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Abstract

There is substantial evidence that polyunsaturated fatty acids (PUFAs) such as n-3 and n-6 fatty acids (FAs) play an important role in prevention of atherosclerosis. In vitro and in vivo studies focusing on the interactions between monocytes and endothelial cells have explored the molecular effects of FAs on these interactions. Epidemiological surveys, followed by large, randomized, control trials have demonstrated a reduction in major cardiovascular events with supplementation of n-3 FAs in secondary prevention settings. The evidence of beneficial effects specific to patients with peripheral artery disease (PAD) remains elusive, and is the focus of this review.

Keywords

lipids; nutrition; peripheral arterial disease; peripheral vascular diseases; review

Introduction

Peripheral artery disease (PAD) is a condition with significant impact on the health of our society. Population-based studies suggest that PAD affects more than 12% of people aged > 65 years, and more than 20% of those aged > 75 years.[1] A more recent study suggests that in a primary care population, nearly one-third of patients > 70 years suffer from PAD.[2] Overall, the public is poorly informed about PAD, with major knowledge gaps as to the definition of PAD, the risk factors and symptoms of the disease, as well as the associated risks of amputation or mortality.[3] In the International Reduction of Atherothrombosis for Continued Health (REACH) Registry, PAD treatment was associated with higher annual mean medication and hospitalization costs than coronary artery disease (CAD) or cerebrovascular disease (CVD).[4] The need for cost-effective therapies to prevent and treat PAD is great.

Nutritional intake of n-3 fatty acids (FAs) has long been recognized to correlate with cardiovascular health. Greenland Eskimos who consume a diet rich in whale, seal, and fish have a very low incidence of CAD,[5] pointing toward the ability of n-3 FAs, a compound found in fish and fish oils, to reduce cardiovascular risk.[5,6] In fact, n-3 FAs have been considered to act as natural HMG-CoA reductase and ACE enzyme inhibitors, anti-arrhythmic, anti-hypertensive, anti-atherosclerotic, anti-inflammatory, cytoprotective, and cardioprotective molecules.[7] Although several randomized trials and meta-analyses have demonstrated beneficial effects in CAD, this type of evidence remains sparse for PAD. The importance and timeliness of this clinical research question was highlighted by a recent editorial.[8] In this review, we summarize the cellular, physiological, and clinical evidence reporting possible associations of n-3 FAs and PAD, and discuss the controversy surrounding the dietary intake of n-6 FAs.

Historical perspectives

Human diet has changed substantially over the past 10,000 years, particularly in Western countries. Important societal changes that began with the development of agriculture and animal husbandry have been followed more recently

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by the industrial revolution, agribusiness, and modern food-processing techniques. Hypotheses abound that these profound nutritional changes in food quality have led to many of the diseases of our Western civilization, including atherosclerosis (CAD, CVD, PAD), diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), colon cancer, essential hypertension, obesity, diverticulosis, and dental caries.[9–11] These conditions have appeared in the last 100 years and are still unknown in some of the remaining hunter-gatherer populations whose lifestyles reflect those of the pre-agricultural human beings.[10] These hypotheses are balanced, however, by the fact that the life expectancy of our population has increased.

Compared to the Paleolithic period, our Western diet is characterized by (1) an increase in energy intake and decrease in energy expenditure; (2) a decrease in protein, antioxidants, and calcium intake; (3) a decrease in complex carbohydrates and fibers; (4) an increase in processed cereal grains and a decrease in fruits and vegetables; (5) an increase in saturated fat (SFA), *trans* fat and n-6 FAs; and (6) a decrease in n-3 FAs.[12] Two main technological processes have led to the changes in FA consumption: animal husbandry and agri-business, practices of processing cheap and altered food stocks. In addition, the increase in grain harvests and the subsequent feeding of grain (primarily corn) to cattle have led to 'marbled meat' made up of excessive triglyceride fat accumulation in muscle interfascicular adipocytes, which contain an increase in SFA content, more n-6 FAs, and less n-3 FAs.[13] Furthermore, with the advent of the oil-seed processing industry ca. 1950, there was a significant increase in the total intake of vegetable cooking oil high in n-6 FAs.

Overall, these societal and environmental changes have directly increased the dietary level of n-6 FAs at the expense of lowered levels of n-3 FAs, leading to n-6:n-3 dietary ratios close to 15:1, compared with hunter-gatherer diets, which were estimated to be around 2:1.[13,14] The total intake of n-3 FAs in the US is 1.6 g/day (0.7% of energy intake).[14] Of this, α-linolenic acid (ALA) accounts for 1.4 g/day and only 0.1–0.2 g/day comes from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The conversion of ALA to EPA and DHA, the more biologically active molecules, remains low (0.2–15%).[15,16] The average US intake of linoleic acid (LA) is 14.8 g/day,[17] while arachidonic acid (AA) is consumed at the amount of 0.15 g/day. The conversion of LA to AA is ~0.2%.[18] It is likely that the higher ratio of n-6:n-3 impacts processes in atherogenesis at the molecular, physiological, and clinical levels. These concepts are reviewed in the next sections.

