affect several intermediate determinants of cardiovascular risk. At doses of 3 g per day or more, these substances generally reduce hypertriglyceridemia in humans<sup>27,28</sup> without changing cholesterol levels substantially.<sup>29</sup> The n-3 fatty acids are also associated with decreased levels of markers and mediators of inflammation such as the cytokines interleukin-1 $\beta$  and tumor necrosis factor  $\alpha$ .<sup>30,31</sup> Studies have shown that increased intake of n-3 fatty acids is associated with a small reduction in blood pressure (approximately 2 to 3 mm Hg systolic and 1 to 2 mm Hg diastolic)<sup>32</sup> and with a reduction in the resting heart rate (approximately 3 beats per minute).<sup>33</sup> Other studies suggest that the intake of n-3 fatty acids is associated with improved cardiac diastolic fill-



**Figure 2. "Orthodox Pathways" of Polyunsaturated Fatty Acid Metabolism.**

Metabolic pathways involve reactions catalyzed by conversion through cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and lipoxygenases to eicosanoids, which include prostaglandins, thromboxanes, and leukotrienes in different cell types. Arachidonic acid is the precursor of the prostanoids of the 2 series (including prostacyclin [PGI<sub>2</sub>] and thromboxane A<sub>2</sub> [TXA<sub>2</sub>]), whereas eicosapentaenoic acid (EPA) is the precursor of prostanoids of the 3 series (prostaglandin I<sub>3</sub> [PGI<sub>3</sub>] and thromboxane A<sub>3</sub> [TXA<sub>3</sub>]). Increasing the content of n-3 fatty acids in the membranes reduces the production of prostanoids in general, but it also favors the synthesis of TXA<sub>3</sub> in platelets, which, unlike arachidonic acid-derived TXA<sub>2</sub>, has been reported to have weak platelet-aggregating and vasoconstricting activity (although recent studies have challenged this observation<sup>15</sup>). In endothelial cells, however, the main products of arachidonic acid and those of EPA, PGI<sub>2</sub> and PGI<sub>3</sub> are virtually equipotent in their antiaggregative and vasodilating properties, and EPA does not appreciably interfere with arachidonic acid metabolism as a substrate for COX-2.<sup>15</sup> In leukocytes and monocytes, arachidonic acid and EPA are substrates of 5-lipoxygenase (5-LOX) for the synthesis of leukotrienes. Leukotriene B<sub>4</sub> (LTB<sub>4</sub>), derived from arachidonic acid, has potent chemotactic and leukocyte-activating properties, whereas the cysteinyl leukotrienes C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub> (LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>) have vasoconstrictive effects and increase vascular permeability. Through 5-LOX, EPA gives rise to leukotrienes of the 5 series, which have weaker proinflammatory and vasoconstrictive activities. Final products are labeled in bold, and the cells shown are the respective main production sites. The abbreviation cPLA<sub>2</sub> denotes cytosolic phospholipase A<sub>2</sub>, EET epoxyeicosatrienoic acid, GSH glutathione, HPETE hydroperoxy-eicosatetraenoic acid, LTA<sub>4</sub> leukotriene A<sub>4</sub>, LTA<sub>5</sub> leukotriene A<sub>5</sub>, LTB<sub>5</sub> leukotriene B<sub>5</sub>, LTC<sub>5</sub> leukotriene C<sub>5</sub>, LTD<sub>5</sub> leukotriene D<sub>5</sub>, LTE<sub>5</sub> leukotriene E<sub>5</sub>, PAF platelet-activating factor, PGA<sub>3</sub> prostaglandin A<sub>3</sub>, PGD<sub>2</sub> prostaglandin D<sub>2</sub>, PGE<sub>2</sub> prostaglandin E<sub>2</sub>, PGF<sub>2α</sub> prostaglandin F<sub>2α</sub>, PGF<sub>3α</sub> prostaglandin F<sub>3α</sub>, PGG<sub>2</sub> prostaglandin G<sub>2</sub>, and PGH<sub>2</sub> prostaglandin H<sub>2</sub>.

ing,<sup>33</sup> modulation of autonomic function (thus increasing heart-rate variability),<sup>34</sup> and increased baroreceptor control,<sup>35</sup> which in turn reduce the risk of fatal arrhythmias. Some data suggest that n-3 fatty acids improve insulin sensitivity<sup>36</sup> and mildly inhibit platelet function,<sup>37,38</sup> though the overall effects on hemostasis appear to be modest. Despite a mild increase in the bleeding time, clinically important bleeding, originally described in Eskimos,<sup>4</sup> has not been substantiated in other groups.<sup>39,40</sup> A short course of n-3 fatty acid supplementation has been reported to improve endothelial function<sup>41</sup> and to reduce features of inflammatory atherosclerotic plaque.<sup>42</sup>

#### ANIMAL MODELS

Experimental studies in animal models fall broadly into two categories: studies exploring the progression of vascular disease or thrombosis, and studies of antiarrhythmic effects. In several animal models, n-3 fatty acids improved endothelial function and diminished atheromas, but such results are inconsistent, probably reflecting differences in the species used and the study design.<sup>43</sup>

In Langendorff preparations of perfused rabbit heart<sup>44</sup> and in feeding experiments in rats<sup>45</sup> and monkeys,<sup>46</sup> n-3 fatty acids were associated with an increased arrhythmogenic threshold. In a canine model of sudden death due to exercise-induced ventricular fibrillation, the intravenous infusion of an emulsion of n-3 fatty acid concentrate<sup>47</sup> or isolated EPA, DHA, or ALA plus serum albumin<sup>48</sup> prevented ventricular fibrillation (see the Supplementary Appendix).

