Essential fatty acids in health and chronic disease[1,2]

Artemis P Simopoulos

ABSTRACT Human beings evolved consuming a diet that contained about equal amounts of n−3 and n−6 essential fatty acids. Over the past 100–150 y there has been an enormous increase in the consumption of n−6 fatty acids due to the increased intake of vegetable oils from corn, sunflower seeds, safflower seeds, cottonseed, and soybeans. Today, in Western diets, the ratio of n−6 to n−3 fatty acids ranges from ≈20–30:1 instead of the traditional range of 1–2:1. Studies indicate that a high intake of n−6 fatty acids shifts the physiologic state to one that is prothrombotic and proaggregatory, characterized by increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time. n−3 Fatty acids, however, have antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory properties. These beneficial effects of n−3 fatty acids have been shown in the secondary prevention of coronary heart disease, hypertension, type 2 diabetes, and, in some patients with renal disease, rheumatoid arthritis, ulcerative colitis, Crohn disease, and chronic obstructive pulmonary disease. Most of the studies were carried out with fish oils [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)]. However, α-linolenic acid , found in green leafy vegetables, flaxseed, rapeseed, and walnuts, desaturates and elongates in the human body to EPA and DHA and by itself may have beneficial effects in health and in the control of chronic diseases. *Am J Clin Nutr* 1999;70(suppl): 560S–9S.

KEY WORDS Essential fatty acids, eicosapentaenoic acid, EPA, docosahexaenoic acid, DHA, linoleic acid, LA, α-linolenic acid, ALA, coronary heart disease, diabetes, hypertension,ventricular premature complexes

INTRODUCTION

Over the past 20 y many studies and clinical investigations have been carried out on the metabolism of polyunsaturated fatty acids (PUFAs) in general and on n−3 fatty acids in particular. Today we know that n−3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer (1–7). Research has been done in animal models, tissue cultures, and human beings. The original observational studies have given way to controlled clinical trials. Great progress has taken place in our knowledge of the physiologic and molecular mechanisms of the various fatty acids in health and disease. Specifically, their beneficial effects have been shown in the prevention and management of coronary heart disease (8, 9), hypertension (10–12), type 2 diabetes (13, 14), renal disease (15, 16), rheumatoid arthritis (17), ulcerative colitis (18), Crohn disease (19), and chronic obstructive pulmonary disease (20). However, this review focuses on the evolutionary aspects of diet, the biological effects of n−6 and n−3 fatty acids, and the effects of dietary α-linolenic acid (ALA) compared with long-chain n−3 derivatives on coronary heart disease and diabetes.

EVOLUTIONARY ASPECTS OF DIET

On the basis of estimates from studies in Paleolithic nutrition and modern-day hunter-gatherer populations, it appears that human beings evolved consuming a diet that was much lower in saturated fatty acids than is today's diet (21). Furthermore, the diet contained small and roughly equal amounts of n−6 and n−3 PUFAs (ratio of 1–2:1) and much lower amounts of *trans* fatty acids than does today's diet (**Figure 1**) (21, 22). The current Western diet is very high in n−6 fatty acids (the ratio of n−6 to n−3 fatty acids is 20–30:1) because of the indiscriminate recommendation to substitute n−6 fatty acids for saturated fats to lower serum cholesterol concentrations (23). Intake of n−3 fatty acids is much lower today because of the decrease in fish consumption and the industrial production of animal feeds rich in grains containing n−6 fatty acids, leading to production of meat rich in n−6 and poor in n−3 fatty acids (24). The same is true for cultured fish (25) and eggs (26). Even cultivated vegetables contain fewer n−3 fatty acids than do plants in the wild (27, 28). In summary, modern agriculture, with its emphasis on production, has decreased the n−3 fatty acid content in many foods: green leafy vegetables, animal meats, eggs, and even fish.

BIOLOGICAL EFFECTS OF n−6 AND n−3 FATTY ACIDS

Linoleic acid (LA; 18:2n−6) and ALA (18:3n−3) and their long-chain derivatives are important components of animal and plant cell membranes. When humans ingest fish or fish oil, the ingested eicosapentaenoic acid (EPA; 20:5n−3) and docosahexaenoic acid (DHA; 22:6n−3) partially replace the n−6 fatty acids [especially arachidonic acid (AA; 20:4n−6)] in cell membranes, especially those of platelets, erythrocytes, neutrophils, monocytes and liver cells (reviewed in 1). As a result, ingestion of

[1] From The Center for Genetics, Nutrition and Health, Washington, DC.

[2] Address reprint requests to AP Simopoulos, The Center for Genetics, Nutrition and Health, Washington, DC 20009. E-mail: cgnh@bellatlantic.net.

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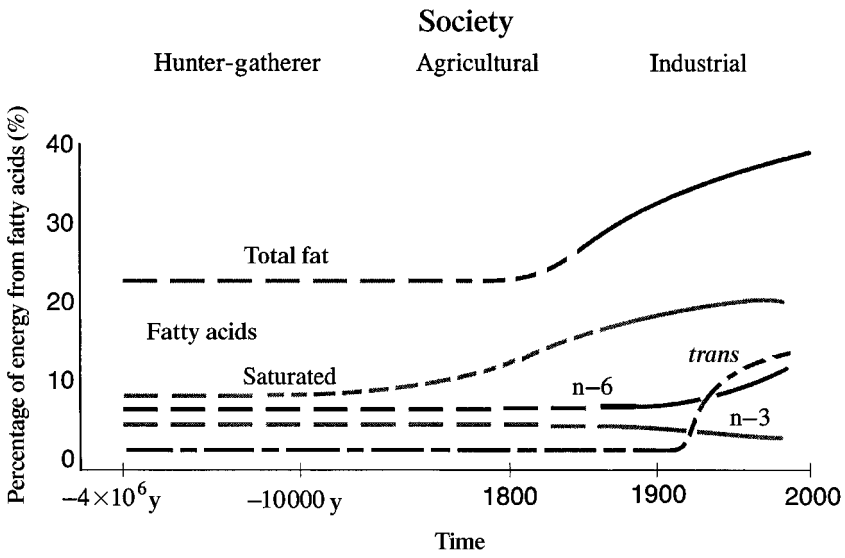

FIGURE 1. Hypothetical scheme of the relative percentages of fat and different fatty acid families in human nutrition as extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 y. *trans* Fatty acids, the result of the hydrogenation process, have increased dramatically in the food supply during this century (22).

EPA and DHA from fish or fish oil leads to *1*) decreased production of prostaglandin E_2 metabolites; *2*) decreased concentrations of thromboxane A_2, a potent platelet aggregator and vasoconstrictor; *3*) decreased formation of leukotriene B_4, an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence; *4*) increased concentrations of thromboxane A_3, a weak platelet aggregator and vasoconstrictor; *5*) increased concentrations of prostacyclin PGI3, leading to an overall increase in total prostacyclin by increasing PGI3 without decreasing PGI2 (both PGI2 and PGI3 are active vasodilators and inhibitors of platelet aggregation); and *6*) increased concentrations of leukotriene B_5, a weak inducer of inflammation and chemotactic agent (29, 30).