Molecular and biological responses

FAs are part of the larger group of lipids including fats, waxes, sterols, fat-soluble vitamins, phospholipids, and others. Lipids are generally classified as fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides.[19] Lipids play an important role in energy storage, structure of the cell membrane, and signaling pathways. With regards to FAs, animal fats are stored in the body as triglycerides, compounds in which three FA molecules (the acyl group) are linked to a glycerol molecule by an ester bond. There are three main categories of FAs: (1) the SFAs, (2) the mono-unsaturated fatty acids (MUFAs), and (3) the poly-unsaturated fatty acids (PUFAs) (Figure 1). The PUFAs (all of which are essential FAs) are further subdivided into n-6 PUFAs and n-3 PUFAs. They are referred to as n-3 FAs and n-6 FAs in this article.

The names of PUFAs are derived from their molecular structure, with the n-3 FA family having the first double-bond on the third carbon, counting from the terminal methyl end of the FA, and the n-6 FA family having the first double-bond on the sixth carbon, counting from the terminal methyl end of the FA. These differences give these FAs different biochemical properties (Table 1). The chemical nomenclature describes the number of carbon atoms and the number of double-bounds in the carbon chain. For example, a C18:3 PUFA has 18 carbon atoms and three double-bonds in the carbon chain.

The most prevalent n-3 FA is ALA, an n-3 FA (C18:3) that is found in large quantities in flaxseed oil (about 55%) and to lesser extents in canola (about 10%) and unhydrogenated soybean oil (about 7%). A very small fraction (< 5%, perhaps < 1%) of ingested ALA is converted into EPA (20:5), one of the long-chain n-3 FAs, and even less is converted into the 22-carbon DHA. EPA and DHA are found in fish oil, which is derived from the tissues and gut of oily fish (as compared to whitefish, which contain oil mostly in the liver). Fish, like other animals, do not manufacture EPA and DHA; they accumulate these molecules from feeding on plant species such as microalgae. Hence, n-3 FAs can be considered as 'phyto oils' which are simply harvested from fish.

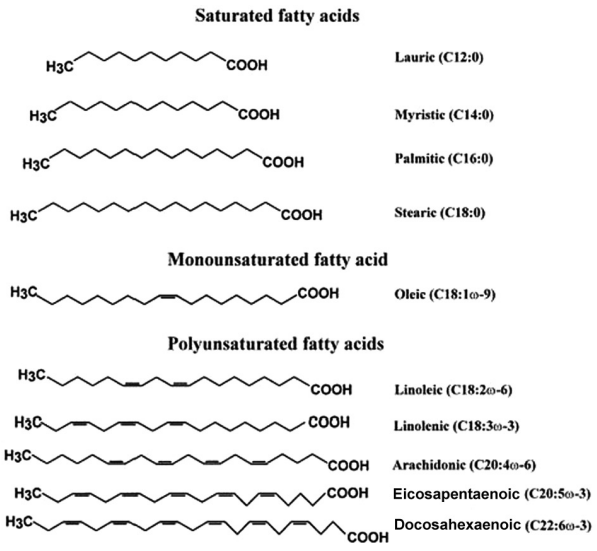

Figure 1. Chemical structure of different fatty acids including saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids.

Table 1. Differences between n-3 and n-6 fatty acids

	n-6 Fatty acids	n-3 Fatty acids
Molecular structure	First double-bond on the 6th carbon counting from the methyl end (the 'n'th carbon)	First double-bond on the 3rd carbon counting from the methyl end (the 'n'th carbon)
Types	Linoleic acid (LA) [C18:2] Arachidonic acid (AA) [C20:4]	α-Linolenic acid (ALA) [C18:3] Eicosapentaenoic acid (EPA) [C20:5] Docosahexaenoic acid (DHA) [C22:6]
Food sources	Corn oil (LA) Soybean oil (LA) Safflower oil (LA) Sunflower oil (LA) Poultry (AA) Meats (AA)	Flaxseed oil (ALA) Canola oil (ALA) Soybean oil (ALA) Oily fish (EPA/DHA) Fish oil capsules (EPA/DHA)
Examples of end mediators	Lipoxins Aspirin-triggered lipoxins Thromboxanes Prostacyclins Leukotrienes	Resolvins type D Resolvins type E Protectins

The biological effects of n-3 FAs are divided in three broad categories, recently reviewed by De Caterina:[20] direct effects on ion channels modulating arrhythmias, direct effects on membranes requiring incorporation into cell phospholipids, and effects mediated by the release of bioactive mediators. The latter two are discussed here.