#### EPIDEMIOLOGIC STUDIES

In 25 studies involving a total of 280,000 participants, there was an inverse association between fish consumption and morbidity or mortality from coronary heart disease.<sup>49,50</sup> The first epidemiologic observations in Greenland Inuits<sup>51,52</sup> (with an estimated mean n-3 fatty acid intake of up to about 15 g per day<sup>52</sup>) suggested that a nutritional factor was associated with cardiovascular protection. This observation was subsequently confirmed in natives of Northern Canada and Alaska who were living traditionally,<sup>4,5,53,54</sup> and in Japanese,<sup>55</sup> Western, and Chinese populations<sup>49,56,57</sup> (Table 1

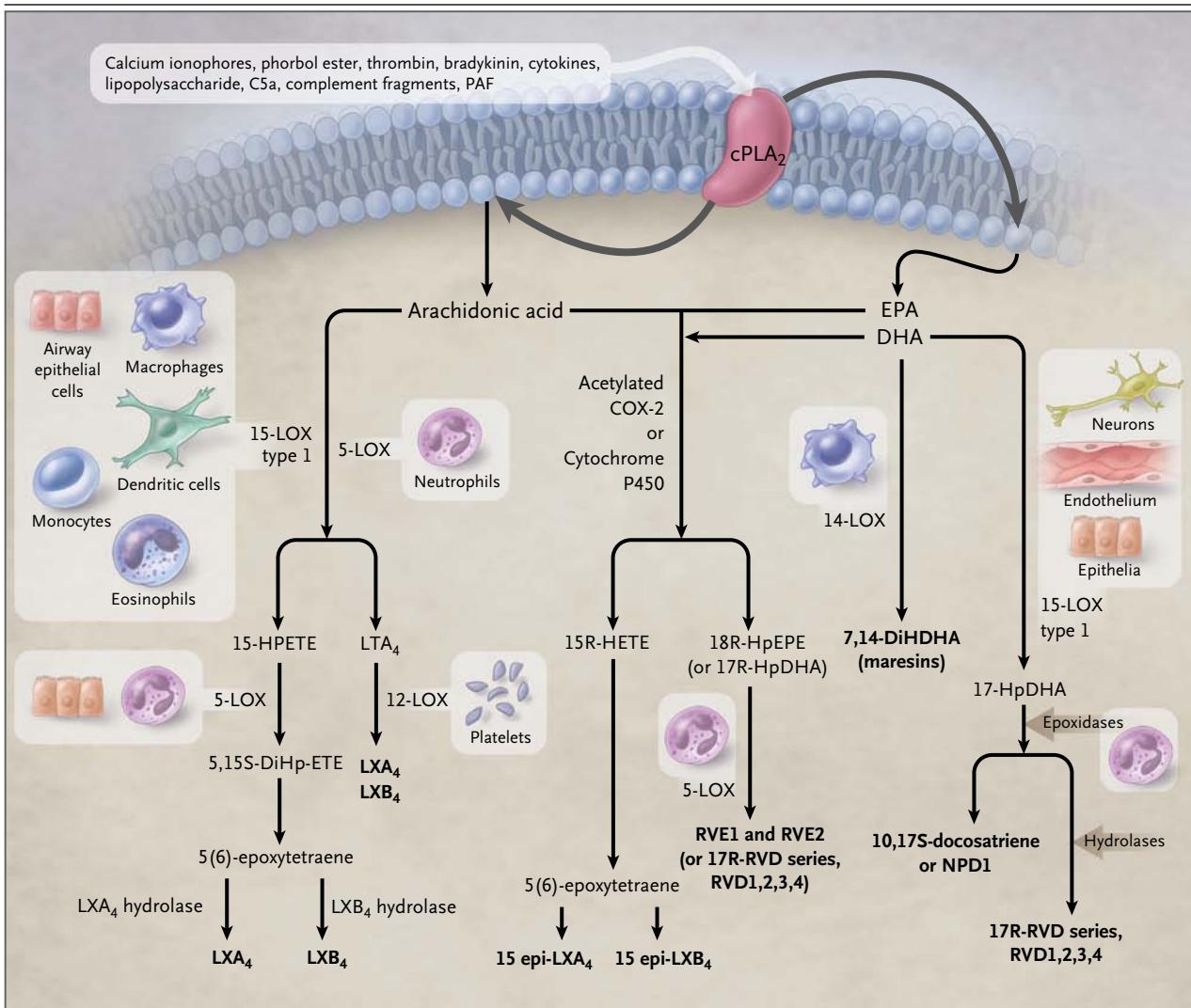
in the Supplementary Appendix). Blood levels of n-3 fatty acids also appear to correlate inversely with death from cardiovascular causes<sup>58,59</sup> and total mortality.<sup>60</sup>

#### CLINICAL TRIALS

##### SECONDARY PREVENTION OF CARDIOVASCULAR DISEASE

In the Diet and Reinfarction Trial,<sup>60,61</sup> a total of 2033 male survivors of myocardial infarction were randomly assigned in an open-label fashion to receive or not receive one of the following three dietary recommendations: a reduction in fat intake with an increase in the ratio of polyunsaturated fatty acids to saturated fat, an increase in the intake of cereal fiber, and an increase in the intake of oily fish (200 to 400 g per week, providing an additional 500 to 800 mg of n-3 fatty acids per day). Participants who declined to eat fish were allowed to take fish-oil capsules (900 mg of EPA-DHA per day). There was a 29% reduction in the rate of death from any cause over a 2-year period in the group of persons who received advice on increasing fish consumption.<sup>61,62</sup> This study was novel in showing that dietary advice and subsequent changes in fish intake might influence mortality, but the dietary intervention was complex, the study design was unavoidably open-label, and the study lacked power to detect a true difference in mortality between the two groups.

