MINIREVIEW

The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases

ARTEMIS P. SIMOPOULOS[1]

The Center for Genetics, Nutrition and Health, Washington, DC 20009

Several sources of information suggest that human beings evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids (EFA) of ~1 whereas in Western diets the ratio is 15/1–16.7/1. Western diets are deficient in omega-3 fatty acids, and have excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved and their genetic patterns were established. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio, as is found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a lower omega-6/omega-3 ratio), exert suppressive effects. In the secondary prevention of cardiovascular disease, a ratio of 4/1 was associated with a 70% decrease in total mortality. A ratio of 2.5/1 reduced rectal cell proliferation in patients with colorectal cancer, whereas a ratio of 4/1 with the same amount of omega-3 PUFA had no effect. The lower omega-6/omega-3 ratio in women with breast cancer was associated with decreased risk. A ratio of 2–3/1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5/1 had a beneficial effect on patients with asthma, whereas a ratio of 10/1 had adverse consequences. These studies indicate that the optimal ratio may vary with the disease under consideration. This is consistent with the fact that chronic diseases are multigenic and multifactorial. Therefore, it is quite possible that the therapeutic dose of omega-3 fatty acids will depend on the degree of severity of disease resulting from the genetic predisposition. A lower ratio of omega-6/omega-3 fatty acids is more desirable in reducing the risk of many of the chronic diseases of high prevalence in Western societies, as well as in the developing countries. Exp Biol Med 233:674–688, 2008

[1] To whom correspondence should be addressed at The Center for Genetics, Nutrition and Health, 2001 S Street, NW, Suite 530, Washington, DC 20009. E-mail: cgnh@bellatlantic.net

DOI: 10.3181/0711-MR-311
1535-3702/08/2336-0674$15.00

Key words: balanced omega-6/omega-3 ratio; dietary omega-3 fatty acids; inflammation; cardiovascular disease; chronic diseases; diet-gene interactions

Introduction

The interaction of genetics and environment, nature, and nurture is the foundation for all health and disease. In the last two decades, using the techniques of molecular biology, it has been shown that genetic factors determine susceptibility to disease and environmental factors determine which genetically susceptible individuals will be affected (1–6). Nutrition is an environmental factor of major importance. Using the tools of molecular biology and genetics, research is defining the mechanisms by which genes influence nutrient absorption, metabolism and excretion, taste perception, and degree of satiation; and the mechanisms by which nutrients influence gene expression. Whereas major changes have taken place in our diet over the past 10,000 years since the beginning of the Agricultural Revolution, our genes have not changed. The spontaneous mutation rate for nuclear DNA is estimated at 0.5% per million years. Therefore, over the past 10,000 years there has been time for very little change in our genes, perhaps 0.005%. In fact, our genes today are very similar to the genes of our ancestors during the Paleolithic period 40,000 years ago, at which time our genetic profile was established (7). Humans today live in a nutritional environment that differs from that for which our genetic constitution was selected. Studies on the evolutionary aspects of diet indicate that major changes have taken place in our diet, particularly in the type and amount of essential fatty acids and in the antioxidant content of foods (7–11) (Fig. 1).

Today industrialized societies are characterized by 1) an

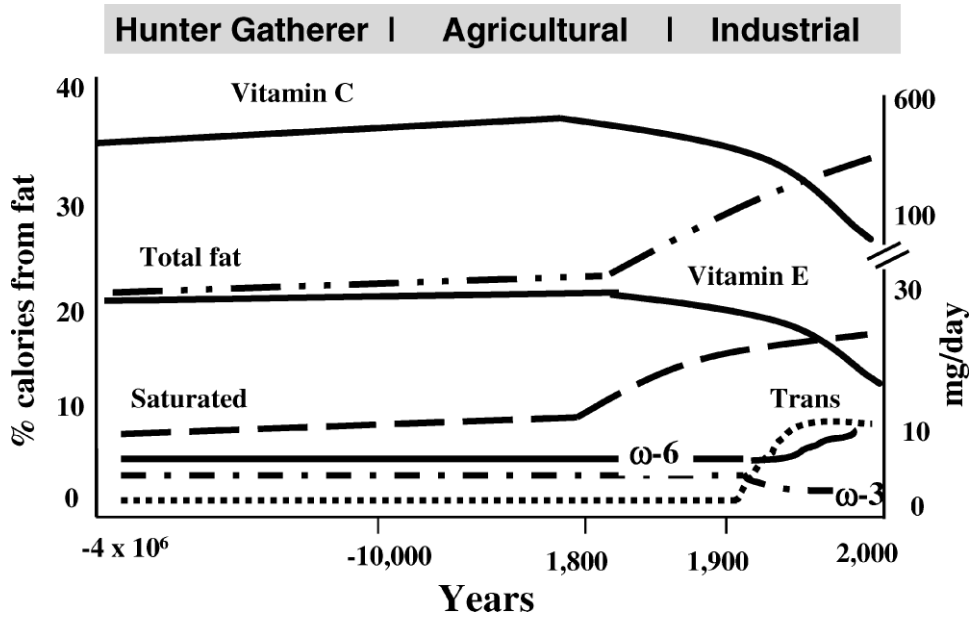

Figure 1. Hypothetical scheme of fat, fatty acid (ω6, ω3, trans and total) intake (as percent of calories from fat) and intake of vitamins E and C (mg/d). Data were extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 years (9).

increase in energy intake and decrease in energy expenditure; 2) an increase in saturated fat, omega-6 fatty acids and trans fatty acids, and a decrease in omega-3 fatty acid intake; 3) a decrease in complex carbohydrates and fiber; 4) an increase in cereal grains and a decrease in fruits and vegetables; and 5) a decrease in protein, antioxidants and calcium intake (7, 9, 12–15) (Tables 1 and 2). The increase in *trans* fatty acids is detrimental to health as shown in Table 3 (17). In addition, *trans* fatty acids interfere with the desaturation and elongation of both omega-6 and omega-3 fatty acids, thus further decreasing the amount of arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid availability for human metabolism (18).

Table 1. Estimated Omega-3 and Omega-6 Fatty Acid Intake in the Late Paleolithic Period (g/d)[a,b,c]

Plants	
LA	4.28
ALA	11.40
Animals	
LA	4.56
ALA	1.21
Total	
LA	8.84
ALA	12.60
Animal	
AA (ω6)	1.81
EPA (ω3)	0.39
DTA (ω6)	0.12
DPA (ω3)	0.42
DHA (ω3)	0.27
Ratios of ω6/ω3	
LA/ALA	0.70
AA+DTA/EPA+DPA+DHA	1.79
Total ω6/ω3	0.79[b]

[a] Data from Eaton *et al.* (13).
[b] Assuming an energy intake of 35:65 of animal:plant sources.
[c] LA, linoleic acid; ALA, linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetranoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

The beneficial health effects of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were described first in the Greenland Eskimos who consumed a high seafood diet and had low rates of coronary heart disease, asthma, type 1 diabetes mellitus, and multiple sclerosis. Since that observation, the beneficial health effects of omega-3 fatty acids have been extended to include benefits related to cancer, inflammatory bowel disease, rheumatoid arthritis, and psoriasis (19).