Incorporation of n-3 FAs into the cell membranes leads to several molecular and cellular events. At the molecular level, several effects have been reported, including: (1) a reduction in production of cytokines (interleukin-1 (IL-1) and tumor necrosis factor (TNF) in lipopolysaccharide (LPS)-stimulated monocytes);[21] (2) a reduction in the production of platelet-derived growth factor (PDGF)-A and -B protein and mRNA;[22,23] (3) a decrease in tissue factor production by monocytes;[24] (4) an increase in the bioavailability of endothelial nitric oxide;[25] (5) down-regulation of gene expression of monocyte-chemoattractant protein-1 (MCP)-1,[26,27] a monocyte-chemoattractant protein; and (6) inhibition of IL-8.[27] Several of the anti-inflammatory effects of n-3 FAs seem to be at least partly mediated through peroxisome proliferator-activated receptor (PPAR)-alpha.[28] The nuclear factor (NF)-κB[27,28] system of transcription factors, which controls the coordinated expression of adhesion molecules and of leukocyte-specific chemoattractants upon cytokine stimulation,[29,30] also appears to be involved,[31] with a decrease in nuclear translocation of the NF-κB subunit p65.[32] Other nuclear factors are likely to be involved and are reviewed elsewhere in greater detail.[33,34] It also appears that n-3 FAs lead to a reduction in expression of endothelial adhesion molecules (vascular cell adhesion protein-1 (VCAM-1), E-selectin, and intercellular adhesion molecule-1 (ICAM-1)).[35–37] The consequences of such changes include a reduction in leukocyte adhesion to the endothelium, a critical early step in atherogenesis.[28,32,36] Recent studies have also suggested a decrease in the migration of neutrophils across the endothelium.[38] Several of their actions may be related to cell membrane phospholipid makeup and cell signaling.[39,40] Furthermore, it appears that n-3 FAs may modulate the cellular and structural composition of the atherosclerotic plaque, in a manner to reduce rupture or ulceration[41] and overall regression of the plaque.[42]

Related to the release of a bioactive mediator, and also at the level of the cell membrane, there is evidence that in the presence of n-3 FAs, prostaglandin (PG) D_3 replaces PGD_2,[38] leading to competitive inhibition at the COX-2 level. This likely leads to replacement of AA with the prostanoid derivatives of EPA, potentially less pro-thrombotic and vasoconstrictive than AA derivatives.[43] More recently, Serhan et al. discovered novel mechanisms in inflammation related to the 'resolution-phase interaction products' (resolvins) pathways.[44–46] This new paradigm describes the transition from acute to chronic inflammation as involving the loss of endogenously operative resolution processes. This response aims to re-establish homeostasis through resolution of an acute inflammatory response. Lipid autacoids such as n-3 and n-6 FAs are at the core of these responses.[47] As described by Stables and Gilroy, the classic tale of inflammation describes a process of resolution of inflammation that is passive, mediated by a decrease in the pro-inflammatory cytokines, prostaglandins, and oxygen species, with no active contribution of pro-resolving lipid mediators.[48] An alternative pathway for inflammation now describes resolution as an active process involving changes in the phenotypes of cells such as endothelial cells, macrophages, and monocytes leading to the production of lipid mediators such as resolvins, protectins, lipoxins, aspirin-triggered lipoxins, and newly identified maresins (Table 2). Hence, while cyclooxygenase (COX) and lipoxygenase (LOX)-derived lipid mediators such as the prostaglandins and leukotrienes promote inflammation,[49] anti-inflammatory and pro-resolving lipid mediators such as lipoxins and resolvins[45] are generated to actively turn off inflammation. Lipoxins and resolvins are thought to be bioactive products of n-3 and n-6 FAs.[44,50] Of interest, it was recently demonstrated that plasma levels of pro-resolving mediator 15-epimeric lipoxin are significantly

Table 2. New concepts on resolution of inflammation

	Classic view regarding inflammation	Alternative view regarding resolution of inflammation
Resolution is…	A passive process	An active process
Lipid mediators	No active participation	Active participation
Critical components	Decrease cytokines Decrease prostaglandins Decrease oxygen species	Resolvins Protectins Lipoxins Aspirin-triggered lipoxins Maresins

lower in patients with symptomatic PAD than in healthy volunteers, suggesting a 'resolution deficit' in PAD.[51]