Because of the increased amounts of n−6 fatty acids in the Western diet, the eicosanoid metabolic products from AA, specifically prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins, are formed in larger quantities than those formed from n−3 fatty acids, specifically EPA. The eicosanoids from AA are biologically active in small quantities and if they are formed in large amounts, they contribute to the formation of thrombi and atheromas; the development of allergic and inflammatory disorders, particularly in susceptible people; and cell proliferation. Thus, a diet rich in n−6 fatty acids shifts the physiologic state to one that is prothrombotic and proaggregatory, with increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time. Bleeding time is shorter in groups of patients with hypercholesterolemia (31), hyperlipoproteinemia (32), myocardial infarction, other forms of atherosclerotic disease, type 2 diabetes, obesity, and hypertriglyceridemia. Atherosclerosis is a major complication in type 2 diabetes patients. Bleeding time is longer in women than in men and in younger than in older persons. There are ethnic differences in bleeding time that appear to be related to diet. As shown in **Table 1,** the higher the ratio of n–6 to n−3 fatty acids in platelet phospholipids, the higher is the death rate from cardiovascular disease (33). As the ratio of n−6 PUFAs to n−3 PUFAs increases, the prevalence of type 2 diabetes also increases (13; **Figure 2**).

The hypolipidemic, antithrombotic, and antiinflammatory effects of n−3 fatty acids have been studied extensively in animal models, tissue cultures, and cells (**Table 2**; 34). As expected, earlier studies focused on mechanisms that involve eicosanoid metabolites. More recently, however, the effects of fatty acids on gene expression have been investigated and this focus of interest has led to studies at the molecular level (**Tables 3** and **4**). Previous studies have shown that fatty acids, whether released from membrane phospholipids by cellular phospholipases or made available to the cell from the diet or other aspects of the extracellular environment, are important cell-signaling molecules. They can act as second messengers or substitute for the classic second messengers of the inositide phospholipid and cyclic AMP signal transduction pathways (48). They can also act as modulator molecules mediating responses of the cell to extracellular signals (48). It has been shown that fatty acids rapidly and directly alter the transcription of specific genes (49).

Effects of dietary ALA compared with long-chain n−3 fatty acid derivatives on physiologic indexes

Several clinical and epidemiologic studies have been conducted to determine the effects of long-chain n−3 PUFAs on various physiologic indexes (7). Whereas the earlier studies were conducted with large doses of fish or fish-oil concentrates, more

TABLE 1
Ethnic differences in fatty acid concentrations in thrombocyte phospholipids and percentage of all deaths from cardiovascular disease[1]

	Europe and United States	Japan	Greenland Eskimos
		%	
Arachidonic acid (20:4n−6)	26	21	8.3
Eicosapentaenoic acid (20:5n−3)	0.5	1.6	8.0
n−6:n−3	50	12	1
Mortality from cardiovascular disease	45	12	7

[1] Data modified from reference 33.

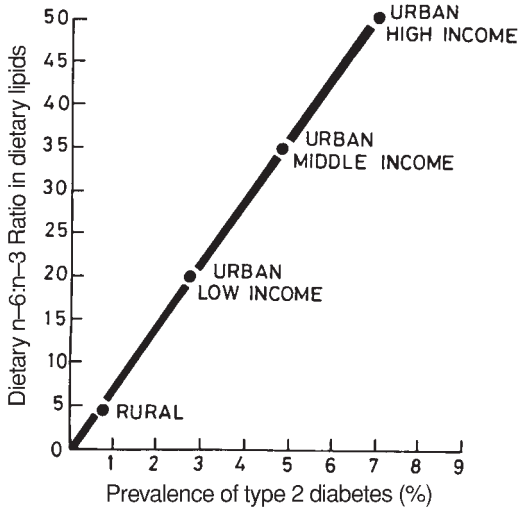

FIGURE 2. Relation between the ratio of n−6 to n−3 fatty acids in dietary lipids in the Indian diet and the prevalence of type 2 diabetes (13).

recent studies have used lower doses (50). ALA, the precursor of n−3 fatty acids, can be converted to long-chain n−3 PUFAs and can therefore be substituted for fish oils. The minimum intake of long-chain n−3 PUFAs needed for beneficial effects depends on the intake of other fatty acids. Dietary amounts of LA as well as the ratio of LA to ALA appear to be important for the metabolism of ALA to long-chain n−3 PUFAs. Indu and Ghafoorunissa (50) showed that while keeping the amount of dietary LA constant, 3.7 g ALA appears to have biological effects similar to those of 0.3 g long-chain n−3 PUFA with conversion of 11 g ALA to 1 g long-chain n−3 PUFA. Thus, a ratio of 4 (15 g LA:3.7 g ALA) is appropriate for conversion. In human studies, Emken et al (51) showed that the conversion of deuterated ALA to longer-chain metabolites was reduced by ≈50% when dietary intake of LA was increased from 4.7% to 9.3% of energy as a result of the known competition between n−6 and n−3 fatty acids for desaturation.

Indu and Ghafoorunissa (50) further indicated that increasing dietary ALA increases EPA concentrations in plasma phospholipids after both 3 and 6 wk of intervention. Dihomo-γ-linolenic acid (20:3n−6) concentrations were reduced but AA concentrations were not altered. The reduction in the ratio of long-chain n−6 PUFAs to long-chain n−3 PUFAs was greater after 6 wk than after 3 wk. Indu and Ghafoorunissa were able to show antithrombotic effects by reducing the ratio of n−6 to n−3 fatty acids with ALA-rich vegetable oil. After ALA supplementation there was an increase in long-chain n−3 PUFA in plasma and platelet phospholipids and a decrease in platelet aggregation. ALA supplementation did not alter triacylglycerol concentrations. As shown by others, only long-chain n−3 PUFAs have triacylglycerol-lowering effects (52).

In Australian studies, ventricular fibrillation in rats was reduced with canola oil as much or even more efficiently than with fish oil, an effect attributable to ALA (53). Further studies should be able to show whether this result is a direct effect of ALA per se or occurs as a result of its desaturation and elongation to EPA and DHA.

The diets of Western countries have contained increasingly larger amounts of LA, which has been promoted for its cholesterol-lowering effect. It is now recognized that dietary LA favors oxidative modification of LDL cholesterol (54, 55), increases platelet response to aggregation (56), and suppresses the immune system (57). In contrast, ALA intake is associated with inhibitory effects on the clotting activity of platelets, on their response to thrombin (58, 59), and on the regulation of AA metabolism (60). In clinical studies, ALA contributed to lowering of blood pressure (61). In a prospective study, Ascherio et al (62) showed that ALA is inversely related to the risk of coronary heart disease in men.