Whereas evolutionary maladaptation leads to reproductive restriction (or differential fertility), the rapid changes in our diet, particularly the last 150 years, are potent promoters of chronic diseases such as atherosclerosis, essential hypertension, obesity, diabetes, arthritis and other autoimmune diseases, and many cancers, especially cancer of the breast (20), colon (21), and prostate (22). In addition to diet, sedentary lifestyles and exposure to noxious substances interact with genetically controlled biochemical processes leading to chronic disease.

In this review, I discuss the importance of the balance of omega-6 and omega-3 essential fatty acids in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, osteoporosis, other inflammatory and autoimmune disorders, cancer and mental health, and the mechanisms involved.

Table 2. Late Paleolithic and Currently Recommended Nutrient Composition for Americans

	Late Paleolithic[a]	FNB-IOM 1989 recommendations[a]	FNB-IOM 2005 recommendations[b]
Total dietary energy, (%)			
Protein	33	12	10–35
Carbohydrate	46	58	45–65
Fat	21	30	20–35
Alcohol	~0	–	–
P/S ratio[c]	1.41	1.00	–
Cholesterol (mg)	520	300	<300
Fiber (g)	100–150	30–60	38
Sodium (mg)	690	1100–3300	<2300
Calcium (mg)	1500–2000	800–1600	1000–1300
Ascorbic acid (mg)	440	60	75

[a] Modified from Eaton *et al.* (13).
[b] Data from DRI Tables on the internet: http://www.iom.edu/CMS/3788/4574.aspx
[c] P/S, polyunsaturated to saturated fat.

Imbalance of Omega-6/Omega-3

Food technology and agribusiness provided the economic stimulus that dominated the changes in the food supply (23, 24). From per capita quantities of foods available for consumption in the U.S. national food supply in 1985, the amount of EPA is reported to be about 50 mg per capita/day and the amount of DHA is 80 mg per capita/day. The two main sources are fish and poultry (25). It has been estimated that the present Western diet is "deficient" in omega-3 fatty acids with a ratio of omega-6 to omega-3 of 15–20/1, instead of 1/1 as is the case with wild animals and presumably human beings (7–11, 13, 26–28) (Table 4).

Before the 1940s cod-liver oil was ingested mainly by children as a source of vitamin A and vitamin D with the usual dose being a teaspoon. Once these vitamins were synthesized, consumption of cod-liver oil was drastically decreased, contributing further to the decrease of EPA and DHA intake. Table 5 shows ethnic differences in fatty acid concentrations in thrombocyte phospholipids, the ratios of omega-6/omega-3 fatty acids, and percentage of all deaths from cardiovascular disease (16).

An absolute and relative change of omega-6/omega-3 in the food supply of Western societies has occurred over the last 150 years. A balance existed between omega-6 and omega-3 for millions of years during the long evolutionary history of the genus Homo, and genetic changes occurred partly in response to these dietary influences. During evolution, omega-3 fatty acids were found in all foods consumed: meat, wild plants, eggs, fish, nuts and berries (29–38). Studies by Cordain *et al*. (39) on wild animals confirm the original observations of Crawford and Sinclair *et al*. (27,40). However, rapid dietary changes over short periods of time as have occurred over the past 100–150 yr is a totally new phenomenon in human evolution (13, 15, 41–43) (Table 6).

Table 3. Adverse Effects of Trans Fatty Acids[a]

Decrease or inhibit
Decrease or inhibit incorporation of other fatty acids into cell membranes
Decrease high-density lipoprotein (HDL)
Inhibit delta-6 desaturase (interfere with elongation and desaturation of essential fatty acids)
Decrease serum testosterone (in male rats)
Cross the placenta and decrease birth weight (in humans)
Increase
Low-density lipoprotein (LDL)
Platelet aggregation
Lipoprotein (a) [Lp(a)]
Body weight
Cholesterol transfer protein (CTP)
Abnormal morphology of sperm (in male rats)

[a] Modified from reference 17.

Biological Effects and the Omega-6/Omega-3 Ratio

There are two classes of essential fatty acids (EFA), omega-6 and omega-3. The distinction between omega-6 and omega-3 fatty acids is based on the location of the first double bond, counting from the methyl end of the fatty acid molecule. In the omega-6 fatty acids, the first double bond is between the 6th and 7th carbon atoms and for the omega-3 fatty acids the first double bond is between the 3rd and 4th

Table 4. Ratios of Dietary Omega-6:Omega-3 Fatty Acids in the Late Paleolithic Period and in Current Western Diets (United States) (g/d)[a]

	Paleolithic	Western
LA:ALA	0.70	18.75
AA+DTA:EPA+DPA+DHA	1.79	3.33
Total	0.79	16.74

[a] LA, linoleic acid; ALA, linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetranoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. Reprinted with permission from reference (15).

Table 5. Ethnic Differences in Fatty Acid Concentrations in Thrombocyte Phospholipids and Percentage of All Deaths from Cardiovascular Disease[a]

	Europe and United States (%)	Japan (%)	Greenland Eskimos (%)
Arachidonic acid (20:4ω6)	26	21	8.3
Eicosapentaenoic acid (20:5ω3)	0.5	1.6	8.0
Ratio of ω6/ω3	50	12	1
Mortality from cardiovascular disease	45	12	7

[a] Data modified from reference 16.

carbon atoms. Monounsaturates are represented by oleic acid, an omega-9 fatty acid, which can be synthesized by all mammals including humans. Its double bond is between the 9th and 10th carbon atoms.

Omega-6 and omega-3 fatty acids are essential because humans, like all mammals, cannot make them and must obtain them in their diet. Omega-6 fatty acids are represented by linoleic acid (LA; 18:2ω6) and omega-3 fatty acids by α-linolenic acid (ALA; 18:3ω3). LA is plentiful in nature and is found in the seeds of most plants except for coconut, cocoa, and palm. ALA on the other hand is found in the chloroplasts of green leafy vegetables, and in the seeds of flax, rape, chia, perilla and in walnuts. Both EFA are metabolized to longer-chain fatty acids of 20 and 22 carbon atoms. LA is metabolized to arachidonic acid (AA; 20:4ω6), and LNA to EPA (20:5ω3) and DHA (22:6ω3), increasing the chain length and degree of unsaturation by adding extra double bonds to the carboxyl end of the fatty acid molecule (Fig. 2).

Humans and other mammals, except for carnivores such as lions, can convert LA to AA and ALA to EPA and DHA, but it is slow (44). This conversion was shown by using deuterated ALA (45). There is competition between omega-6 and omega-3 fatty acids for the desaturation enzymes. However, both Δ-4 and Δ-6 desaturases prefer omega-3 to omega-6 fatty acids (44, 46, 47). But, a high LA intake interferes with the desaturation and elongation of ALA (45, 48). Trans fatty acids interfere with the desaturation and elongation of both LA and ALA. Δ-6 desaturase is the limiting enzyme and there is some evidence that it decreases with age (44). Premature infants (49), hypertensive individuals (50), and some diabetics (51) are limited in their ability to make EPA and DHA from ALA. These findings are important and need to be considered when making dietary recommendations. EPA and DHA are found in the oils of fish, particularly fatty fish. AA is found predominantly in the phospholipids of grain-fed animals and eggs.