Physiological studies

The clinically relevant benefits of fish-oil and n-3 supplementation (discussed below) are thought to be at least partially due to improvement in endothelial function and reduction in inflammation. Supporting evidence of this proposition includes an improvement in endothelial function in healthy volunteers (both acute and chronic administration of n-3 FAs; response measured with flow-mediated brachial artery vasodilation (FMD), laser Doppler imaging, or strain-gauge phlethysmography),[52–54] in obese adolescents (3-month treatment with n-3 FAs; response measured with peripheral artery tonometry),[55] and in patients with chronic heart failure (6-week treatment with n-3 FAs; response measured by venous occlusion strain-gauge plethysmography).[56] Furthermore, a reduction in inflammation as measured by C-reactive protein (CRP) was found in healthy individuals,[57–63] male smokers,[64] obese adolescents,[55] and in patients with rheumatoid arthritis[65] or CAD.[66] However, these benefits are more controversial in the elderly population. One prospective study conducted in a nursing home population, where a diet enriched with mackerel was given to the patients, increased FMD compared with a control group.[67] Conversely, a study assessing the postprandial response to a single fish oil-enriched meal in older versus younger men found no improvement in FMD in the older men and a significant increase in FMD in younger men.[68] Clinically, n-3 FAs reduce serum triglycerides at pharmacological doses (typically 3 g of EPA/DHA).[69–71]

Clinical evidence

Trials in coronary artery disease

Studies report that n-3 FAs derived from fish oil reduce cardiovascular disease,[6,72] with a high tissue ratio of n-3:n-6 FAs leading to a reduced risk of coronary events.[73] Greenland Eskimos, who consume a diet very high in n-3 FAs, have a very low incidence of CAD.[5] Several different clinical trials have investigated the effects of fish consumption or fish oil supplementation for the prevention of cardiovascular events. Patients from three large trials, the Diet and Reinfarction trial (DART trial), the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico trial (GISSI-Prevenzione trial), and the Japan EPA Lipid Intervention Study (JELIS trial) account for 95% of individuals demonstrating an overall decrease in total mortality, decrease in cardiovascular death, decrease in sudden cardiac death, and a reduction in non-fatal cardiovascular events.[74–76] These trials are described in great detail in several well-written reviews and meta-analyses.[20,77–84] The omega-3 index, used in some CAD trials, is defined as the percentage of EPA + DHA in red blood cells (RBCs) and reflects FA dietary intake. It has been proposed as a marker and risk factor for CAD, especially sudden cardiac death.[85–89] To the authors' knowledge, this marker has not been correlated thus far with PAD.

Marik and Varon, in their meta-analysis on the beneficial cardiovascular effects of n-3 FAs, identified 11 prospective, randomized, placebo-controlled clinical trials evaluating clinical cardiovascular endpoints.[77] These studies included 39,044 patients with recent myocardial infarction (MI), with an implanted cardioverter defibrillator, patients with heart failure (HF), PAD, and hypercholesterolemia. The main dose used was 1.8 g/day for 2.2 ± 1.2 years. The authors reported an overall reduction in the risk of cardiovascular death, sudden cardiac death (SCD), all-cause mortality, and non-fatal cardiovascular events. The authors concluded that dietary supplementation with n-3 FAs should be considered in the secondary prevention of cardiovascular events.

It is worth mentioning that the OMEGA trial, not included in this most recent meta-analysis, became the first randomized study to assess the effects of highly purified n-3 acid ethyl ester at 1 g/day for 1 year (460 mg EPA, 380 mg DHA), in addition to the current guideline therapy. It was a double-blinded, multicenter trial that randomized a total of 3851 patients.[90] The primary endpoint was SCD and the secondary endpoints included total mortality and non-fatal events. There were no significant differences between the two groups in both the primary or secondary endpoints. Although the findings were unexpected in view of the results from other large trials, it is possible that with current therapies including statins, anti-platelets, angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, aspirin with or without a second anti-platelet drug if indicated, and beta-blocker, the effects of fish oil have less of an impact. However, a more likely explanation is that the study was underpowered (with the initial power calculations based on the GISSI-Prevenzione trial – a different era with regard to CAD medical therapy), the dose was too low to see a real effect, and fish consumption post-MI increased from 30% to 45%, which could have blunted a response difference.