ALA is not equivalent in its biological effects to the long-chain n−3 fatty acids found in marine oils. EPA and DHA are more

TABLE 2
Effects of n−3 fatty acids on factors involved in the pathophysiology of atherosclerosis and inflammation[1]

Factor	Function	Effect of n−3 fatty acid on factor concentrations
Arachidonic acid	Eicosanoid precursor, aggregates platelets, and stimulates white blood cells	↑
Thromboxane A_2	Platelet aggregation, vasoconstriction, increases intracellular Ca^{2+}	↓
Prostacyclin	Prevents platelet aggregation, vasodilator, increases cyclic AMP	↑
Leukotriene B_4	Neutrophil chemoattractant increases intracellular Ca^{2+}	↓
Tissue plasminogen activator	Increases endogenous fibrinolysis	↑
Fibrinogen	Blood clotting factor	↓
Red blood cell deformability	Decreases tendency to thrombosis and improves oxygen delivery to tissues	↑
Platelet activating factor	Activates platelets and white blood cells	↓
Platelet-derived growth factor	Chemoattractant and mitogen for smooth muscles and macrophages	↓
Oxygen free radicals	Causes cellular damage, enhances LDL uptake via the scavenger pathway, stimulates arachidonic acid metabolism	↓
Lipid hydroperoxides	Stimulates eicosanoid formation	↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil oxygen free radical formation, lymphocyte proliferation, and platelet activating factor; expresses intercellular adhesion molecule 1 on endothelial cells; and inhibits plasminogen activator and thus is procoagulant	↓
Endothelial-derived relaxation factor	Reduces arterial vasoconstrictor response	↑
VLDL	Related to LDL and HDL concentrations	↓
HDL	Decreases the risk of coronary heart disease	↑
Lipoprotein (a)	Atherogenic and thrombogenic	↓
Triacylglycerols and chylomicrons	Contribute to postprandial lipemia	↓

[1] Data from Weber and Leaf (34). ↑, increases; ↓, decreases.

TABLE 3
Effects of polyunsaturated fatty acids on several genes encoding enzyme proteins involved in lipogenesis, glycolysis, and glucose transport[1]

Function, gene, and reference	Linoleic acid	α-Linolenic acid	Arachidonic acid	Eicosapentaenoic acid	Docosahexaenoic acid
Hepatic cells					
Lipogenesis					
FAS (35–38)	↓	↓	↓	↓	↓
S14 (35–38)	↓	↓	↓	↓	↓
SCD1 (39)	↓	↓	↓	↓	↓
SCD2 (40)	↓	↓	↓	↓	↓
ACC (38)	↓	↓	↓	↓	↓
ME (38)	↓	↓	↓	↓	↓
Glycolysis					
G6PD (41)	↓				
GK (41)	↓	↓	↓	↓	↓
PK (42)	—	↓	↓	↓	↓
Mature adiposites					
Glucose transport					
GLUT4 (43)	—	—	↓	↓	—
GLUT1 (43)	—	—	↑	↑	—

[1]FAS, fatty acid synthase; SCD, steroyl-CoA desaturase; ACC, acetyl CoA carboxylase; ME, malic enzyme; G6PD, glucose-6-phosphate dehydrogenase; GK, glucokinase; PK, pyruvate kinase; GLUT, glucose transporter; ↓, suppresses or decreases; ↑, induces or increases.

rapidly incorporated into plasma and membrane lipids and produce more rapid effects than does ALA. Relatively large reserves of LA in body fat, as are found in vegans or in the diet of omnivores in Western societies, would tend to slow down the formation of long-chain n−3 fatty acids from ALA. Therefore, the role of ALA in human nutrition becomes important in terms of long-term dietary intake. One advantage of the consumption of ALA over n−3 fatty acids from fish is that the problem of insufficient vitamin E intake does not exist with high intake of ALA from plant sources.

Coronary heart disease

Most epidemiologic studies and clinical trials using n−3 fatty acids in the form of fish or fish oil have been carried out in patients with coronary heart disease. However, studies have also been carried out on the effects of ALA in normal subjects and in patients with myocardial infarction (9). The effects of long-chain n−3 fatty acids (EPA and DHA) on factors involved in the pathophysiology of atherosclerosis and inflammation are shown in Table 2 (34).

The hypolipidemic effects of n−3 fatty acids are similar to those of n−6 fatty acids, provided that they replace saturated fats in the diet. n−3 Fatty acids have the added benefit of consistently lowering serum triacylglycerol concentrations, whereas the n−6 fatty acids do not and may even increase them (63).

Another important consideration is the finding that during chronic fish-oil feeding postprandial triacylglycerol concentrations decrease. Furthermore, Nestel (64) reported that consumption of high amounts of fish oil blunted the expected rise in plasma cholesterol concentrations in humans. These findings are consistent with the low rate of coronary artery disease found in fish-eating populations (65). Studies in humans have shown that fish oils reduce the rate of hepatic secretion of VLDL triacylglycerol (66–69). In normolipidemic subjects, n−3 fatty acids prevent and rapidly reverse carbohydrate-induced hypertriglyceridemia (67). There is also evidence from kinetic studies that fish oil increases the fractional catabolic rate of VLDL (66, 68, 69).

The effects of different doses of fish oil on thrombosis and bleeding time were investigated by Saynor et al (70). A dose of 1.8 g EPA/d did not result in any prolongation in bleeding time, but 4 g/d increased bleeding time and decreased platelet count with no adverse effects. In human studies, there has never been a case of clinical bleeding, even in patients undergoing angioplasty, while the patients were taking fish oil supplements (71).

Coronary artery bypass grafting

Coronary artery bypass grafting is an important treatment alternative in the management of coronary artery disease. The Shunt Occlusion Trial (SOT) was a randomized, controlled study that assessed the effect of dietary supplementation with fish oil rich in n−3 fatty acids on 1-y graft occlusion rates in patients undergoing coronary artery bypass grafting (72). A total of 610 patients were randomly assigned to receive 4 g fish-oil concentrate/d or to a control group. Patients continued their antithrombotic treatment. The primary endpoint was 1-y graft patency assessed by angiography in 95% of patients. The n−3 fatty acid supplementation significantly reduced the incidence of vein-graft occlusion. An inverse relation between relative change in serum phospholipid n−3 fatty acids and vein-graft occlusions was observed (72). This beneficial effect on vein-graft patency may be due to antithrombotic as well as antiatherosclerotic properties of n−3 fatty acids. It is unlikely that the effect is directly linked to serum lipoproteins, because serum cholesterol concentrations were not altered by fish-oil supplementation and there was no association between the reduction in serum triacylglycerol and vein-graft patency. Their effects may most likely be due to the influence of n−3 fatty acids on cellular processes locally in the vessel wall.

The effect of n−3 supplementation on the incidence of restenosis after coronary angioplasty has been addressed in several clinical studies and the results so far are equivocal (73, 74). However, the pathophysiology of coronary restenosis is different from that of vein-graft occlusion, and the 2 conditions are not analogous. The results of the SOT suggest that patients undergoing coronary bypass surgery should be encouraged to consume high amounts of n−3 fatty acids (72).

Relations of dietary intake and cell membrane concentrations of long-chain n23 PUFAs with risk of primary cardiac arrest

In a population-based controlled study, Siscovick et al (75) assessed the effect of dietary intake of EPA and DHA from seafood on primary cardiac arrest risk. All case and control subjects were free of prior clinical heart disease and major comorbidity and did not use fish-oil supplements. Information on the dietary intake of n−3 PUFAs from seafood during the prior month was obtained from the spouses of the subjects. Blood specimens were analyzed to determine the fatty acid composition of red blood cell membranes. The data showed that dietary intake of n−3 PUFAs from seafood was associated with reduced risk of primary cardiac arrest compared with no fish intake; 5.5 g n−3 fatty acids/mo or the equivalent of 1 fatty fish meal/wk was associated with a 50% reduction in the risk of primary cardiac arrest. A 5.0% increase in n−3 PUFAs in red blood cell membrane phospholipids was associated with a 70% reduction in the risk of primary cardiac arrest.