LA, ALA, and their long-chain derivatives are important components of animal and plant cell membranes. In mammals and birds, the n-3 fatty acids are distributed selectively among lipid classes. ALA is found in triglycerides, in cholesteryl esters, and in very small amounts in phospholipids. EPA is found in cholesteryl esters, triglycerides, and phospholipids. DHA is found mostly in phospholipids. In mammals, including humans, the cerebral cortex, retina, and testis and sperm are particularly rich in DHA. DHA is one of the most abundant components of the brain's structural lipids. DHA, like EPA, can be derived only from direct ingestion or by synthesis from dietary EPA or ALA.

Table 6. Omega-6:Omega-3 Ratios in Various Populations

Population	ω6/ω3	Reference
Paleolithic	0.79	(13)
Greece prior to 1960	1.00–2.00	(15)
Current Japan	4.00	(41)
Current India, rural	5–6.1	(42)
Current United Kingdom and northern Europe	15.00	(43)
Current United States	16.74	(13)
Current India, urban	38–50	(42)

Mammalian cells cannot convert omega-6 to omega-3 fatty acids because they lack the converting enzyme, omega-3 desaturase. LA, the parent omega-6 fatty acid, and ALA, the parent omega-3 fatty acid, and their long-chain derivatives are important components of animal and plant cell membranes (Fig. 2). These two classes of EFA are not interconvertible, are metabolically and functionally distinct, and often have important opposing physiological functions. When humans ingest fish or fish oil, the EPA and DHA from the diet partially replace the omega-6 fatty acids, especially AA, in the membranes of probably all cells, but especially in the membranes of platelets, erythrocytes, neutrophils, monocytes, and liver cells (reviewed in references 8, 52). Whereas cellular proteins are genetically determined, the polyunsaturated fatty acid (PUFA) composition of cell membranes is to a great extent dependent on the dietary intake. AA and EPA are the parent compounds for eicosanoid production (8) (Tables 7–8, Fig. 3).

Because of the increased amounts of omega-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 omega-3 fatty acids, specifically EPA (8). The eicosanoids from AA are biologically active in very small quantities and, if they are formed in large amounts, they contribute to the formation of thrombus and atheromas; to allergic and inflammatory disorders, particularly in susceptible people; and to proliferation of cells. Thus, a diet rich in omega-6 fatty acids shifts the physiological state to one that is prothrombotic and proaggregatory, with increases in blood viscosity, vasospasm, and vasocontriction and decreases in bleeding time. Bleeding time is decreased in groups of patients with hypercholesterolemia, hyperlipoproteinemia, myocardial in-

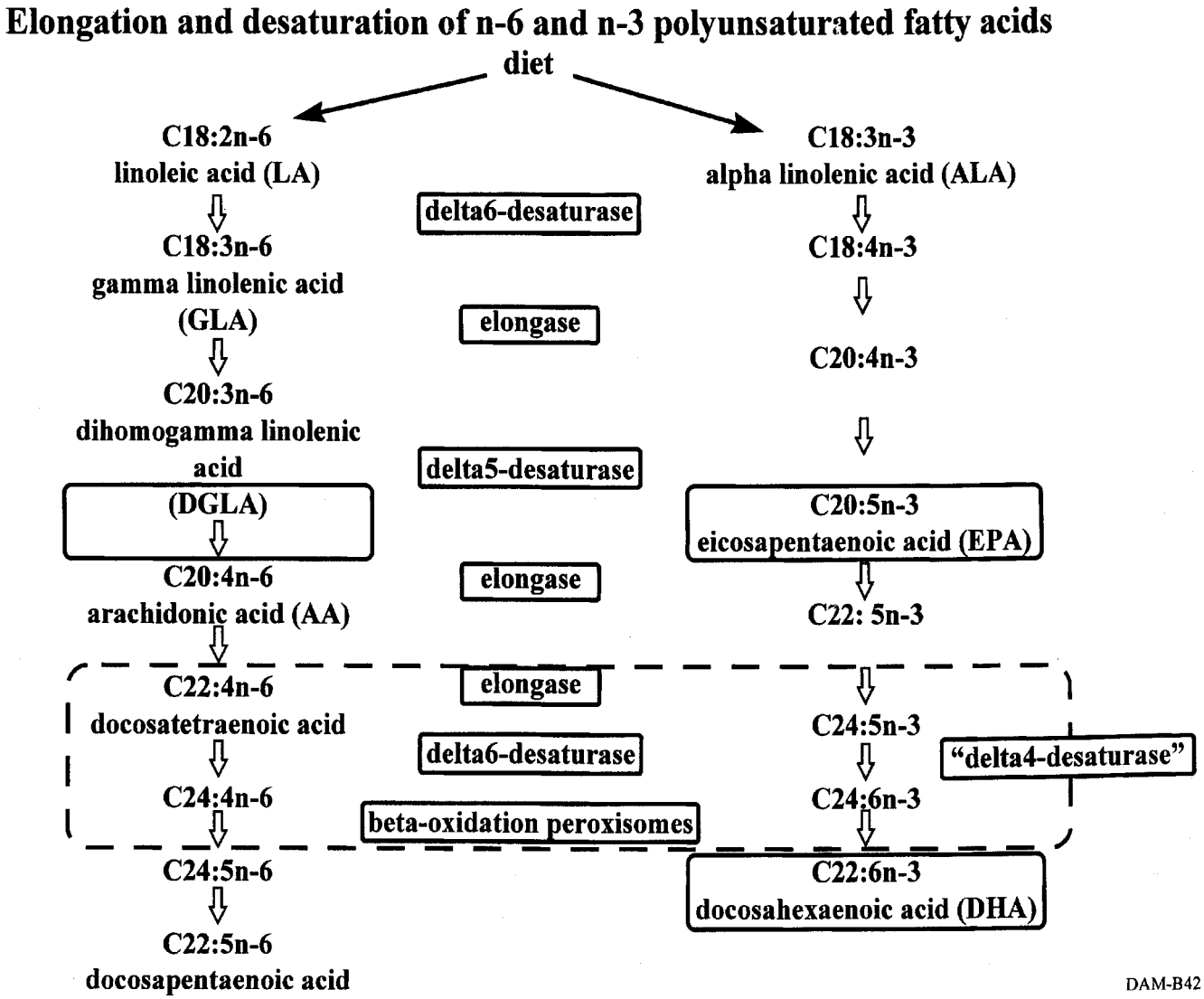

Figure 2. Elongation and desaturation of omega-6 and omega-3 polyunsaturated fatty acids.

farction, other forms of atherosclerotic disease, and diabetes (obesity and hypertriglyceridemia). Bleeding time is longer in women than in men and longer in young than in old people. There are ethnic differences in bleeding time that appear to be related to diet.