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione trial<sup>29</sup> randomly assigned 11,324 survivors of recent myocardial infarction (within the previous 3 months) to receive n-3 polyunsaturated fatty acids (1 g per day, in 2836 patients), vitamin E (300 mg per day, in 2830 patients), both (in 2830 patients), or neither (in 2828 patients [the control group]) for 3.5 years. No placebos were used in the study. Among the patients who received n-3 fatty acids alone, as compared with the control group, there was a 15% reduction in the composite primary end point of death, nonfatal myocardial infarction, or nonfatal stroke ( $P < 0.02$ ), with a 20% reduction in the rate of death from any cause ( $P < 0.01$ ) and a 45% reduction in the rate of sudden death, an end point adjudicated by a committee whose members were unaware of the group assignments ( $P < 0.001$ ), whereas the incidence of



**Figure 3. Novel Pathways of Polyunsaturated Fatty Acid Metabolism.**

When released from membrane phospholipids, n-3 fatty acids can give rise to a host of novel eicosanoid derivatives through metabolism catalyzed by several lipoxygenases, modified cyclooxygenases, and the cytochrome P450 system, producing hydroxy- and hydroperoxy-derivatives, as well as lipoxins, maresins, protectins. These compounds are typically produced during the resolution of inflammation and most are produced by transcellular metabolism; therefore they are generically termed “resolution-phase interaction products” (resolvins). Final products are labeled in bold, and the cells shown are the respective main production sites. COX-2 denotes cyclooxygenase-2, cPLA<sub>2</sub> cytosolic phospholipase A<sub>2</sub>, DHA docosahexaenoic acid, DiHDHA dihydroxy-docosahexaenoic acid, DiHp-ETE dihydroperoxy-eicosatetraenoic acid, EPA eicosapentaenoic acid, HETE hydroxy-eicosatetraenoic acid, HpDHA hydroperoxy-docosahexaenoic acid, HpEPE hydroperoxy-eicosapentaenoic acid, HPETE hydroperoxy-eicosatetraenoic acid, 5-LOX 5-lipoxygenase, 12-LOX 12-lipoxygenase, 14-LOX 14-lipoxygenase, 15-LOX 15-lipoxygenase, LTA<sub>4</sub> leukotriene A<sub>4</sub>, LXA<sub>4</sub> lipoxin A<sub>4</sub>, LXB<sub>4</sub> lipoxin B<sub>4</sub>, NPD1 neuroprotectin D1, and RV resolvins.

myocardial infarction was not significantly reduced. Vitamin E provided no additional benefit.<sup>29</sup> Survival curves diverged early after randomization. Total mortality was significantly reduced after only 3 months of treatment (relative risk, 0.59), and the rate of sudden death after only 4 months (relative

risk, 0.47).<sup>63</sup> However, the study was open-label and had a high dropout rate (>25%), limiting its generalizability.

Burr et al.<sup>64</sup> reported on a trial involving 3114 male patients of general practitioners in south Wales. These patients, who were younger than

70 years of age and had angina pectoris, were randomly assigned to four groups that received different advice about food. 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. After 3 to 9 years, there was no reduction in mortality in the groups of patients who received dietary advice on increasing fish consumption. Furthermore, no other effects were attributable to advice regarding fruit. Contrary to the prespecified hypothesis, the risk of death from cardiac causes was higher among patients who were advised to consume oily fish than among those who were not so advised (adjusted hazard ratio, 1.26; 95% confidence interval [CI], 1.00 to 1.58;  $P=0.047$ ), and the risk of sudden death from cardiac causes was even greater (1.54; 95% CI, 1.06 to 2.23;  $P=0.02$ ). The apparent excess risk occurred largely in the subgroup of patients who received fish-oil capsules. There was no evidence that this excess risk was due to interactions with other medications that the participants were receiving.<sup>64</sup> However, there were several problems during the conduct of this trial, including the inability to check long-term adherence to the assigned treatments and between-group differences in changes in concomitant medications and health behaviors.<sup>65</sup>

In the Japan Eicosapentaenoic Acid (EPA) Lipid Intervention Study (JELIS; ClinicalTrials.gov number, NCT00231738),<sup>66</sup> the long-term use of pure EPA was tested in an open-label trial for efficacy in the prevention of major coronary events in Japanese patients with hypercholesterolemia. A total of 18,645 patients with total cholesterol levels of 252 mg per deciliter or higher ( $\geq 6.5$  mmol per liter) were randomly assigned to receive either 1800 mg of EPA per day with statins (9326 patients) or statins alone (9319 patients). The primary end point was a major coronary event, defined as sudden death from cardiac causes, fatal or nonfatal myocardial infarction, unstable angina pectoris, angioplasty, stenting, or coronary-artery bypass grafting; these outcomes were adjudicated by investigators who were unaware of the group assignments. At a mean follow-up of 4.6 years, the primary end point occurred in 262 patients in the EPA group (2.8%) and in 324 patients in the control

group (3.5%) — a 19% relative reduction in major coronary events ( $P=0.01$ ).<sup>66</sup> There was also a significant reduction in stroke.<sup>67</sup> The level of low-density lipoprotein cholesterol after treatment decreased similarly in both groups and thus was not a major factor in the observed effect. Nonfatal coronary events were also significantly reduced in the EPA group, but sudden death from cardiac causes and death from coronary causes were not. The reduction in events associated with EPA was similar in patients with and those without a history of coronary artery disease, but it was significant only in the former group, with a very low number needed to treat (19).<sup>68</sup> The JELIS study showed the efficacy of EPA in a population that did not have high cholesterol levels but did have other risk factors, including elevated triglyceride levels, low levels of high-density lipoprotein cholesterol, and impaired glucose tolerance. The lack of an effect on the incidence of sudden death probably reflects the high background n-3 fatty acid intake in the Japanese population.