Serum fatty acids and coronary heart disease risk

Simon et al (76) examined the relation between serum fatty acids and coronary heart disease by conducting a nested case-control study in 94 men with incident coronary heart disease and 94 healthy control men who were enrolled in the usual-care group of the Multiple Risk Factor Intervention Trial between December 1973 and February 1976. The results are consistent with other evidence indicating that saturated fatty acids are directly correlated, and n−3 PUFAs are inversely correlated, with coronary heart disease. Because these associations were present after adjustment for blood lipid concentrations, other mechanisms, such as a direct effect on blood clotting, may be involved.

Effects of dietary fish oil on ventricular premature complexes

In a prospective, double-blind, placebo-controlled study, Sellmayer et al (77) tested the potential antiarrhythmic effects of dietary supplementation with fish oil rich in DHA and EPA in patients with spontaneous ventricular premature complexes (VPCs). The patients were eligible if they had a minimum of 2000 VPCs/24 h as measured by 24-h Holter monitoring. The patients had moderate-to-low-grade ventricular arrhythmias in the absence of severe myocardial pump failure and were randomly assigned to receive either fish oil (cod liver oil) or placebo sunflower oil. Vitamin E (10 μg α-tocopherol/L) was added as antioxidant in both oils.

A daily dose of 0.9 g EPA, 1.5 g DHA, or 5 g LA was taken for 16 wk, by which time tissue concentrations of n−3 fatty acids would have reached steady state. Compliance was checked by a phone call after 1 wk, a visit after 8 wk, and serum phospholipid fatty acid analysis after 16 wk. Serum concentrations of EPA and DHA increased significantly (>2-fold and 1.7-fold, respectively; $P < 0.001$), whereas concentrations of AA and LA decreased slightly. No changes in the fatty acid composition of the placebo groups were noted. VPCs were reduced by >70% in 44% of patients ($n = 15$) after fish oil compared with 15% of patients ($n = 5$) in the placebo group. The results showed that moderate intake of fish oil has antiarrhythmic effects that lead to a reduction of VPCs in nearly half of patients with frequent ventricular arrhythmia. In a similar study by Danish investigators, patients who received fish oil had a nonsignificant reduction in VPCs and those who received corn oil had no reduction (78).

Antiarrhythmic effects of n−3 fatty acids

The antiarrhythmic effects of n−3 fatty acids are supported by 2 clinical intervention trials. The results of the Diet and Reinfarction Trial (DART) strongly support the role of fish or fish oil in decreasing total mortality and sudden death in patients with 1 episode of myocardial infarction (8). The estimated dose of EPA was ≈0.3 g/d in the fish-advice group and 0.1 g/d in the control group (8). In the study by de Lorgeril et al (9), the estimated intake of ALA was 2 g/d in the intervention group and 0.6 g/d in the control group. Experimental studies suggest that intake of 3–4 g ALA/d is equivalent to 0.3 g EPA/d with respect to its effect on the EPA content of plasma phospholipids (50).

The studies of McLennan (53), Charnock (79) and McLennan et al (80) showed that n−3 fatty acids, more so than n−6 PUFAs, can prevent ischemia-induced fatal ventricular arrhythmias in experimental animals. Kang and Leaf (81, 82) showed that n−3 fatty acids make the heart cells less excitable by modulating the conductance of the sodium and other ion channels. The clinical studies of Burr et al (8) and de Lorgeril et al (9, 83) further support the role of n−3 fatty acids in the prevention of sudden death due to ventricular arrhythmias which, in the United States, account for 50–60% of the mortality from acute myocardial

TABLE 4
Effects of polyunsaturated fatty acids on several genes encoding enzyme proteins involved in cell growth, early gene expression, adhesion molecules, inflammation, β-oxidation, and growth factors[1]

Function, gene, and reference	Linoleic acid	α-Linolenic acid	Arachidonic acid	Eicosapentaenoic acid	Docosahexaenoic acid
Cell growth and early gene expression					
c-fos (44)	—	—	↑	↓	↓
Egr-1 (44)	—	—	↑	↓	↓
Adhesion molecules					
VCAM-1 mRNA (45)[2]	—	—	↑	[3]	↓
Inflammation					
IL-1β (46)	—	—	↑	↓	↓
β-oxidation					
Acyl-CoA oxidase (38)[4]	↑	↑	↑	↑↑	↑
Growth factors					
PDGF (47)	—	—	↑	↓	↓

[1] VCAM, vascular cell adhesion molecule; IL, interleukin; PDGF, platelet-derived growth factor; ↓, suppresses or decreases; ↑, induces or increases.
[2] Monounsaturated fatty acids (MUFAs) also suppress VCAM1 mRNA, but to a lesser degree than does docosahexaenoic acid (DHA).
[3] Eicosapentaenoic acid has no effect by itself but enhances the effect of DHA.
[4] MUFAs also induce acyl-CoA oxidase mRNA.

infarction and causes 250 000 deaths/y. The intervention studies of Sellmayer et al (77) and Christensen et al (78), showing a decrease in the rate of VCPs and the case control study by Siscovick et al (75) reporting an inverse relation between fish consumption and sudden death, provide further evidence for the antiarrhythmic effects of fish-oil ingestion.

The studies by Burr et al (8) and de Lorgeril et al (9, 83) clearly showed that in the secondary prevention of coronary heart disease, increasing n−3 fatty acids from fish or fish-oil supplements (8) and by increasing ALA in the diet (9, 83) reduced the incidence of sudden death significantly [by 29% (8), by 70% after 2 y (9), and by 37% at 5-y follow up (83)]. Intake of EPA in the Burr study was ≈0.3 and 0.1 g/d in the experimental and control groups, respectively. ALA intake in the study of de Lorgeril et al was ≈2.0 and 0.6 g/d in the intervention and control group, respectively. Thus, the dietary intervention in the Burr study was not very different from the intervention in the de Lorgeril study. In both studies there was no change in lipid concentration, suggesting that the beneficial effects of n−3 fatty acids were due to their antithrombotic and antiarrhythmic effects.

A study comparing the fatty acid composition of serum cholesterol esters in subjects in Crete, Greece, and Zutphen, Netherlands, reported that the Cretans had higher concentrations of 18:1n−9, much lower concentrations of LA and unexpectedly high concentrations of ALA (84). ALA in the Cretan diet comes from purslane, walnuts, and other wild green leafy plants. Similarly, the population of Kohama Island, Japan, which has the longest life expectancy in the world and the lowest coronary heart disease mortality rate, has high concentrations of plasma ALA (85). In Japan, the dietary sources of ALA are mainly canola (35% of all oils consumed) and soybean oils. Thus, the 2 populations documented to have the greatest life expectancies in the world (Japanese and Cretans) both appear to have high intakes of ALA. The dietary ratio of LA to ALA in the de Lorgeril trial was 4:1. This ratio permits the desaturation and elongation from ALA to 20:5n−3, as shown by Indu and Ghafoorunissa (50).