Mechanisms

Linoleic Acid Increases Low-Density Lipoprotein Oxidation and Severity of Coronary Atherosclerosis. Oxidative modification increases the atherogenicity of low-density lipoprotein (LDL) cholesterol. Oxidized LDL is taken up by scavenger receptors that do not recognize unmodified LDL leading to foam cell formation. Diets enriched with LA increase the LA content of LDL and its susceptibility to oxidation (53, 54). Reaven *et al.* (55) showed that a LA-enriched diet especially affects oxidation of small, dense LDL. Louheranta *et al.* (56) showed that as the percent of energy intake from LA increased from the lower quartile 2.9% to the highest 6.4% so did the LDL oxidation. In their study, the average energy from LA was 4.6%. In another small cross-sectional study, enhanced susceptibility of LDL to oxidize was associated with severity of coronary atherosclerosis (57).

Linoleic Acid Inhibits Eicosapentaenoic Acid Incorporation from Dietary Fish Oil Supplements in Human Subjects. Cleland *et al.* showed that LA inhibits EPA incorporation from dietary fish oil supplements in human subjects (58). Thirty healthy male subjects were randomly allocated into one of two treatment groups. One group was on a high LA and low saturated fatty acid diet, whereas the other group was on a low LA and low saturated fat diet. The difference in the low LA and low saturated fatty acid diet was made up with monounsaturated fatty acids (olive oil). After a 3-week run-in period, the subjects consumed a fish oil supplement containing 1.6 g EPA and 0.32 g DHA per day. After four weeks of fish oil

Table 7. Effects of Ingestion of EPA and DHA from Fish or Fish Oil

Decreased production of prostaglandin E_2 (PGE_2) metabolites
A decrease in thromboxane A_2, a potent platelet aggregator and vasoconstrictor
A decrease in leukotriene B_4 formation, an inducer of inflammation, and a powerful inducer of leukocyte chemotaxis and adherence
An increase in thromboxane A_3, a weak platelet aggregator and weak vasoconstrictor
An increase in prostacyclin PGI_3, leading to an overall increase in total prostacyclin by increasing PGI_3 without a decrease in PGI_2, both PGI_2 and PGI_3 are active vasodilators and inhibitors of platelet aggregation
An increase in leukotriene B_5, a weak inducer of inflammation and a weak chemotactic agent

supplementation, the incorporation of EPA in neutrophil membrane phospholipids was highest in the lowest LA group, indicating that the ingestion of omega-6 fatty acids within the diet is an important determinant of EPA incorporation into neutrophil membranes. This study also shows that monounsaturated fatty acids, in this case olive oil, do not interfere with EPA incorporation.

Decreasing Linoleic Acid with Constant α-Linolenic Acid in Dietary Fats Increases (Omega-3) Eicosapentaenoic Acid in Plasma Phospholipids in Healthy Men. Liou *et al*. carried out a study in which decreasing levels of LA with constant ALA led to increases of EPA in plasma phospholipids in healthy men (59). The omega-6/omega-3 dietary ratio varied between 10/1 to 4/0 of LA/ALA. It is unfortunate that the authors did not have a lower ratio of 2–1/1 omega-6/omega-3, which is closer to the ratio on which humans evolved. At a ratio of 1/1, Zampelas *et al*. showed a decrease in C-reactive protein (CRP), which Liou *et al*. at a ratio of 4/1 did not show (60).

A Lower Omega-6/Omega-3 Ratio as Part of a Mediterranean Diet Decreases Vascular Endothelial Growth Factor. Ambring *et al*. studied the ratio of serum phospholipid omega-6 to omega-3 fatty acids, the number of leukocytes and platelets, and vascular endothelial growth factor (VEGF) in healthy subjects on an ordinary Swedish diet and on a Mediterranean-inspired diet that was high in fish and flaxseed oil (61). This is a very interesting and important study, because it clearly showed that the serum phospholipid ratio of omega-6/omega-3 fatty acids was substantially lowered after the Mediterranean diet versus the Swedish diet. The omega-6/omega-3 ratio was 4.72 ± 0.19 on the Swedish diet and 2.60 ± 0.19 on the Mediterranean diet ($P < 0.0001$). There was no change in CRP or interleukin-6 (IL-6), but the total number of leukocytes was 10% lower after the Mediterranean diet, the total number of platelets was 15% lower, and so was the serum VEGF, 206 ± 25 pg/mL versus 237 ± 30 on the Swedish diet ($P = 0.0014$). The authors concluded that "A Mediterranean-inspired diet reduces the number of platelets and leukocytes and VEGF concentrations in healthy subjects. This may be linked to higher serum concentrations of omega-3 fatty acids, which promote a favorable composition of phospholipids." These findings are consistent with our studies on the traditional diet of Greece prior to 1960 that was rich in ALA, EPA and DHA and balanced in the omega-6/omega-3 ratio, which distinguished it from other Mediterranean diets (62, 63), by being similar in the omega-6/omega-3 ratio to the diet on which human beings evolved (7–13, 26–28).

As the Omega-6/Omega-3 Ratio Decreases, So Does the Platelet Aggregation. Freese *et al*. compared the effects of two diets rich in monounsaturated fatty acids, differing in their LA/ALA ratio on platelet aggregation in human volunteers (64). Both diets were similar in saturated, monounsaturated and polyunsaturated fatty acids. The results showed that platelet aggregation in vitro decreases as the ratio of LA/ALA decreases in diets rich in monounsaturated fatty acids.

The higher the ratio of omega-6/omega-3 fatty acids in platelet phospholipids, the higher the death rate from cardiovascular disease (16). Excessive amounts of omega-6 PUFA and a very high omega-6/omega-3 ratio, as is found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a lower omega-6/omega-3 ratio), exert suppressive effects (65).

Plasma Omega-6/Omega-3 Ratio and Inflammatory Markers. Ferrucci *et al*. studied the relationship of plasma PUFA to circulating inflammatory markers in 1123 persons aged 20–98 years in a community-based sample (66). The total omega-3 fatty acids were independently associated with lower levels of pro-inflammatory markers [IL-6, IL-1ra, tumor necrosis factor-α (TNFα), CRP], and higher anti-inflammatory markers [soluble IL-6r, IL-10, transforming growth factor-α (TGFα)] independent of confounders. The omega-6/omega-3 ratio was a strong negative correlate of IL-10. The authors concluded, "Omega-3 fatty acids are beneficial in patients affected by diseases characterized by active inflammation."