The GISSI Heart Failure Study (GISSI-HF, NCT00336336)<sup>69</sup> was a randomized, double-blind, placebo-controlled trial testing whether fish oil could reduce morbidity and mortality in a large population of patients with symptomatic chronic heart failure (New York Heart Association class II, III, or IV) of any cause who were considered to be at high risk for sudden cardiac death and were receiving standard treatment. Patients were randomly assigned to receive n-3 fatty acids at a dose of 1 g per day (in 3494 patients) or placebo (in 3481 patients) and were followed for a median of 3.9 years. In a secondary analysis, patients were also randomly assigned, in an open-label design, to receive either rosuvastatin at a dose of 20 mg per day or placebo. The primary end points were the time to death and a composite of the time to death or admission to the hospital for cardiovascular reasons. A total of 955 patients in the fish-oil group died from any cause, as compared with 1014 patients in the placebo group (27% vs. 29%; adjusted hazard ratio, 0.91; 95.5% CI, 0.83 to 1.00;  $P=0.04$ ). A total of 1981 patients in the fish-oil group (57%) and 2053 patients in the placebo group (59%) died or were admitted to the hospital for cardiovascular reasons (adjusted hazard ratio, 0.92; 99% CI, 0.85 to 1.00;  $P=0.009$ ). On the basis of the results, 56 patients would need to be treated for a median of 3.9 years to avoid 1 death, and

44 would need to be treated to avoid 1 death or admission to the hospital for cardiovascular reasons.<sup>69</sup> Thus, the GISSI-HF trial confirmed the reduction in mortality seen in the GISSI-Prevenzione trial, although the extent of the reduction was smaller. However, no other pharmacologic agent tested in contemporary trials has been shown to reduce mortality among patients with symptomatic chronic heart failure. Indeed, the rosuvastatin-versus-placebo comparison in the GISSI-HF study did not show the efficacy of statin therapy.

The recent randomized, double-blind, multicenter Alpha Omega trial<sup>70</sup> included 4837 patients with previous myocardial infarction (median elapsed time, 3.7 years). The patients were 60 to 80 years of age, and 78% were men. They received one of four trial margarines: a margarine supplemented with a combination of EPA and DHA (with a targeted additional daily intake of 400 mg of EPA-DHA — a dose less than half that used in the GISSI-Prevenzione and GISSI-HF trials), a margarine supplemented with ALA (targeted additional daily intake of 2 g of ALA), a margarine supplemented with EPA-DHA and ALA, or a placebo margarine. The primary end point was the rate of major fatal and nonfatal cardiovascular events and cardiac interventions. Data were analyzed according to the intention-to-treat principle. In patients who received the EPA-DHA supplement, plasma levels of EPA and DHA increased (by much less than the levels obtained in the GISSI-Prevenzione trial). Levels of EPA, but not of DHA, also increased in the ALA group, suggesting some conversion of ALA to EPA. A major cardiovascular event occurred in 671 patients (13.9%) during the follow-up period. Neither EPA-DHA nor ALA reduced the primary end point (hazard ratio with EPA-DHA, 1.01; 95% CI, 0.87 to 1.17;  $P=0.93$ ; hazard ratio with ALA, 0.91; 95% CI, 0.78 to 1.05;  $P=0.20$ ). In a prespecified subgroup analysis involving female participants, ALA, as compared with placebo and EPA-DHA alone, was associated with a reduction in the rate of major cardiovascular events that did not achieve significance (hazard ratio, 0.73; 95% CI, 0.51 to 1.03;  $P=0.07$ ). The adverse-event rates did not differ significantly among the study groups.<sup>70</sup> The outcome of the EPA-DHA supplementation in this study is difficult to interpret because the supplementation dose was small and its effects might have been obscured by the larger amount of ALA administered in half the groups in the comparison. In this instance, the factorial de-

sign of the study was inappropriate for the two nonindependent study drugs tested. Furthermore, the study was underpowered to detect differences between each of the four study groups.

Other studies, each involving fewer than 600 patients, have addressed the use of n-3 fatty acids to prevent restenosis after coronary angioplasty, atrial fibrillation, or other clinical conditions, with mortality as the end point. The results are inconclusive, because these studies together accounted for less than 4% of all deaths reported in trials of n-3 fatty acids.<sup>71-80</sup>

#### PREVENTION OF ARRHYTHMIAS IN PATIENTS WITH IMPLANTABLE CARDIOVERTER-DEFIBRILLATORS

Since several studies showed that n-3 fatty acids reduce the rates of death from cardiac causes and sudden death, the hypothesis that n-3 fatty acids work largely by preventing life-threatening cardiac arrhythmias gained increasing attention.<sup>47,81,82</sup> Three double-blind, randomized, placebo-controlled intervention studies involving patients with implantable cardioverter-defibrillators investigated the direct effects of fish oil on ventricular tachyarrhythmias<sup>77,79,80</sup>; none convincingly showed that supplementation with n-3 polyunsaturated fatty acids prevented discharges from implantable cardioverter-defibrillators. However, a meta-analysis of these three trials (involving a total of 1148 patients)<sup>83</sup> suggested that patients with underlying coronary artery disease, in whom triggered ectopic beats and prolonged action potentials are predominant proarrhythmogenic mechanisms, might benefit, whereas patients with heart failure, in whom arrhythmias are mostly caused by reentry, might not. These studies were small, and the hypothesized relative risk reduction, 20% at best, would require several thousand patients in order to be conclusive.