Diabetes

Type 2 diabetes is a multigenic, multifactorial disorder. Several environmental factors contribute to insulin resistance (**Table 5**; 86–88). The importance of genetic predisposition and its interaction with diet and exercise in the development of type 2 diabetes has been known since the time of Hippocrates, but progress in the genetics of type 2 diabetes has been slow until recently (89). The observation that fatty acids can regulate gene expression in adipose cells introduces a link between the composition of diets and the hyperplastic and hypertrophic responses of white adipose tissue. The characterization of fatty acid–responsive genes may also produce some clues about the development of an insulin-resistant state and cell hypertrophy (90). Type 2 diabetes is characterized by hyperglycemia in the presence of insulin resistance, hypertriglyceridemia, and the development of vascular complications. Men and women with type 2 diabetes have 3-fold and 5-fold higher cardiovascular mortality, respectively, than the non-diabetic population, and this higher risk is also carried by nondiabetic first-degree relatives of type 2 diabetes subjects. Clustering of atherogenic (eg, dyslipidemia, hypertension) and thrombotic (eg, plasminogen activator inhibitor 1, factor VII, and fibrinogen) risk factors in association with insulin resistance may explain the higher risk. EPA and DHA decrease plasminogen activator inhibitor 1, factor VII, and serum fibrinogen concentrations (reviewed in 91).

TABLE 5
Effects of environmental factors on insulin action[1]

Increases in body weight reduce insulin action (although in some patients, insulin resistance precedes the obese state).
Physical activity reduces insulin resistance.
Weight loss reduces insulin resistance.
Alcohol reduces insulin resistance.
Saturated fat intake increases insulin resistance in animals.
Linoleic acid (18:2n−6) in muscle phospholipids is positively correlated with hyperinsulinemia.
Long-chain polyunsaturated fatty acids (carbon chain length 20–22) in muscle phospholipids are inversely related to hyperinsulinemia and positively related to insulin sensitivity.

[1] Modified from reference 86.

Clustering of hypertension, lipid abnormalities, obesity, and diabetes in one patient may be the result of a shared genetic background for these disorders (92, 93). Apo E 4,3 and apo E 4,4 phenotypes in patients with central obesity were shown to be associated with high insulin concentrations and abnormal insulin-to-glucose ratios (94). Therefore, in selecting patients for clinical intervention, apo E polymorphism needs to be taken into consideration because women with the apo E 3,2 phenotype are less susceptible, and those with apo E 4,3 and apo E 4,4 phenotypes are more susceptible to hyperinsulinemia and related abnormalities characteristic of insulin resistance, including hypertension (94).

In 1993, Borkman et al (95) showed that hyperinsulinemia and insulin resistance are inversely associated with the amount of 20- and 22-carbon fatty acids in muscle cell membrane phospholipids in patients with coronary heart disease and in normal volunteers. Such decreases in 20- and 22-carbon fatty acid concentrations could occur as a result of *1*) low dietary intake of 20- and 22-carbon fatty acids (current Western diets are characterized by low amounts of EPA and DHA relative to LA and AA); *2*) high dietary intake of *trans* fatty acids, which interfere with the desaturation and elongation of LA and ALA and thus lower AA, EPA, and DHA concentrations; *3*) genetic defects of Δ6 and Δ5 desaturase; *4*) genetic defects that interfere with the transport or binding of 20- and 22-carbon fatty acids, such as in intestinal fatty acid–binding protein; *5*) high dietary intake of LA, which leads to decreased production of AA and interferes with the desaturation and elongation of ALA to EPA and DHA; or *6*) increased catabolism of AA, which reduces the number of available 20- and 22-carbon atoms (**Figure 3**; 86, 87, 96).

An increase in 20- and 22-carbon PUFAs, ie, AA, EPA, and DHA, leads to increases in membrane fluidity, the number of insulin receptors, and insulin action. In humans, the ratio of n–6 to saturated fatty acids in serum phospholipids correlates with insulin sensitivity. *trans* Fatty acids are incorporated into cell membrane phospholipids, resulting in decreased fluidity of membranes and binding of insulin to its receptor, leading to impaired insulin action, insulin resistance, and hyperinsulinemia. In animal experiments, isomeric *trans* fatty acids increased LA and lowered AA concentrations in tissue phospholipids, indicating inhibition of Δ6 desaturase.

About 23 studies have been conducted on the effects of n−3 fatty acids in patients with type 2 diabetes (97). In most studies,

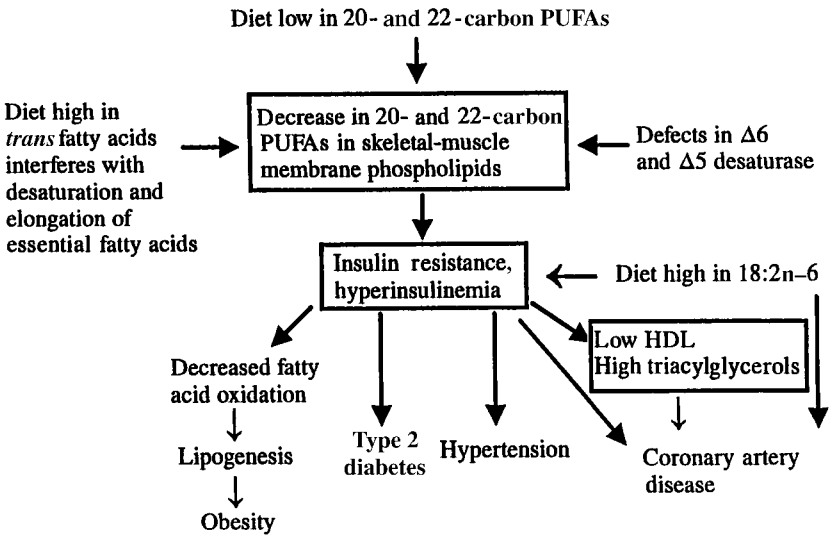

FIGURE 3. Hypothetical scheme of the effects of dietary 20- and 22-carbon polyunsaturated fatty acids (PUFAs) on the composition of the 20- and 22-carbon PUFAs in skeletal-muscle membrane phospholipids, and their relations with insulin resistance, hyperinsulinemia, and chronic disease (96).

fish-oil consumption lowered serum triacylglycerol concentrations significantly, but in some studies plasma glucose concentrations rose. In many of these studies, however, the number of subjects was small and the dose of n−3 fatty acids was >3 g/d and controls were lacking. Intake of 3 g n−3 fatty acids/d decreased triacylglycerol concentrations significantly. At intakes of 3 g n−3 fatty acids/d, only one study showed an increase in blood glucose concentrations.

In a randomized, double-blind, placebo-controlled, crossover trial, patients with type 2 diabetes consumed 6 g n−3 fatty acids (EPA and DHA)/d for 6 mo in addition to their usual oral therapy (14). Fasting serum glucose concentrations increased by 11% during the n−3 fatty acid phase and by 8% during the placebo phase (olive oil), showing a nonsignificant net increase of 3%. Similarly, there was no significant change in glycated hemoglobin concentrations. However, fasting triacylglycerol concentrations decreased by 43%, which is a highly significant change. This study is the largest and longest reported placebo-controlled trial of the effect of n−3 fatty acids on type 2 diabetes. It showed convincingly that n−3 fatty acid intake, along with oral therapy for diabetes, can lower triacylglycerol concentrations with no adverse effects on glycemic control.