The Balance of Omega-6/Omega-3 Fatty Acids Is Important for Health: The Evidence from Gene Transfer Studies

Further support for the need to balance the omega-6/omega-3 EFA comes from the studies of Kang *et al*. (67, 68), which clearly show the ability of both normal rat cardiomyocytes and human breast cancer cells in culture to form all the omega-3's from omega-6 fatty acids when fed the cDNA encoding omega-3 fatty acid desaturase obtained from the roundworm *Caenorhabditis elegans* (*C. elegans*). The omega-3 desaturase efficiently and quickly converted the omega-6 fatty acids that were fed to the cardiomyocytes in culture to the corresponding omega-3 fatty acids. Thus,

Table 8. Effects of Omega-3 Fatty Acids on Factors Involved in the Pathophysiology of Atherosclerosis and Inflammation

Factor	Function	Effect of n-3 fatty acid
Arachidonic acid	Eicosanoid precursor; aggregates platelets; stimulates white blood cells	↓
Thromboxane A_2	Platelet aggregation; vasoconstriction; increase of intracellular Ca^{++}	↓
Prostacyclin ($PGI_{2/3}$)	Prevent platelet aggregation; vasodilation; increase cAMP	↑
Leukotriene (LTB_4)	Neutrophil chemoattractant; increase of intracellular Ca^{++}	↓
Fibrinogen	A member of the acute phase response; and a blood clotting factor	↓
Tissue plasminogen activator	Increase endogenous fibrinolysis	↑
Platelet activating factor (PAF)	Activates platelets and white blood cells	↓
Platelet-derived growth factor (PDGF)	Chemoattractant and mitogen for smooth muscles and macrophages	↓
Oxygen free radicals	Cellular damage; enhance LDL uptake via scavenger pathway; stimulate arachidonic acid metabolism	↓
Lipid hydroperoxides	Stimulate eicosanoid formation	↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil O_2 free radical formation; stimulate lymphocyte proliferation; stimulate PAF; express intercellular adhesion molecule-1 on endothelial cells; inhibit plasminogen activator, thus, procoagulants	↓
Interleukin-6	Stimulates the synthesis of all acute phase proteins involved in the inflammatory response: C-reactive protein; serum amyloid A; fibrinogen; α_1-chymotrypsin; and haptoglobin	↓
C-reactive protein (CRP)	An acute phase reactant and an independent risk factor for cardiovascular disease	↓
Endothelial-derived relaxation factor	Reduces arterial vasoconstrictor response	↑
Insulin function		Increases sensitivity to insulin
VLDL	Related to LDL and HDL level	↓
HDL	Decreases the risk for coronary heart disease	↑
Lp(a)	Lipoprotein(a) is a genetically determined protein that has atherogenic and thrombogenic properties	↓
Triglycerides and chylomicrons	Contribute to postprandial lipemia	↓

omega-6 LA was converted to omega-3 ALA and AA was converted to EPA, so that at equilibrium, the ratio of omega-6 to omega-3 PUFA was close to 1/1. Further studies demonstrated that the cancer cells expressing the omega-3 desaturase underwent apoptotic death whereas the control cancer cells with a high omega-6/omega-3 ratio continued to proliferate (69). More recently, Kang, *et al.* showed that transgenic mice and pigs expressing the *C. elegans fat-1* gene encoding an omega-3 fatty acid desaturase are capable of producing omega-3 from omega-6 fatty acids, leading to enrichment of omega-3 fatty acids with reduced levels of omega-6 fatty acids in almost all organs and tissues, including muscles and milk, with no need of dietary omega-3 fatty acid supply (70–72). This discovery provides a unique tool and new opportunities for omega-3 research, and raises the potential of production of *fat-1* transgenic livestock as a new and ideal source of omega-3 fatty acids to meet the human nutritional needs. Furthermore, the transgenic mouse model is being used widely by scientists for the study of chronic diseases and for the study of mechanisms of the beneficial effects of omega-3 fatty acids (73).

Omega-3 Fatty Acids and Gene Expression

Previous studies have shown that fatty acids 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 classical second messengers of the inositide phospholipid and the cyclic AMP signal transduction pathways. They can also act as modulator molecules mediating responses of the cell to extracellular signals. Recently it has been shown that fatty acids rapidly and directly alter the transcription of specific genes (74). In the case of genes involved in inflammation, such as IL-1β, EPA and DHA suppress IL-1β mRNA whereas AA does not, and the same effect appears in studies on growth-related early response gene expression and growth factor (74). In the case of vascular cell adhesion molecule (VCAM), AA has a modest suppressing effect relative to DHA. The latter situation may explain the protective effect of fish oil toward colonic carcinogenesis, since EPA and DHA did not stimulate protein kinase C. PUFA regulation of gene expression extends beyond the liver and includes genes

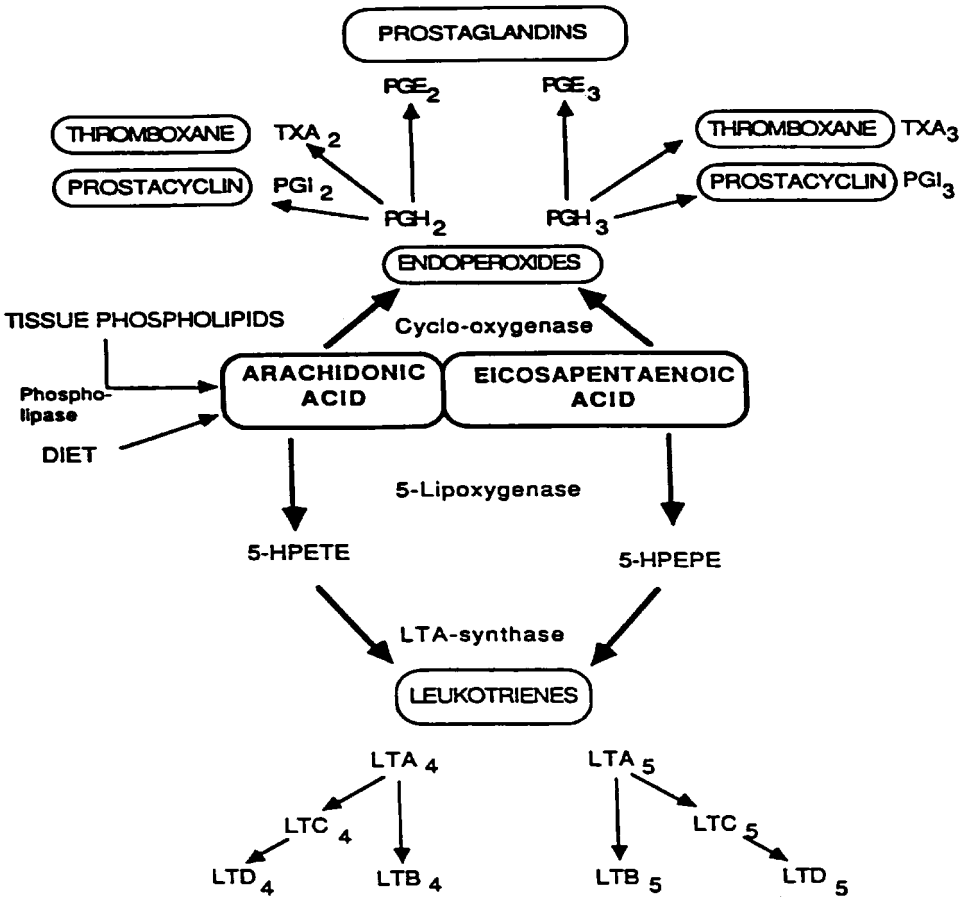

Figure 3. Oxidative metabolism of arachidonic acid and eicosapentaenoic acid by the cyclooxygenase and 5-lipoxygenase pathways. 5-HPETE denotes 5-hydroperoxyeicosatetranoic acid and 5-HPEPE denotes 5-hydroxyeicosapentaenoic acid.

such as adipocyte glucose transporter-4, lymphocyte stearoyl-CoA desaturase 2 in the brain, peripheral monocytes (IL-1β, and VCAM-1) and platelets [platelet derived growth factor (PDGF)]. Whereas some of the transcriptional effects of PUFA appear to be mediated by eicosanoids, the PUFA suppression of lipogenic and glycolytic genes is independent of eicosanoid synthesis, and appears to involve a nuclear mechanism directly modified by PUFA.