Small, mostly uncontrolled studies have shown that fish oil may be useful in reducing the incidence of supraventricular arrhythmias, including atrial fibrillation.<sup>84</sup>

#### COMPARISONS OF EPA, DHA, AND ALA

The favorable effects of fish oils were originally attributed primarily to EPA. However, more DHA than EPA accumulates in the body, and DHA has broadly similar biologic effects. Only in the past 10 years have sufficient quantities of purified EPA or DHA become available for controlled trials, and these trials suggest some differential prop-

erties (see the Supplementary Appendix). Only one large trial<sup>66</sup> selectively administered EPA. Some studies have suggested that ALA may have biologic properties that are independent of its conversion to EPA and DHA,<sup>44,48,85</sup> although this is controversial.<sup>86</sup>

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#### BACKGROUND DIET AND RATIO OF N-6 TO N-3 FATTY ACIDS

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Western diets are low in n-3 fatty acids but have high amounts of n-6 fatty acids, such as those in poultry, meat, and most vegetable oils, as compared with early human diets.<sup>87</sup> Some investigators have speculated that larger amounts of n-6 fatty acids, such as those in today's Western diets, may promote many diseases of modern life, including cardiovascular disease. Therefore, it has been hypothesized that lowering the intake of n-6 polyunsaturated fatty acids (decreasing the ratio of n-6 to n-3 fatty acids, no matter how it is defined) would have favorable effects.<sup>88</sup> This idea has been disputed, since epidemiologic studies in Western populations have generally shown that the intake of n-6 fatty acids has a favorable inverse relationship with morbidity and mortality from cardiovascular causes,<sup>86,89</sup> although the effect is weaker than that seen with n-3 fatty acids. Small studies involving varying intakes of dietary polyunsaturated fatty acids (mostly n-6 polyunsaturated fatty acids) showed that the intake paralleled reciprocal changes in saturated fatty acids.<sup>90</sup> Randomized, controlled trials have not provided clear evidence that dietary intake of foods rich in pure n-6 fatty acids reduces the risk of coronary heart disease.<sup>91</sup> Data from randomized, controlled trials are lacking to test the hypothesis that a selective lowering of the intake of n-6 fatty acids, without altering the intake of saturated or n-3 fatty acids, will be beneficial.

Much less controversial is the possibility that, given the existence of quite different background n-3 fatty acid intakes, one can expect varying outcomes from studies involving different populations that received similar amounts of n-3 fatty acids. For example, studies of an increased intake of n-3 fatty acids showed an association with a reduction in the risk of sudden death in Western populations,<sup>29</sup> but this has not been seen in the Japanese population,<sup>66</sup> which has a much higher dietary intake of fish and a very low baseline rate of sudden death.

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#### FISH CONTAMINANTS AND COUNTERBALANCED EFFECTS OF N-3 FATTY ACIDS

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Although contaminants in pharmaceutical preparations of fish oil can be controlled easily, this is not true of contaminants in seafood. The presence of such contaminants, especially mercury, has direct implications for dietary recommendations at the population level.<sup>92</sup> If long-term mercury exposure were to increase cardiovascular risk, the relevant question for recommendations regarding fish consumption would be the balance of the relative harm and benefits. Most current epidemiologic evidence suggests that the benefits of fish consumption outweigh the harm.<sup>93</sup>

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#### CONCLUSIONS

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On the basis of currently available evidence, the American Heart Association (AHA) has recommended<sup>94</sup> that all adults eat fish (particularly fatty fish) at least twice a week, as well as vegetables containing plant-derived n-3 fatty acids (ALA). The AHA also suggests that patients with documented coronary heart disease consume approximately 1 g of EPA and DHA (combined) per day, from oily fish or fish-oil capsules (after consultation with a physician). The AHA recommendations also state that EPA-DHA supplements may be useful in patients with severe hypertriglyceridemia (>500 mg of triglycerides per deciliter [5.6 mmol per liter]), for whom effective doses are higher: 2 to 4 g of EPA-DHA per day to lower triglyceride levels by 20% to 40%. The AHA advises caution with respect to contaminants and notes that many species of fish are low in methylmercury<sup>95</sup> and that fish-oil supplements are free of methylmercury.