Also notable was the 2007 publication of the results of the JELIS trial.[76] This study used a prospective, randomized open-label, blinded endpoint evaluation and recruited 18,645 patients with total cholesterol of 6.5 mmol/l or greater between 1996 and 1999 (both a primary and secondary prevention trial). The patients were randomized to either 1800 mg of EPA daily with statin ($n = 9326$) or statin alone ($n = 9319$), with a 5-year follow-up. Analysis was done in an intention-to-treat fashion, with the primary endpoint being any major coronary event (sudden cardiac death, fatal and non-fatal MI, and other non-fatal events including unstable angina, angioplasty, stenting or coronary artery bypass grafting). At 4.6 years, there was a 19% relative reduction in major coronary events ($p = 0.011$), with the primary endpoint occurring in 2.8% of the patients in the EPA group and 3.5% of patients in the control group. Serum low-density lipoprotein (LDL) cholesterol was not a significant factor in a reduction of risk for major coronary events. The EPA group had significantly reduced unstable angina and non-fatal coronary events, but no change was seen in sudden cardiac death and coronary death. In the secondary prevention subgroup (patients with a history of CAD), major coronary events were reduced by 19% ($p = 0.048$). In the primary prevention subgroup, EPA reduced major coronary events by 18% but this was not significant. More interestingly, in a later sub-study analysis of the JELIS trial, n-3 supplementation was associated with a 55% reduction in major coronary events in the PAD subgroup, compared with an 18% relative reduction in the patients without PAD, corresponding to a numbers needed to treat (NNT) of 11 and pointing to a significant benefit to the PAD population of EPA supplementation (hazard ratio (95% confidence interval (CI)): 0.44 (0.19–0.97); $p = 0.041$).[91] However, the JELIS trial was an open-label trial that was not focused on PAD, and was done in a Japanese population that eats a significant amount of fish at baseline. A related editorial entitled 'Eicosapentaenoic acid as the gold standard for patients with peripheral artery disease?' summarized some of these major questions.[8] The evidence from studies focused on PAD is described below.

Trials in peripheral artery disease

Although there is some evidence suggesting that consumption of an n-3 FA diet may be associated with a decreased prevalence of PAD,[92] the effects of n-3 FAs on PAD have not been as thoroughly investigated as their effects in the CAD patient population. In a cross-sectional study using the National Health and Nutrition Examination Survey, Lane et al. demonstrated that better nutrition (particularly a higher consumption of n-3 FAs) was associated with a reduced prevalence of PAD in the US population, irrespective of traditional cardiovascular risk factors.[92] Using patients with PAD sampled from the Edinburgh Artery Study, Leng et al. attempted to determine the levels of plasma FAs in patients with PAD versus control subjects.[93] In 113 cases and 122 control subjects, FA levels were measured in three plasma fractions (triglyceride, cholesteryl ester, and phospholipid), and smoking habits and dietary antioxidant intake were determined by questionnaire. AA, EPA, DHA, and docosapentaenoic acid (DPA; 20:5; n-3) were significantly lower in cases than in controls ($p < 0.01$). By logistic regression adjustment for smoking and vitamin C intake, DPA (odds ratio (95% CI): 0.19 (0.08–0.56); $p < 0.01$) and AA (odds ratio: 0.44 (0.19–0.98); $p < 0.05$) remained significantly related to the presence of disease. Only DPA reduced the risk associated with smoking. The authors concluded that in subjects with PAD compared with healthy controls, the largest benefits occurred in FAs of the n-3 series, particularly DPA.

Several smaller prospective studies have aimed to assess the effects of fish oil and n-3 FAs on different parameters related to PAD. They are summarized in Table 3. In a double-blinded, randomized, placebo-controlled study, Woodcock et al. assessed the effects of 1.8 g/day of n-3 FAs for 7 weeks in 19 patients with intermittent claudication (IC). They assessed walking distance, Doppler ultrasound, ankle–brachial index (ABI), platelets, blood viscosity, cholesterol, and triglycerides. The authors demonstrated an overall decrease in blood viscosity and serum triglycerides, but no significant changes in other parameters.[94]

Gans et al., in a double-blinded, randomized fashion, also studied the effects of fish oil supplementation in patients with IC. Thirty-two patients were randomized to 3 g/day of n-3 FAs versus placebo for 4 months. The authors were interested in outcomes including blood pressure, ABI, pain-free and maximal walking distance, blood and plasma viscosity, cholesterol and triglycerides. They demonstrated a decrease in blood viscosity, improvement in lipid profile, but no changes in walking distance or ABIs.[95]

Mori et al. performed a study of 32 patients with symptomatic and angiographically demonstrated PAD.[96] The randomized, controlled, double-blinded trial provided 4 g/day of n-3 FAs (EPA and DHA) or placebo for 4 weeks. Endpoints of interest included cholesterol, triglycerides, and platelet count. Fish oil treatment reduced serum triglyceride levels, but increased total cholesterol levels. Platelet aggregation was significantly reduced after fish oil treatment.