Linoleic Acid and Arachidonic Acid Increase Atherogenesis: Evidence from Diet-Gene Interactions: Genetic Variation and Omega-6 and Omega-3 Fatty Acid Intake in the Risk for Cardiovascular Disease

As discussed above, leukotrienes are inflammatory mediators generated from AA by the enzyme 5-lipoxygenase. Since atherosclerosis involves arterial inflammation, Dwyer *et al*. hypothesized that a polymorphism in the 5-lipoxygenase gene promoter could relate to atherosclerosis in humans, and that this effect could interact with the dietary intake of competing 5-lipoxygenase substrates (75). The study consisted of 470 healthy middle-aged women and men from the Los Angeles Atherosclerosis study, randomly sampled. The investigators determined 5-lipoxygenase (5-LO) genotypes, carotid-artery intima-media thickness, markers of inflammation, CRP, IL-6, dietary AA, EPA, DHA, LA, and ALA with the use of six 24-hour recalls of food intake. The results showed that 5-LO variant genotypes were found in 6.0% of the cohort. Mean intima-media thickness adjusted for age, sex, height and racial or ethnic group was increased by 80 ± 19 μm from among the carriers of two variant alleles as compared with the carrier of the common (wild-type) allele. In multivariate analysis, the increase in intima-media thickness among carriers of two variant alleles (62 μm, $P < 0.001$) was similar in this cohort to that associated with diabetes (64 μm, $P < 0.01$) the strongest common cardiovascular risk factor. Increased dietary AA significantly enhanced the apparent atherogenic effect of genotype, whereas increased dietary intake of omega-3 fatty acids EPA and DHA blunted this effect. Furthermore, the plasma level of CRP of two variant alleles was increased by a factor of 2, as compared with that among carriers of the common allele. Thus, genetic variation of 5-LO identifies a subpopulation with increased risk for atherosclerosis. The diet-gene interaction further suggests that dietary omega-6 fatty acids promote, whereas marine omega-3 fatty acids EPA and DHA inhibit leukotriene-

mediated inflammation that leads to atherosclerosis in this subpopulation.

The prevalence of variant genotypes did differ across racial and ethnic groups with higher prevalence among Asians or Pacific Islanders (19.4%), blacks (24.0%) and other racial or ethnic groups (18.2%) than among Hispanic subjects (3.6%) and non-Hispanic whites (3.1%). Increased intima-media thickness was significantly associated with intake of both AA and LA among carriers of the two variant alleles, but not among carriers of the common alleles. In contrast, the intake of marine omega-3 fatty acids was significantly and inversely associated with intima-media thickness only among carriers of the two variant alleles. Diet-gene interactions were specific to these fatty acids and were not observed for dietary intake of monounsaturated, saturated fat, or other measured fatty acids. The study constitutes evidence that genetic variation in an inflammatory pathway—in this case the leukotriene pathway—can trigger atherogenesis in humans. These findings could lead to new dietary and targeted molecular approaches for the prevention and treatment of cardiovascular disease according to genotype, particularly in the populations of non-European descent (6).

Clinical Intervention Studies and the Omega-6/Omega-3 EFA Balance

The Lyon Heart Study was a dietary intervention study in which a modified diet of Crete (the experimental diet) was compared with the prudent diet or Step I American Heart Association Diet (the control diet) (76–79). The experimental diet provided a ratio of LA to ALA of 4/1. This ratio was achieved by substituting olive oil and canola (oil) margarine for corn oil. Since olive oil is low in LA whereas corn oil is high, 8% and 61% respectively, the ALA incorporation into cell membranes was increased in the low LA diet. Cleland *et al.* (58) have shown that olive oil increases the incorporation of omega-3 fatty acids whereas the LA from corn oil competes. In the Lyon Heart Study, the ratio of 4/1 of LA/ALA led to a 70% decrease in total mortality at the end of two years (76).

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) Prevenzione Trial participants were on a traditional Italian diet plus 850–882 mg of omega-3 fatty acids at a ratio of 2/1 EPA to DHA (80). The supplemented group had a decrease in sudden cardiac death by 45%. Although there are no dietary data on total intake for omega-6 and omega-3 fatty acids, the difference in sudden death is most likely due to the increase of EPA and DHA and a decrease of AA in cell membrane phospholipids. Prostaglandins derived from AA are proarrhythmic, whereas the corresponding prostaglandins from EPA are not (81). In the Diet and Reinfarction Trial (DART), Burr *et al.* reported a decrease in sudden death in the group that received fish advice or took fish oil supplements relative to the group that did not (82). Similar results have been obtained by Singh *et al.* (83, 84). Except for the Lyon Heart Study, most of the cardiovascular disease omega-3 fatty acids supplementation trials did not attempt to modify the consumption of other fat components, and specifically did not seek to reduce the intake of omega-6 fatty acids despite the fact that there is convincing support for such studies. The differences in the omega-6/omega-3 ratio in the background diets and the dose of EPA and DHA could be an important factor in studies with conflicting results in intervention trials on the role of EPA and DHA in patients with ventricular arrhythmias in which a beneficial effect was shown by Leaf *et al.* (85) but not by Raitt *et al.* (86).

Yokoyama *et al.* investigated the effects of EPA on major coronary events in hypercholesterolemic patients in a randomized open label, blinded analysis (87). Patients were randomly assigned to receive either 1800 mg of EPA with statin or statin only in a 5-year follow up. The results showed that EPA is a promising treatment for prevention of major coronary events, and especially nonfatal coronary events, in Japanese hypercholesterolemic patients. This is a very important finding because the Japanese already have a high fish intake. These findings further support the data from the study by Iso *et al.* that showed, compared with a modest fish intake of once a week or about 20 g/d, a higher intake was associated with substantially reduced risk of coronary heart disease, primarily nonfatal cardiac events, among middle aged persons (88).