The n-3 fatty acids continue to attract interest as a possible addition to available lifestyle measures and medications for the prevention of cardiovascular disease, but important gaps in knowledge remain. Data are lacking from clinical and mechanistic studies of the putative benefits of n-3 fatty acids for both primary and secondary prevention.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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## REFERENCES

- Leaf A, Weber PC. Cardiovascular effects of n-3 fatty acids. *N Engl J Med* 1988;318:549-57.
- Bang HO, Dyerberg J, Nielsen AB. Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. *Lancet* 1971;1:1143-5.
- Dyerberg J, Bang HO, Aagaard O. Alpha-linolenic acid and eicosapentaenoic acid. *Lancet* 1980;1:199.
- Kromann N, Green A. Epidemiological studies in the Upernavik district, Greenland: incidence of some chronic diseases 1950-1974. *Acta Med Scand* 1980;208:401-6.
- Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* 1978;2:117-9.
- Burr G, Burr M. A new deficiency disease produced by rigid exclusion of fat from diet. *J Biol Chem* 1929;82:345-67.
- Idem*. On the nature and role of the fatty acids essential in nutrition. *J Biol Chem* 1930;86:587-621.
- Faulkner WJ, Flint LM Jr. Essential fatty acid deficiency associated with total parenteral nutrition. *Surg Gynecol Obstet* 1977;144:665-7.
- Department of Health, Committee on Medical Aspect of Food Policy. Nutritional aspects of cardiovascular disease: report of the Cardiovascular Review Group. London: The Stationery Office, 1994.
- Fats and fatty acids in human nutrition: proceedings of the Joint FAO/WHO Expert Consultation: November 10-14, 2008: Geneva, Switzerland. *Ann Nutr Metab* 2009;55:5-300.
- Hussein N, Ah-Sing E, Wilkinson P, Leach C, Griffin BA, Millward DJ. Long-chain conversion of [<sup>13</sup>C]linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men. *J Lipid Res* 2005;46:269-80.
- Sprecher H. Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochim Biophys Acta* 2000;1486:219-31.
- Kang ZB, Ge Y, Chen Z, et al. Adenoviral gene transfer of Caenorhabditis elegans n-3 fatty acid desaturase optimizes fatty acid composition in mammalian cells. *Proc Natl Acad Sci U S A* 2001;98:4050-4.
- Lai L, Kang JX, Li R, et al. Generation of cloned transgenic pigs rich in omega-3 fatty acids. *Nat Biotechnol* 2006;24:435-6.
- Wada M, DeLong CJ, Hong YH, et al. Enzymes and receptors of prostaglandin pathways with arachidonic acid-derived versus eicosapentaenoic acid-derived substrates and products. *J Biol Chem* 2007;282:22254-66.
- Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* 2008;8:349-61.
- Serhan CN, Yang R, Martinod K, et al. Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med* 2009;206:15-23.
- Woodcock SR, Marwitz AJ, Bruno P, Branchaud BP. Synthesis of nitrolipids: all four possible diastereomers of nitrooleic acids: (E)- and (Z)-, 9- and 10-nitro-octadec-9-enoic acids. *Org Lett* 2006;8:3931-4.
- Leaf A, Xiao YF, Kang JX, Billman GE. Membrane effects of the n-3 fish oil fatty acids, which prevent fatal ventricular arrhythmias. *J Membr Biol* 2005;206:129-39.
- De Caterina R, Madonna R, Zucchi R, La Rovere MT. Antiarrhythmic effects of omega-3 fatty acids: from epidemiology to bedside. *Am Heart J* 2003;146:420-30. [Errata, *Am Heart J* 2004;147:874, IN1.]
- De Caterina R, Massaro M. Omega-3 fatty acids and the regulation of expression of endothelial pro-atherogenic and pro-inflammatory genes. *J Membr Biol* 2005;206:103-16.
- Massaro M, Habib A, Lubrano L, et al. The omega-3 fatty acid docosahexaenoate attenuates endothelial cyclooxygenase-2 induction through both NADP(H) oxidase and PKCepsilon inhibition. *Proc Natl Acad Sci U S A* 2006;103:15184-9.
- Ma DW, Seo J, Davidson LA, et al. n-3 PUFA alter caveolae lipid composition and resident protein localization in mouse colon. *FASEB J* 2004;18:1040-2.
- Li Q, Wang M, Tan L, et al. Docosahexaenoic acid changes lipid composition and interleukin-2 receptor signaling in membrane rafts. *J Lipid Res* 2005;46:1904-13.
- Massaro M, Basta G, Lazzarini G, et al. Quenching of intracellular ROS generation as a mechanism for oleate-induced reduction of endothelial activation and early atherogenesis. *Thromb Haemost* 2002;88:335-44.
- Oh DY, Talukdar S, Bae EJ, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 2010;142:687-98.
- von Schacky C. A review of omega-3 ethyl esters for cardiovascular prevention and treatment of increased blood triglyceride levels. *Vasc Health Risk Manag* 2006;2:251-62.
- Sadovsky R, Kris-Etherton P. Prescription omega-3 acid ethyl esters for the treatment of very high triglycerides. *Postgrad Med* 2009;121:145-53.
- Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999;354:447-55. [Errata, *Lancet* 2001;357:642, 2007;369:106.]
- Endres S, Ghorbani R, Kelley VE, et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 1989;320:265-71.
- Calder PC. Immunomodulation by omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2007;77:327-35.
- Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ. Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials. *J Hypertens* 2002;20:1493-9.
- Mozaffarian D. Fish, n-3 fatty acids, and cardiovascular haemodynamics. *J Cardiovasc Med (Hagerstown)* 2007;8:Suppl 1:S23-S26.
- Christensen JH, Skou HA, Fog L, et al. Marine n-3 fatty acids, wine intake, and heart rate variability in patients referred for coronary angiography. *Circulation* 2001;103:51-7.
- Radaelli A, Cazzaniga M, Viola A, et al. Enhanced baroreceptor control of the cardiovascular system by polyunsaturated fatty acids in heart failure patients. *J Am Coll Cardiol* 2006;48:1600-6.
- De Caterina R, Madonna R, Bertolotto A, Schmidt EB. n-3 Fatty acids in the treatment of diabetic patients: biological rationale and clinical data. *Diabetes Care* 2007;30:1012-26.
- Kristensen SD, Iversen AM, Schmidt EB. n-3 Polyunsaturated fatty acids and coronary thrombosis. *Lipids* 2001;36:Suppl:S79-S82.
- Lev EI, Solodky A, Harel N, et al. Treatment of aspirin-resistant patients with omega-3 fatty acids versus aspirin dose escalation. *J Am Coll Cardiol* 2010;55:114-21.
- DeCaterina R, Giannessi D, Mazzone A, et al. Vascular prostacyclin is increased in patients ingesting omega-3 polyunsaturated fatty acids before coronary artery bypass graft surgery. *Circulation* 1990;82:428-38.
- Harris WS. Expert opinion: omega-3 fatty acids and bleeding — cause for concern? *Am J Cardiol* 2007;99:44C-46C.
- Goodfellow J, Bellamy MF, Ramsey MW, Jones CJ, Lewis MJ. Dietary supplementation with marine omega-3 fatty acids improve systemic large artery endothelial function in subjects with hypercholesterolemia. *J Am Coll Cardiol* 2000;35:265-70.
- Thies F, Garry JM, Yaqoob P, et al. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003;361:477-85.
- Zampolli A, Falk E, De Caterina R. Anti-atherogenic effects of omega-3 fatty acids. In: De Caterina R, Libby P, eds. Endothelial dysfunctions and vascular disease. Oxford, England: Blackwell Publishing, 2007:286-99.
- Murnaghan MF. Effect of fatty acids on the ventricular arrhythmia threshold in the isolated heart of the rabbit. *Br J Pharmacol* 1981;73:909-15.