Leng et al. performed a randomized, controlled trial on the effects of γ-linolenic and EPA treatment over 2 years in 84 men and 36 women with lower limb atherosclerosis presenting with IC.[97] The patients were treated with 1.68 g of γ-linolenic acid and 0.27 g of EPA per day versus placebo. The outcomes measured included fatal and non-fatal cardiovascular events, cholesterol, blood and plasma viscosity, blood pressure, ABI, and pain-free walking distance. The authors found that treatment produced a statistically significant reduction in systolic blood pressure, but no other significant benefits except from a trend toward fewer coronary events in patients taking FAs.

Carrero et al. conducted a study of 60 patients with IC and randomized them in a double-blinded, controlled fashion to skimmed milk containing 200 mg EPA and 130 mg of DHA plus oleic acid, folic acid, and vitamins or to a placebo-milk.[98] The patients received the treatment or placebo for 12 months. The outcomes of interest included ABI, pain-free walking distance, triglycerides, and cholesterol.

Table 3. Previous studies on n-3 fatty acids and patients with peripheral artery disease

Authors	Year	Type of study	Patients	*n*	Fish oil treatment (PUFA content)	Variables measured	Findings	Ref.
Woodcock et al.	1984	Double-blind, randomized, placebo-controlled study	M/F IC	19	1.8 g/day × 7 weeks (1.8 g EPA)	–Blood viscosity –TG –Cholesterol –ABI –Walking distance	–Decrease in blood viscosity –Decrease in TG –No change in other parameters	94
Gans et al.	1990	Double-blind, randomized, placebo-controlled study	M/F IC	32	3 g/day × 4 months (1.8 g EPA + 1.2 g DHA)	–Walking distance –ABI –SBP –Blood viscosity –Fibrinogen –Lipids	–Decrease in viscosity –Improvement in lipid profile –No change in walking distance or ABIs	95
Mori et al.	1992	Double-blind, randomized, placebo-controlled study	M PAD based on symptoms and angiogram	32	15 g/day × 1 month (2.8 g EPA + 1.8 g DHA)	–Cholesterol –Triglycerides –Platelet count	–Increase in cholesterol –Decrease in triglycerides –Decrease in platelet aggregation	96
Leng et al.	1998	Double-blind, randomized, placebo-controlled study	M/F IC	120 (total)	1.95 g/day × 2 years[a] (1.68 g γ-linolenic acid + 0.27 g EPA)	–Serum cholesterol and lipoprotein concentrations –Hemostatic and rheological variables –Walking distance –SBP –ABI –Non-fatal coronary events –Death	–No change lipids –Higher hematocrit in treatment group –Lower SBP	97
Carrero et al.	2005	Double-blind, randomized, placebo-controlled study	M IC and controls	60	Enriched dairy product × 12 months	–Cholesterol –Walking distance –ABI	–Decrease in cholesterol –Increase in walking distance –Increase in ABI	98
Conway et al.	2005	Double-blind, randomized, placebo-controlled study	M/F IC	50	10 g/day fish oils × 16 weeks (1.7 g EPA + 1.15 g DHA)	–QOL –ABI –Pain-free walking distance –Walking distance	–No change in QOL, ABI or absolute walking distance –Increase initial walking distance	99

Table 3. (*Continued*)

Authors	Year	Type of study	Patients	*n*	Fish oil treatment (PUFA content)	Variables measured	Findings	Ref.
Luu et al.	2007	Prospective study	IC and controls	16	6 g/day × 12 weeks (1.02 g EPA + 0.69 g DHA)	–Monocyte's ability to induce recruitment using monocyte (from PAD patients) and endothelial cell co-cultures	–No change in recruitment of monocytes in PAD –Less recruitment of monocytes in controls after dietary supplementation	104
Madden et al.	2007	Prospective study	M IC	69	6 g/day × 12 weeks (1.02 g EPA + 0.69 g DHA)	–Walking distance –ABI	–Increase in walking distance to first pain and total walking distance –Increase in ABI	102
Schiano et al.	2008	Single-blind, randomized trial	M/F IC	32	2 g/day × 3 months (EPA:DHA 0.9:1.5)	–Endothelial function –Inflammatory function (CRP, myeloperoxidase)	–Improvement in FMD –Reduction in soluble thrombomodulin –No change in inflammation	101
Madden et al.	2009	Prospective study	M IC and controls	205	6 g/day × 12 weeks (1.02 g EPA + 0.69 g DHA)	–CD44 and CD22v3 expression	–Reduction in CD44 –Increase in CD44v3 expression	103

[a]Two capsules BID × 2 weeks, then three capsules BID for remainder of trial; capsules made of linolenic acid (280 mg) and EPA (45 mg).