The importance of balancing the LA (omega-6) to ALA (omega-3) ratio was shown in a randomized, controlled, 3-diet, 3-period crossover study in which 22 hypercholesterolemic subjects were assigned to 3 experimental diets: a diet high in ALA (ALA diet; 6.5% of energy) a diet high in LA (LA diet; 12.6% of energy), and an average American diet (AAD) for 6 weeks (89). Serum IL-6, IL-1β, and TNF-α concentrations and the production of IL-6, IL-1β, and TNF-α by peripheral blood mononuclear cells (PBMCs) were measured. The ratio of omega-6/omega-3 was 10/1 in AAD, 4.1/1 in the LA diet and 2/1 in the ALA diet. The results showed that on the ALA diet, IL-6, IL-1β, and TNF-α production by PBMCs and serum TNF-α concentrations were lower ($P < 0.05$ and $P < 0.08$ respectively) than with the LA diet or AAD. PBMC production of TNF-α was inversely correlated with ALA ($P = 0.07$) and with eicosapentaenoic acid ($P = 0.03$) concentrations in PBMC lipids with the ALA diet. Changes in serum ALA were inversely correlated with changes in TNF-α produced by PBMCs ($P < 0.05$). In this study the increased intake of dietary ALA elicited anti-inflammatory effects by inhibiting IL-6, IL-1β, and TNF-α production in cultured PBMCs. Changes in PBMC ALA and EPA derived from ALA are associated with beneficial changes in TNF-α release. The cardioprotective effects of ALA are mediated in part by a reduction in the production of inflammatory cytokines, IL-6, IL-1β, and TNF-α (60, 90). Other studies, in which the ratio of LA/ALA was not balanced, failed to decrease CRP or IL-6, IL-1β, or TNF-α (91), leading to wrong conclusions that

LA is not inflammatory, despite the fact that it has been shown by Toborek *et al*. in 2002 (92). These results are important because they strongly suggest that the ALA intake at a ratio of 1–2/1—which is simple to implement, as shown by Paschos *et al*. (90) and Zampelas *et al*. (60)—could lead to an anti-inflammatory state, which is beneficial to health and reduce the risk for heart disease beyond that achieved from lower LDL concentrations alone.

Studies carried out in India indicate that the higher ratio of 18:2ω6 to 18:3ω3 equaling 20/1 in their food supply led to increases in the prevalence of non-insulin diabetes mellitus (NIDDM) in the population, whereas a diet with a ratio of 6/1 prevented the increase in NIDDM (93).

James and Cleland have reported beneficial effects in patients with rheumatoid arthritis (94) and Broughton has shown beneficial effects in patients with asthma by changing the background diet (95). James and Cleland evaluated the potential use of omega-3 fatty acids within a dietary framework of an omega-6/omega-3 ratio of 3–4/1 by supplying 4 gm of EPA+DHA and using flaxseed oil rich in ALA. In their studies, the addition of 4 gm EPA and DHA in the diet produced a substantial inhibition of production of IL-1β and TNFα when mononuclear cell levels of EPA were equal or greater than 1.5% of total cell phospholipid fatty acids which correlated with a plasma phospholipid EPA level equal to or greater than 3.2%. These studies suggest the potential for complementarity between drug therapy and dietary choices that increased intake of omega-3 fatty acids and decreased intake of omega-6 fatty acids may lead to drug-sparing effects. Therefore, future studies need to address the fat composition of the background diet, and the issue of concurrent drug use. A diet rich in omega-3 fatty acids and poor in omega-6 fatty acids provides the appropriate background biochemical environment in which drugs function.

Asthma is a mediator driven inflammatory process in the lungs and the most common chronic condition in childhood. The leukotrienes and prostaglandins are implicated in the inflammatory cascade that occurs in asthmatic airways. There is evidence of airway inflammation even in newly diagnosed asthma patients within 2–12 months after their first symptoms (96). Among the cells involved in asthma are mast cells, macrophages, eosinophils, and lymphocytes. The inflammatory mediators include cytokines and growth factors (peptide mediators) as well as the eicosanoids, which are the products of AA metabolism, which are important mediators in the underlying inflammatory mechanisms of asthma (Table 8; Fig. 3). Leukotrienes and prostaglandins appear to have the greatest relevance to the pathogenesis of asthma. The leukotrienes are potent inducers of bronchospasm, airway edema, mucus secretion, and inflammatory cell migration, all of which are important to the asthmatic symptomatology. Broughton *et al*. (95) studied the effect of omega-3 fatty acids at a ratio of omega-6/omega-3 of 10/1 to 5/1 in an asthmatic population in ameliorating methacholine-induced respiratory distress. With low omega-3 ingestion, methacholine-induced respiratory distress increased. With high omega-3 fatty acid ingestion, alterations in urinary 5-series leukotriene excretion predicted treatment efficacy and a dose change in >40% of the test subjects (responders) whereas the nonresponders had a further loss in respiratory capacity. A urinary ratio of 4-series to 5-series of <1 induced by omega-3 fatty acid ingestion may predict respiratory benefit.

Bartram *et al*. (97, 98) carried out two human studies in which fish oil supplementation was given in order to suppress rectal epithelial cell proliferation and prostaglandin E_2 (PGE_2) biosynthesis. This was achieved when the dietary omega-6/omega-3 ratio was 2.5/1 but not with the same absolute level of fish oil intake and an omega-6/omega-3 ratio of 4/1. More recently, Maillard *et al*. reported their results on a case control study (99). They determined omega-3 and omega-6 fatty acids in breast adipose tissue and relative risk of breast cancer. They concluded, "Our data based on fatty acid levels in breast adipose tissue (which reflect dietary intake) suggest a protective effect of omega-3 fatty acids on breast cancer risk and support the hypothesis that the balance between omega-3 and omega-6 fatty acids plays a role in breast cancer." In a case-control study in Shanghai, China on the relationship between fatty acids and breast cancer, Shannon *et al*. concluded, "Our results support a positive effect of omega-3 fatty acids on breast cancer risk and provide additional evidence for the importance of evaluating the ratio of fatty acids when evaluating diet and breast cancer risk (20)."

Osteoporosis represents a major challenge to health care services, particularly with increases in the elderly population worldwide. Bone mineral accrual during childhood and adolescence plays a vital role in preventing osteoporosis. The identification of factors influencing peak bone mass is important for the prevention of osteoporosis and related fractures. Genetic factors are responsible for about 70% of the variance in bone mass (100, 101). Other factors include nutrition, physical activity, and body mass index (BMI) (102). Animal studies have shown that dietary intake of long-chain omega-3 fatty acids may influence both bone formation and bone resorption (103, 104) and an increase in periosteal bone formation (105, 106).