45. McLennan PL, Abeywardena MY, Charnock JS. Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion. *Am Heart J* 1988;116:709-17.
46. McLennan PL, Bridle TM, Abeywardena MY, Charnock JS. Dietary lipid modulation of ventricular fibrillation threshold in the marmoset monkey. *Am Heart J* 1992;123:1555-61.
47. Billman GE, Kang JX, Leaf A. Prevention of ischemia-induced cardiac sudden death by n-3 polyunsaturated fatty acids in dogs. *Lipids* 1997;32:1161-8.
48. *Idem*. Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs. *Circulation* 1999;99:2452-7.
49. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 2002;106:2747-57.
50. Calder PC. n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin Sci (Lond)* 2004;107:1-11.
51. Sinclair HM. The diet of Canadian Indians and Eskimos. *Proc Nutr Soc* 1953;12:69-82.
52. Bang HO, Dyerberg J, Hj orne N. The composition of food consumed by Greenland Eskimos. *Acta Med Scand* 1976;200:69-73.
53. Bjerregaard P, Dyerberg J. Mortality from ischaemic heart disease and cerebrovascular disease in Greenland. *Int J Epidemiol* 1988;17:514-9.
54. Newman WP, Middaugh JP, Propst MT, Rogers DR. Atherosclerosis in Alaska natives and non-natives. *Lancet* 1993;341:1056-7.
55. Yano K, MacLean CJ, Reed DM, et al. A comparison of the 12-year mortality and predictive factors of coronary heart disease among Japanese men in Japan and Hawaii. *Am J Epidemiol* 1988;127:476-87.
56. von Schacky C. Omega-3 fatty acids and cardiovascular disease. *Curr Opin Clin Nutr Metab Care* 2004;7:131-6.
57. *Idem*. Omega-3 fatty acids and cardiovascular disease. *Curr Opin Clin Nutr Metab Care* 2007;10:129-35.
58. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA* 1995;274:1363-7.
59. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002;346:1113-8.
60. Pottala JV, Garg S, Cohen BE, Woolley MA, Harris WS. Blood eicosapentaenoic and docosahexaenoic acids predict all-cause mortality in patients with stable coronary heart disease: the Heart and Soul study. *Circ Cardiovasc Qual Outcomes* 2010;3:406-12.
61. Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and Reinfarction Trial (DART). *Lancet* 1989;2:757-61.
62. Burr ML, Sweetham PM, Fehily AM. Diet and reinfarction. *Eur Heart J* 1994;15:1152-3.
63. Marchioli R, Barzi F, Bomba E, et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* 2002;105:1897-903.
64. Burr ML, Ashfield-Watt PA, Dunstan FD, et al. Lack of benefit of dietary advice to men with angina: results of a controlled trial. *Eur J Clin Nutr* 2003;57:193-200.
65. Ness AR, Ashfield-Watt PA, Whiting JM, Smith GD, Hughes J, Burr ML. The long-term effect of dietary advice on the diet of men with angina: the diet and angina randomized trial. *J Hum Nutr Diet* 2004;17:117-9.
66. Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 2007;369:1090-8.
67. Tanaka K, Ishikawa Y, Yokoyama M, et al. Reduction in the recurrence of stroke by eicosapentaenoic acid for hypercholesterolemic patients: subanalysis of the JELIS trial. *Stroke* 2008;39:2052-8. [Erratum, *Stroke* 2008;39(9):e149.]
68. Matsuzaki M, Yokoyama M, Saito Y, et al. Incremental effects of eicosapentaenoic acid on cardiovascular events in statin-treated patients with coronary artery disease. *Circ J* 2009;73:1283-90.
69. Tavazzi L, Maggioni AP, Marchioli R, et al. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;372:1223-30.
70. Kromhout D, Giltay EJ, Geleijnse JM. n-3 Fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med* 2010;363:2015-26.
71. Nielsen GL, Faarvang KL, Thomsen BS, et al. The effects of dietary supplementation with n-3 polyunsaturated fatty acids in patients with rheumatoid arthritis: a randomized, double blind trial. *Eur J Clin Invest* 1992;22:687-91.
72. Sacks FM, Stone PH, Gibson CM, Silverman DI, Rosner B, Pasternak RC. Controlled trial of fish oil for regression of human coronary atherosclerosis. *J Am Coll Cardiol* 1995;25:1492-8.
73. Leng GC, Lee AJ, Fowkes FG, et al. Randomized controlled trial of gamma-linolenic acid and eicosapentaenoic acid in peripheral arterial disease. *Clin Nutr* 1998;17:265-71.
74. von Schacky C, Angerer P, Kothny W, Theisen K, Mudra H. The effect of dietary omega-3 fatty acids on coronary atherosclerosis: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1999;130:554-62.
75. Johansen O, Brekke M, Seljeflot I, Abdelnoor M, Arnesen H. N-3 fatty acids do not prevent restenosis after coronary angioplasty: results from the CART study: Coronary Angioplasty Restenosis Trial. *J Am Coll Cardiol* 1999;33:1619-26.
76. Durrington PN, Bhatnagar D, Mackness MI, et al. An omega-3 polyunsaturated fatty acid concentrate administered for one year decreased triglycerides in simvastatin treated patients with coronary heart disease and persisting hypertriglyceridaemia. *Heart* 2001;85:544-8.
77. Leaf A, Albert CM, Josephson M, et al. Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. *Circulation* 2005;112:2762-8.
78. Cal  L, Bianconi L, Colivicchi F, et al. N-3 fatty acids for the prevention of atrial fibrillation after coronary artery bypass surgery: a randomized, controlled trial. *J Am Coll Cardiol* 2005;45:1723-8.
79. Raitt MH, Connor WE, Morris C, et al. Fish oil supplementation and risk of ventricular tachycardia and ventricular fibrillation in patients with implantable defibrillators: a randomized controlled trial. *JAMA* 2005;293:2884-91.
80. Brouwer IA, Zock PL, Camm AJ, et al. Effect of fish oil on ventricular tachyarrhythmia and death in patients with implantable cardioverter defibrillators: the Study on Omega-3 Fatty Acids and Ventricular Arrhythmia (SOFA) randomized trial. *JAMA* 2006;295:2613-9.
81. McLennan PL. Myocardial membrane fatty acids and the antiarrhythmic actions of dietary fish oil in animal models. *Lipids* 2001;36:Suppl:S111-S114.
82. Kang JX, Leaf A. Effects of long-chain polyunsaturated fatty acids on the contraction of neonatal rat cardiac myocytes. *Proc Natl Acad Sci U S A* 1994;91:9886-90.
83. Brouwer IA, Raitt MH, Dullemeijer C, et al. Effect of fish oil on ventricular tachyarrhythmia in three studies in patients with implantable cardioverter defibrillators. *Eur Heart J* 2009;30:820-6.
84. Savelieva I, Kourliouros A, Camm J. Primary and secondary prevention of atrial fibrillation with statins and polyunsaturated fatty acids: review of evidence and clinical relevance. *Naunyn Schmiedeberg's Arch Pharmacol* 2010;381:1-13. [Erratum, *Naunyn Schmiedeberg's Arch Pharmacol* 2010;381:383.]
85. De Caterina R, Bernini W, Carluccio MA, Liao JK, Libby P. Structural requirements for inhibition of cytokine-induced endothelial activation by unsaturated fatty acids. *J Lipid Res* 1998;39:1062-70.
86. Skeaff CM, Miller J. Dietary fat and coronary heart disease: summary of evidence from prospective cohort and ran-