Fanaian et al (98) carried out a randomized, controlled study comparing the effectiveness of a low-fat, high-carbohydrate diet (High-CHO) and a high monounsaturated fat diet (High-MUFA) using canola oil [which is high in oleic acid (18:0) and has a LA-to-ALA ratio of 2:1] on insulin resistance, serum lipids, and other variables in 48 male and female patients with type 2 diabetes (average age: 44.2 ± 0.9 y). There were significant reductions in systolic and diastolic blood pressure and fasting plasma glucose and triacylglycerol concentrations, and significant increases in HDL cholesterol and insulin sensitivity in the High-MUFA group compared with the High-CHO group. The investigators concluded that after 1 y, the monounsaturated fat–enriched program was associated with a better metabolic profile in type 2 diabetes patients and that a diet high in monounsaturated fat is more likely to be followed than is a low-fat, high-carbohydrate diet. These results are of special importance to vegetarians.

CONCLUSIONS AND RECOMMENDATIONS

Essential fatty acids, both n−6 (LA) and n−3 (ALA), have been part of our diet since the beginning of human life. Before the agricultural revolution 10 000 y ago humans consumed about equal amounts of both. Over the past 150 y this balance has been upset. Current estimates in Western cultures suggest a ratio of n–6 to n−3 fatty acids of 10–20:1 instead of 1–4:1.

n−6 And n−3 fatty acids are the parent fatty acids for the production of eicosanoids, eg, prostaglandins, thromboxanes, and leukotrienes. Eicosanoids derived from n−6 fatty acids have opposing metabolic properties to those derived from n−3 fatty acids. A balanced intake of both n−6 and n−3 fatty acids is essential for health. There is competition among the enzymes involved in the elongation and desaturation of LA and ALA. A ratio of LA to ALA of 4:1 or less has been shown to be optimal for the elongation of 11 g of ALA to 1 g EPA. This is important for vegetarians because their diets are typically rich in LA and poor in ALA. Because EPA is biologically more active than ALA and high amounts of LA decrease the conversion of ALA to EPA, the optimal intake of LA relative to ALA is crucial for normal metabolism.

Clinical interventions provide further support for the beneficial effects of n−3 fatty acids in the prevention and management of cardiovascular disease, hyperinsulinemia, and possibly type 2 diabetes. n−3 Fatty acids affect coronary heart disease beneficially not by changing serum lipid concentrations, although EPA and DHA do lower triacylglycerol concentrations, but by reducing blood clotting in vessel walls (72, 76) and ventricular arrhythmias (8, 9, 75, 77). Of further interest is the fact that in the secondary prevention of coronary heart disease, the effects of n−3 fatty acids appear to occur within the first 4 mo of intervention (9). It is clear that large, randomized, double-blind, controlled clinical trials to confirm the roles of n−3 fatty acids in the prevention of sudden death and in total mortality are needed. Double-blind, controlled, clinical trials are the gold standard with which to show cause-and-effect relations. In the planning of such trials, it is essential that the patients are stratified by genetic susceptibility, disease entity, severity of disease, and by sex and age. The composition of the diet must remain constant

throughout the intervention period and the ratios of saturated to unsaturated fat and n−6 to n−3 PUFAs must be taken into consideration. Many of the studies reported thus far did not control for many of the above factors. Common protocols need to be established but also modified according to prevailing genetic, dietary, and other environmental factors.

EPA and DHA lower cardiovascular risk factors in patients with type 2 diabetes without adversely affecting glycemic control. There is a need for clinical trials in which cardiovascular disease endpoints are determined and risk factors, such as changes in lipid concentrations and abnormalities in carbohydrate metabolism, are measured. Because diabetes is a multigenic disease, stratification of patients and controls by genetic markers such as apo E or intestinal fatty acid–binding protein is necessary. In developing protocols, it should be considered essential to use a ratio of n−6 to n−3 PUFAs of ≈1–2:1, to keep saturated fat intake low, and to limit *trans* fatty acid intake to ≤2% of total energy intake.