ABI, ankle–brachial index; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; F, female; FMD, flow-mediated brachial artery vasodilation; IC, intermittent claudication; M, male; PAD, peripheral artery disease; QOL, quality of life; SBP, systolic blood pressure; TG, triglycerides.

The authors found that the plasma concentrations of EPA and DHA increased after treatment with supplements. Interestingly, there was a decrease in cholesterol, and a decrease in total homocysteine in patients in which this value was initially high. Furthermore, walking distance and ABIs increased in the treatment group. The authors concluded that the inclusion of certain nutrients in the diet improves cardiovascular health.

Conway et al. conducted a randomized, controlled, double-blinded trial in 35 men and 15 women with IC.[99] The treatment included a much higher concentration of fish oil (10 g/day of EPA and DHA) versus placebo for 16 weeks. The authors assessed quality of life, ABI, and pain-free and walking distance. They found no difference in ABI, absolute walking distance, or quality of life outcomes between the groups. There was, however, an improvement in initial walking distance.

One meta-analysis studied the effects of n-3 FAs in IC.[100] The study identified six randomized controlled trials assessing the effects of n-3 FAs in patients with IC, representing 313 participants. All studies assessed n-3 dietary supplementation versus placebo, with the treatment duration lasting between 4 weeks and 2 years. Blood viscosity decreased; however, there were no significant differences between ABI, systolic blood pressure (SBP), plasma viscosity, or walking distance (pain-free or maximum walking distance). Gastrointestinal side effects were observed in two studies. It is important to note that despite the administration of fish oil or oily fish, which increases the amount of n-3 FAs in the diet, the consumption of n-6 FAs may remain high, thereby minimally altering the ratio of n-6:n-3. The impact of an intervention may then have less of an effect and may explain the discrepancies among the included studies.

More recent studies not included in the above meta-analysis are worth mentioning. Schiano et al., in a single-blinded study, assessed the effects of n-3 supplementation at the dose of 2 g/day in 32 men and women with IC. Their outcomes of interest were endothelial function with FMD and inflammation. They demonstrated significant improvement in FMD, with no changes in inflammatory markers such as CRP.[101]

Lastly, Madden et al., in a prospective study, demonstrated an increase in both ABI and walking distance with 1.7 g of n-3 FAs for 12 weeks in male patients with claudication.[102] Using the same cohort, the authors investigated changes in CD44 expression in the monocytes of patients.[103] CD44 is known to increase cellular recruitment to the endothelium during inflammation. They demonstrated that CD44 expression was decreased in PAD patients after administration of fish oil for 12 weeks. Yet another study by the same group demonstrated that fish oil supplementation reduced the potency of monocytes to stimulate endothelial cells in normal subjects, but not those from patients with PAD.[104]

Fatty acids intake recommendations

The American Heart Association (AHA) Diet and Lifestyle Recommendations specify that the general population should aim to consume fish, especially oily fish, at least twice a week.[105] This should be coupled with the limitation of saturated fat intake to < 7% of energy, *trans* fat to < 1% of energy, and cholesterol to < 300 mg/day.[105] These recommendations are based on findings that the consumption of two servings per week of fish high in EPA and DHA is associated with a reduced risk of both sudden death and death from CAD.[84, 106] Furthermore, this specific requirement for fish consumption may displace the consumption of other foods that are high in saturated and *trans* FAs from the diet (such as fatty meats and full-fat dairy products). For patients without CAD, the AHA also recommends including oils and foods rich in ALA (flaxseed, canola and soybean oils; flaxseeds and walnuts).[106]

For patients with documented CAD, the AHA recommendations suggest consumption of ~1 g of EPA + DHA per day, preferably from oily fish. The AHA also states that EPA + DHA supplements could be considered, in consultation with the patient's physician. For patients with hypertriglyceridemia, the AHA recommends that 2–4 g of EPA + DHA per day should be provided as capsules under a physician's care.[106] More recent evidence suggests doses in the amount of 3–4 g/day for hypertriglyceridemia.[82,107]

No specific recommendations exist for patients with PAD. Since the first n-3 FA advisory, the FDA has ruled that intakes of up to 3 g/day of marine n-3 FAs are 'generally recognized as safe' (GRAS) in the diet.[108] The only prescription drug available in the US, and approved by the FDA, is Lovaza®, an n-3 acid ethyl ester, approved for the treatment of hypertriglyceridemia at 4 g/day.