- domised controlled trials. *Ann Nutr Metab* 2009;55:173-201.
87. Eaton SB, Eaton SB III, Sinclair AJ, Cordain L, Mann NJ. Dietary intake of long-chain polyunsaturated fatty acids during the paleolithic. *World Rev Nutr Diet* 1998; 83:12-23.
88. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)* 2008;233:674-88.
89. Harris WS, Mozaffarian D, Rimm E, et al. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism, Council on Cardiovascular Nursing, and Council on Epidemiology and Prevention. *Circulation* 2009;119:902-7.
90. Jakobsen MU, O'Reilly EJ, Heitmann BL, et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *Am J Clin Nutr* 2009; 89:1425-32.
91. Ramsden CE, Hibbeln JR, Majchrzak SF, Davis JM. n-6 Fatty acid-specific and mixed polyunsaturate dietary interventions have different effects on CHD risk: a meta-analysis of randomised controlled trials. *Br J Nutr* 2010;104:1586-600.
92. Mozaffarian D. Fish, mercury, selenium and cardiovascular risk: current evidence and unanswered questions. *Int J Environ Res Public Health* 2009;6:1894-916.
93. König A, Bouzan C, Cohen JT, et al. A quantitative analysis of fish consumption and coronary heart disease mortality. *Am J Prev Med* 2005;29:335-46.
94. Kris-Etherton PM, Harris WS, Appel LJ. Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. *Arterioscler Thromb Vasc Biol* 2003;23:151-2.
95. Bolger PM, Schwetz BA. Mercury and health. *N Engl J Med* 2002;347:1735-6.

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