REFERENCES

1. Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 1991;54:438–63.
2. Simopoulos AP, Kifer RR, Martin RE, eds. Health effects of polyunsaturated fatty acids in seafoods. Orlando, FL: Academic Press, 1986.
3. Galli C, Simopoulos AP. Dietary ω3 and ω6 fatty acids. Biological effects and nutritional essentiality. New York: Plenum Press, 1989.
4. Simopoulos AP, Kifer RR, Martin RE, Barlow SM, eds. Health effects of ω3 polyunsaturated fatty acids in seafoods. World Rev Nutr Diet 1991;66:1–592.
5. Galli C, Simopoulos AP, Tremoli E, eds. Fatty acids and lipids: biological aspects. World Rev Nutr Diet 1994;75:1–197.
6. Galli C, Simopoulos AP, Tremoli E, eds. Effects of fatty acids and lipids in health and disease. World Rev Nutr Diet 1994;76:1–152.
7. Salem N Jr, Simopoulos AP, Galli C, Lagarde M, Knapp HR, eds. Fatty acids and lipids from cell biology to human disease. Lipids 1996;31(suppl):S1–326.
8. Burr ML, Fehily AM, Gilbert JF, et al. Effect of changes in fat, fish and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 1989;2:757–61.
9. de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean α-linolenic acid–rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454–9.
10. Morris MC, Sacks F, Rosner B. Fish oil to reduce blood pressure: a meta-analysis. Ann Intern Med 1994;120(suppl):10.
11. Appel LF, Miller ED, Seidler AJ, Whelton PK. Diet supplementation with fish oils and blood pressure reduction: a meta-analysis. Ann Intern Med 1994;120(suppl):9.
12. Appel LJ, Miller ER, Seidler AJ, Whelton PK. Does supplementation of diet with "fish oil" reduce blood pressure? A meta-analysis of controlled clinical trials. Arch Intern Med 1993;153:1429–38.
13. Raheja BS, Sadikot SM, Phatak RB, Rao MB. Significance of the n−6/n−3 ratio for insulin action in diabetes. Ann N Y Acad Sci 1993;683:258–71.
14. Connor WE, Prince MJ, Ullmann D, et al. The hypotriglyceridemic effect of fish oil in adult-onset diabetes without adverse glucose control. Ann N Y Acad Sci 1993;683:337–40.
15. De Caterina R, Caprioli R, Giannessi D, et al. n−3 Fatty acids reduce proteinuria in patients with chronic glomerular disease. Kidney Int 1993;44:843–50.
16. Donadio JV Jr, Bergstralh EJ, Offord KP, Spencer DC, Holley KE. A controlled trial of fish oil in IgA nephropathy. Mayo Nephrology Collaborative Group. N Engl J Med 1994;331:1194–9.
17. Kremer JM. Effects of modulation of inflammatory and immune parameters in patients with rheumatic and inflammatory disease receiving dietary supplementation of n−3 and n−6 fatty acids. Lipids 1996;31(suppl):S243–7.
18. Stenson WF, Cort D, Rodgers J, et al. Dietary supplementation with fish oil in ulcerative colitis. Ann Intern Med 1992;116:609–14.
19. Belluzzi A, Brignola C, Campieri M, Pera A, Boschi S, Miglioli M. Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. N Engl J Med 1996;334:1557–60.
20. Shahar E, Folsom AR, Melnick SL, et al. Dietary n−3 polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. Atherosclerosis Risk in Communities Study Investigators. N Engl J Med 1994;331:228–33.
21. Eaton SB, Konner M. Paleolithic nutrition. A consideration of its nature and current implications. N Engl J Med 1985;312:283–9.
22. Simopoulos AP. Evolutionary aspects of diet: fatty acids, insulin resistance and obesity. In: VanItallie TB, Simopoulos AP, eds. Obesity: new directions in assessment and management. Philadelphia: Charles Press, 1995:241–61.
23. Report of the National Cholesterol Education Program Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults. Arch Intern Med 1988;148:36–69.
24. Crawford MA. Fatty acids in free-living and domestic animals. Lancet 1968;1:1329–33.
25. van Vliet T, Katan MB. Lower ratio of n−3 to n−6 fatty acids in cultured than in wild fish. Am J Clin Nutr 1990;51:1–2.
26. Simopoulos AP, Salem N Jr. n−3 Fatty acids in eggs from range-fed Greek chickens. N Engl J Med 1989;321:1412.
27. Simopoulos AP, Salem N Jr. Purslane: a terrestrial source of omega-3 fatty acids. N Engl J Med 1986;315:833 (letter).
28. Simopoulos AP, Norman HA, Gillaspy JE. Purslane in human nutrition and its potential for world agriculture. World Rev Nutr Diet 1995;77:47–74.
29. Weber PC, Fischer S, von Schacky C, Lorenz R, Strasser T. Dietary omega-3 polyunsaturated fatty acids and eicosanoid formation in man. In: Simopoulos AP, Kifer RR, Martin RE, eds. Health effects of polyunsaturated fatty acids in seafoods. Orlando, FL: Academic Press, 1986:49–60.
30. Lewis RA, Lee TH, Austen KF. Effects of omega-3 fatty acids on the generation of products of the 5-lipoxygenase pathway. In: Simopoulos AP, Kifer RR, Martin RE, eds. Health effects of polyunsaturated fatty acids in seafoods. Orlando, FL: Academic Press, 1986:227–38.
31. Brox JH, Killie JE, Osterud B, Holme S, Nordoy A. Effects of cod liver oil on platelets and coagulation in familial hypercholesterolemia (type IIa). Acta Med Scand 1983;213:137–44.
32. Joist JH, Baker RK, Schonfeld G. Increased in vivo and in vitro platelet function in type II- and type IV-hyperlipoproteinemia. Thromb Res 1979;15:95–108.
33. Weber PC. Are we what we eat? Fatty acids in nutrition and in cell membranes: cell functions and disorders induced by dietary conditions. In: Fish fats and your health. Norway: Svanoy Foundation, 1989:9–18. (Report no. 4.)
34. Weber PC, Leaf A. Cardiovascular effects of omega-3 fatty acids. Atherosclerosis risk factor modification by omega-3 fatty acids. World Rev Nutr Diet 1991:66:218–32.
35. Clarke SD, Romsos DR, Leveille GA. Differential effects of dietary methylesters of long chain saturated and polyunsaturated fatty acids on rat liver and adipose tissue lipogenesis. J Nutr 1977;107:1170–80.
36. Clarke SD, Armstrong MK, Jump DB. Nutritional control of rat liver fatty acid synthase and S14 mRNA abundance. J Nutr 1990; 120:218–24.
37. Clarke SD, Jump DB. Fatty acid regulation of gene expression: a unique role for polyunsaturated fats. In: Berdanier C, Hargrove JL, eds. Nutrition and gene expression. Boca Raton, FL: CRC Press, 1993:227–46.
38. Clarke SD, Jump DB. Polyunsaturated fatty acid regulation of hepatic gene transcription. Lipids 1996;31(suppl):S7–11.
39. Ntambi JM. Dietary regulation of stearoyl-CoA desaturase I gene expression in mouse liver. J Biol Chem 1991;267:10925–30.