It is worth mentioning that the most common side effect of fish oil supplementation is a fishy aftertaste. In the GISSI Prevention study, 0.85 g of omega-3 FAs per day for 3.5 years, 3.8% of patients discontinued taking their supplements (compared with 2.1% for the vitamin E group).[75] Gastrointestinal disturbances (4.9%) and nausea (1.4%) were the most commonly reported side effects. In the HARP Research Group study, 12 capsules containing 6 g of omega-3 FAs were given to 41 patients for 2.4 years.[109] Three patients dropped out of the study claiming intolerance to the capsules. In a study by Leaf et al., a 6-month trial provided 275 patients with 6.9 g of EPA/DHA daily.[110] There was no difference between the fish oil and corn oil control groups for adverse events, with gastrointestinal upset reported by 8% of the fish oil patients and 7% in the placebo group. Lastly, a study by von Schacky et al. administered fish oil at a dose of 6 g/day for 3 months, then 3 g/day for 21 months (111 patients).[111] Four fish oil patients and three placebo patients had mild gastrointestinal discomfort, one patient in the fish oil group had an itchy rash (unlikely related to the study medication as per the authors), and one had a minor hematoma after a second angioplasty.

The n-6 controversy

The role of n-6 FAs in outcomes of patients with cardiovascular disease is more controversial. The primary dietary n-6 FA is LA, which has 18 carbons and two double-bonds

(C18:2 n-6). It is found in abundance in liquid vegetable oils, with safflower oil containing about 75% linoleic by weight and corn oil about 50%. AA is found in the greatest amounts in the phospholipids of grain-fed animals and in eggs. Although humans and other mammals (except some carnivores, such as lions) can convert LA to AA, the conversion is slow.[112] However, mammalian cells cannot convert n-6 to n-3 FAs because they lack the converting enzyme, n-3 desaturase.

The n-6 FAs have been found to directly stimulate pro-inflammatory gene expression in endothelial[113] and smooth muscle cells,[114] and augment the effect of pro-inflammatory cytokines. An increase in the LA content of LDL increases the sensitivity to oxidation,[115] and oxidized LDL is involved in atherosclerosis plaque growth. AA is the precursor for an extensive array of eicosanoids (20-carbon FA metabolites), including all of the 2-series prostaglandins, thromboxane A_2, prostacyclin (PGI_2), the 4-series leukotrienes, and a variety of cytochrome P-450 metabolites. These compounds are bioactive and several mediate inflammatory responses, stimulate platelet aggregation, and produce vasoconstriction. It is important to remember that 'pro-inflammatory' FAs are necessary for proper immune functioning. Although in excess and unopposed they may promote atherosclerotic disease and thrombus formation, there is at this time no universal belief or high-level evidence that n-6 promote CAD.

The evidence for a beneficial role of dietary n-6 FAs is less convincing. A Science Advisory Subcommittee of the AHA published a report on the effects of n-6 FAs and the risk of CAD.[17] Based on a few small studies, the report suggests an increase in the consumption of n-6 FAs to 5–10% of energy intake, in order to reduce the risk of CAD related to lower intakes. It states that reducing n-6 FA intake would likely increase more than decrease the risk of CAD.[17] This area has been quite controversial,[12,116–119] with particular debate focused on the quality of evidence from which the recommendations were derived. A more recent meta-analysis by Ramsden et al. reviewing trials of n-6 FAs suggested that the advice regarding increase in n-6 consumption was based on evidence from trials administering both n-3 and n-6 FAs, and not n-6 in isolation,[120] blurring the evidence for the AHA recommendations.[120,121] In fact, the authors state: "Advice to specifically increase n-6 PUFA intake, based on mixed n-3/n-6 RCT data, is unlikely to provide the intended benefits, and may actually increase the risks of CHD and death". Overall, the question of an association or effect of n-6 FAs and CAD remains unsettled. In view of a lack of evidence for lower extremity artery disease, dietary recommendations for n-6 cannot be given for patients with PAD.

Conclusions and future directions

PUFAs, particularly n-3 FAs, have proven to have considerable beneficial health effects, particularly in the secondary prevention of CAD. Although PAD is another atherosclerotic syndrome, the evidence for beneficial effects of n-3 FAs in that population is present, but weaker. We are presently conducting a clinical trial randomizing patients with IC to 4.4 g of EPA/DHA or placebo (NCT01310270), with the primary endpoint being improvement in endothelial function measured with FMD, and the secondary endpoint being a change in the inflammatory profile. We expect this trial to better characterize the effects of dietary supplementation of n-3 FAs in patients with PAD.

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Conflict of interest

None declared.

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