40. DeWillie JW, Farmer SJ. Linoleic acid controls neonatal tissue-specific stearoyl-CoA desaturase mRNA levels. Biochim Biophys Acta 1993;1170:291–5.
41. Jump DB, Clarke SD, Thelen A, Liimatta N. Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. J Lipid Res 1994;35:1076–84.
42. Liimatta M, Towle HC, Clarke SD, Jump DB. Dietary PUFA interfere with the insulin glucose activation of L-Type pyruvate kinase. Mol Endocrinol 1994;8:1147–53.
43. Tebbey PW, McGowan KM, Stephens JM, Buttke TM, Pekata PH. Arachidonic acid down regulates the insulin dependent glucose transporter gene (Glut 4) in 3T3-L1 adipocytes by inhibiting transcription and enhancing mRNA turnover. J Biol Chem 1994;269:639–44.
44. Sellmayer A, Danesch U, Weber PC. Effects of different polyunsaturated fatty acids on growth-related early gene expression and cell growth. Lipids 1996;31:S37–40.
45. De Caterina R, Libby P. Control of endothelial leukocyte adhesion molecules by fatty acids. Lipids 1996;31(suppl):S57–63.
46. Robinson DR, Urakaze M, Huang R, et al. Dietary marine lipids suppress the continuous expression of interleukin 1B gene transcription. Lipids 1996;31(suppl):S23–31.
47. Kaminski WE, Jendraschak E, Kiefl R, von Schacky C. Dietary omega-3 fatty acids lower levels of platelet-derived growth factor mRNA in human mononuclear cells. Blood 1993;81:1871–9.
48. Graber R, Sumida C, Nunez EA. Fatty acids and cell signal transduction. J Lipid Mediat Cell Signal 1994;9:91–116.
49. Clarke SD, Jump DB. Dietary polyunsaturated fatty acid regulation of gene transcription. Annu Rev Nutr 1994;14:83–98.
50. Indu M, Ghafoorunissa. n−3 Fatty acids in Indian diets—comparison of the effects of precursor (alpha-linolenic acid) vs product (long chain n−3 polyunsaturated fatty acids). Nutr Res 1992;12:569–82.
51. Emken EA, Adlot RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and lino lenic acids in young adult males. Biochim Biophys Acta 1994;1213: 277–88.
52. Mantzioris E, James MJ, Gibson RA, Cleland LG. Dietary substitution with an α-linolenic acid–rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. Am J Clin Nutr 1994;59:1304–9.
53. McLennan PL. Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. Am J Clin Nutr 1993;57:207–12.
54. Reaven P, Parthasarathy S, Grasse BJ, et al. Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. Am J Clin Nutr 1991;54:701–6.
55. Abbey M, Belling GB, Noakes M, Hirata F, Nestel PJ. Oxidation of low-density lipoproteins: intraindividual variability and the effect of dietary linoleate supplementation. Am J Clin Nutr 1993;57:391–8.
56. Renaud S. Linoleic acid, platelet aggregation and myocardial infarction. Atherosclerosis 1990;80:255–6.
57. 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.
58. Renaud S, Morazain R, Godsey F, et al. Nutrients, platelet function and composition in nine groups of French and British farmers. Atherosclerosis 1986;60:37–48.
59. Renaud S, Godsey F, Dumont E, Thevenon C, Ortchanian E, Martin JL. Influence of long-term diet modification on platelet function and composition in Moselle farmers. Am J Clin Nutr 1986;43:136–50.
60. Budowski P, Crawford MA. alpha-Linolenic acid as a regulator of the metabolism of arachidonic acid: dietary implications of the ratio of n−6:n−3 fatty acids. Proc Nutr Soc 1985;44:221–9.
61. Berry EM, Hirsch J. Does dietary linoleic acid influence blood pressure? Am J Clin Nutr 1986;44:336–40.
62. Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willett WC. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. BMJ 1996;313:84–90.
63. Phillipson BE, Rothrock DW, Connor WE, Harris WS, Illingworth DR. Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N Engl J Med 1985;312:1210–6.
64. Nestel PJ. Fish oil attenuates the cholesterol-induced rise in lipoprotein cholesterol. Am J Clin Nutr 1986;43:752–7.
65. Pauletto P, Puato M, Angeli MT, et al. Blood pressure, serum lipids, and fatty acids in populations on a lake-fish diet or on a vegetarian diet in Tanzania. Lipids 1996;31(suppl):S309–12.
66. Connor WE. Hypolipidemic effects of dietary omega-3 fatty acids in normal and hyperlipidemic humans: effectiveness and mechanisms. In: Simopoulos AP, Kifer RR, Martin RE, eds. Health effects of polyunsaturated fatty acids in seafoods. Orlando, FL: Academic Press, 1986:173–210.
67. Harris WS, Connor WE, Inkeles SB, Illingworth DR. Omega-3 fatty acids prevent carbohydrate-induced hypertriglyceridemia. Metabolism 1984;33:1016–9.
68. Nestel PJ, Connor WE, Reardon MR, Connor S, Wong S, Boston R. Suppression by diets rich in fish oil of very low density lipoprotein production in man. J Clin Invest 1984;74:72–89.
69. Sanders TAB, Sullivan DR, Reeve J, Thompson GR. Triglyceride-lowering effect of marine polyunsaturates in patients with hypertriglyceridemia. Arteriosclerosis 1985;5:459–65.
70. Saynor R, Verel D, Gillott T. The long term effect of dietary supplementation with fish lipid concentrate on serum lipids, bleeding time, platelets and angina. Atherosclerosis 1984;50:3–10.
71. Dehmer GJ, Pompa JJ, Van den Berg EK, et al. Reduction in the rate of early restenosis after coronary angioplasty by a diet supplemented with n−3 fatty acids. N Engl J Med 1988;319:733–40.
72. Eritsland J, Arnesen H, Gronseth K, Fjeld NB, Abdelnoor M. Effect of dietary supplementation with n−3 fatty acids on coronary artery bypass graft patency. Am J Cardiol 1996;77:31–6.
73. Gapinski JP, VanRuiswyk JV, Heudehert GR, Schectman GS. Prevention of restenosis with fish oils following coronary angioplasty. A meta-analysis. Arch Intern Med 1993;153:1595–601.
74. Leaf A, Jorgensen MB, Jacobs AK, et al. Do fish oils prevent restenosis after coronary angioplasty? Circulation 1994;90:2248–57.
75. 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.
76. Simon JA, Hodgkins ML, Browner WS, Neuhaus JM, Bernert JT Jr, Hulley SB. Serum fatty acids and the risk of coronary heart disease. Am J Epidemiol 1995;142:469–76.
77. Sellmayer A, Witzgall H, Lorenz RL, Weber PC. Effects of dietary fish oil on ventricular premature complexes. Am J Cardiology 1995;76:974–7.
78. Christensen JH, Gustenhoff P, Eilersen E, et al. n−3 Fatty acids and ventricular extrasystoles in patients with ventricular tachyarrhythmias. Nutr Res 1995;15:1–8.
79. Charnock JS. Antiarrhythmic effects of fish oils. World Rev Nutr Diet 1991;66:278–91.
80. 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.
81. 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.
82. Kang JX, Leaf A. Prevention and termination of the β-adrenergic agonist-induced arrhythmias by free polyunsaturated fatty acids in neonatal rat cardiac myocytes. Biochem Biophy Res Comm 1995; 208:629–36.
83. de Lorgeril M, Salen P, Martin J-L, et al. Effect of a Mediterranean type of diet on the rate of cardiovascular complications in patients with coronary artery disease. Insights into the cardioprotective effect of certain nutrients. J Am Coll Cardiol 1996;28:1103–8.
84. Sandker GN, Kromhout D, Aravanis C, et al. Serum cholesteryl ester fatty acids and their relation with serum lipids in elderly men in Crete and the Netherlands. Eur J Clin Nutr 1993;47:201–8.

85. Kagawa Y, Nishizawa M, Suzuki M, et al. Eicosapolyenoic acids of serum lipids of Japanese Islanders with low incidence of cardiovascular diseases. J Nutr Sci Vitaminol (Tokyo) 1982;28:441–53.
86. Simopoulos AP. Is insulin resistance influenced by dietary linoleic acid and trans fatty acids? Free Radic Biol Med 1994;17:367–72.
87. Yam D, Eliraz A, Berry EM. Diet and disease—the Israeli paradox: possible dangers of a high omega-6 polyunsaturated fatty acid diet. Isr J Med Sci 1996;32:1134–43.
88. Tatarannni PA, Baier LJ, Paolisso G, Howard BV, Ravussin E. Role of lipids in development of noninsulin-dependent diabetes mellitus: lessons learned from the Pima Indians. Lipids 1996;31:S267–70.
89. Simopoulos AP, Herbert V, Jacobson B. The healing diet. New York: Macmillan, 1995.
90. Amri EZ, Bertrand B, Ailhaud G, Grimaldi P. Regulation of adipose cell differentiation. I. Fatty acids are inducers of the aP2 gene expression. J Lipid Res 1991;32:1449–56.
91. Simopoulos AP. Part I: Metabolic effects of omega-3 fatty acids and essentiality. In: Spiller GA, ed. Handbook of lipids in human nutrition. Boca Raton, FL: CRC Press, 1996:51–73.
92. Despres J-P, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C. Genetic aspects of susceptibility to obesity and related dyslipidemias. Mol Cell Biochem 1992;113:151–69.
93. Carmelli D, Cardon LR, Fabsitz R. Clustering of hypertension, diabetes, and obesity in adult male twins: same genes or same environments. Am J Hum Genet 1994;55:566–73.
94. Uusitupa MI, Karhunen L, Rissanen A, et al. Apolipoprotein E phenotype modifies metabolic and hemodynamic abnormalities related to central obesity in women. Am J Clin Nutr 1996;64:131–6.
95. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med 1993;328:238–44.
96. Simopoulos AP. Fatty acid composition of skeletal muscle membrane phospholipids, insulin resistance and obesity. Nutr Today 1994;2:12–6.
97. Harris WS. 1996. Do ω3 fatty acids worsen glycemic control in NIDDM? ISSFAL Newsletter 1996;3:6–9.
98. Fanaian M, Szilasi J, Storlien L, Calvert GD. The effect of modified fat diet on insulin resistance and metabolic parameters in type II diabetes. Diabetologia 1996;39(suppl):A7 (abstr).