The dietary ratio of omega-6/omega-3 fatty acids and bone mineral density in older adults was studied in the Rancho Bernardo Study by Weiss *et al*. (107). The study was carried out in 1532 community-dwelling men and women aged 45–90 years, between 1988 and 1992. The average intake of total omega-3 fatty acids was 1.3 g/d and the average ratio of total omega-6/omega-3 fatty acids was 8.4 in men and 7.9 in women. There was a significant inverse association between the ratio of dietary LA to ALA and bone mineral density (BMD) at the hip in 642 men, 564 women not using hormone therapy, and 326 women using hormone therapy. The results were independent of age, body mass index, and lifestyle factors. An increasing ratio of total dietary omega-6/omega-3 fatty acids was also significant

and independently associated with lower BMD at the hip in all women and at the spine in women not using hormone therapy. Thus, the relative amounts of dietary omega-6 and omega-3 fatty acids may play a vital role in preserving skeletal integrity of old age.

The study by Hogstrom *et al*. is unique in that it measured serum fatty acid concentrations rather than use a dietary recall questionnaire to determine fatty acid intake (108). The aim of the 8-year prospective and retrospective study was to investigate a possible role of fatty acids in bone accumulation and the attainment of peak bone mass in young postpubertal men. Key findings show a positive association between omega-3 fatty acids and BMD of the total body and spine, and the accumulation of BMD at the spine between 16 and 22 years of age in the cohort of healthy young men. This study is the first to examine the association between individual PUFAs, BMD, and bone mineral accrual. In summary, BMD of the total body measured at 22 years of age showed a significant negative correlation with serum concentrations of oleic acid and monounsaturated fatty acids, and a significant positive correlation with DHA and omega-3 fatty acids. BMD of the spine measured at 22 years of age showed a positive association with DHA and omega-3 fatty acids. Changes in BMD of the spine between 16 and 22 years of age showed a positive association with DHA and omega-3 fatty acids, and a negative association with the omega-6/omega-3 ratio (108).

The study by Hogstrom *et al*. (108) adds to the growing body of evidence that omega-3 fatty acids are beneficial to bone health. Animal models have suggested that omega-3 fatty acids may attenuate postmenopausal bone loss. Ovariectomized mice fed a diet high in fish oil had significantly less bone loss at the femur and lumbar vertebrae than did ovariectomized mice fed a diet high in omega-6 fatty acids (109). In vitro models using a preosteoblastic cell line, MC3T3-E1, indicated a greater production of the bone-formation markers alkaline phosphatase and osteocalcin after 48 hours of treatment with EPA than after treatment with ALA (110).

Omega-3 fatty acids play an important role in health and disease and favorably affect skeletal growth. The attainment of peak bone mass in adolescence and the prevention of age-related osteoporosis are potential positive effects of omega-3 fatty acids.

Psychologic stress in humans induces the production of proinflammatory cytokines such as interferon gamma (IFNγ), TNFα, IL-6 and IL-1. An imbalance of omega-6 and omega-3 PUFA in the peripheral blood causes an overproduction of proinflammatory cytokines. There is evidence that changes in fatty acid composition are involved in the pathophysiology of major depression (111). Changes in serotonin (5-HT) receptor number and function caused by changes in PUFA provide the theoretical rationale connecting fatty acids with the current receptor and neurotransmitter theories of depression (112–116). The increased C20:4ω6/C20:5ω3 ratio and the imbalance in the omega-6/omega-3 PUFA ratio in major depression may be related to the increased production of proinflammatory cytokines and eicosanoids in that illness (114). There are a number of studies evaluating the therapeutic effect of EPA and DHA in major depression. Stoll and colleagues have shown that EPA and DHA prolong remission, that is, reduce the risk of relapse in patients with bipolar disorder (117, 118).

Kiecolt-Glaser *et al*. studied depressive symptoms, omega-6/omega-3 fatty acid ratio and inflammation in older adults (119). As the dietary ratio of omega-6/omega-3 increased, the depressive symptoms, TNF-α, IL-6, and IL-6 soluble receptor (sIL-6r) increased. The authors concluded that diets with a high omega-6/omega-3 ratio may enhance the risk for both depression and inflammatory diseases.

Dry eye syndrome (DES) is one of the most prevalent conditions. Inflammation of the lacrimal gland, the meibomian gland, and the ocular surface plays a significant role in DES (120, 121). Increased concentration of inflammatory cytokines, such as IL-1, IL-6, and TNFα have been found in tear film in patients with DES (122). Miljanovic *et al*. investigated the relation of dietary intake of omega-3 fatty acids and the ratio of omega-6 to omega-3 with DES incidence in a large population of women participating in the Women's Health Study (123). A higher ratio of omega-6/omega-3 consumption was associated with a significantly increased risk of DES (OR: 2.51; 95% CI: 1.13, 5.58) for $>$15:1 versus $<$4.1 (P for trend = 0.01). These results suggest that a higher dietary intake of omega-3 fatty acids is associated with a decreased incidence of DES in women and a high omega-6/omega-3 ratio is associated with a greater risk.

Age-related macular degeneration (AMD) is the leading cause of vision loss among people 65 and older. Both AMD and cardiovascular disease share similar modifiable factors (124–128). Fish intake has been reported to have protective properties in lowering the risk of AMD (129–133), especially when LA intake was low (129, 130). In a study involving twins, Seddon *et al*. showed that fish consumption and omega-3 fatty acid intake reduce the risk of AMD whereas cigarette smoking increases the risk for AMD (134).

Conclusions and Recommendations

Western diets are characterized by high omega-6 and low omega-3 fatty acid intake, whereas during the Paleolithic period when human's genetic profile was established, there was a balance between omega-6 and omega-3 fatty acids. Therefore, humans today live in a nutritional environment that differs from that for which our genetic constitution was selected.

The balance of omega-6/omega-3 fatty acids is an important determinant in decreasing the risk for coronary heart disease, both in the primary and secondary prevention of coronary heart disease.

Increased dietary intake of LA leads to oxidation of LDL, platelet aggregation, and interferes with the incorporation of EPA and DHA in cell membrane phospholipids.

Both omega-6 and omega-3 fatty acids influence gene expression. EPA and DHA have the most potent anti-inflammatory effects. Inflammation is at the base of many chronic diseases, including coronary heart disease, diabetes, arthritis, cancer, osteoporosis, mental health, dry eye disease and age-related macular degeneration. Dietary intake of omega-3 fatty acids may prevent the development of disease, particularly in persons with genetic variation, as for example in individuals with genetic variants at the 5-LO and the development of coronary heart disease.

Chronic diseases are multigenic and multifactorial. It is quite possible that the therapeutic dose of omega-3 fatty acids will depend on the degree or severity of disease resulting from the genetic predisposition.

In carrying out clinical intervention trials, it is essential to increase the omega-3 and decrease the omega-6 fatty acid intake in order to have a balanced omega-6 and omega-3 intake in the background diet. Both the dietary intake and plasma levels should be determined before and after the intervention study.

1. Simopoulos AP, Childs B (Eds.). Genetic Variation and Nutrition. World Rev Nutr Diet. Basel: Karger, Vol. 63, 1990.
2. Simopoulos AP, Robinson J. The Omega Diet. The Lifesaving Nutritional Program Based on the Diet of the Island of Crete. New York: HarperCollins, 1999.
3. Simopoulos AP, Nestel PJ (Eds.). Genetic Variation and Dietary Response. World Rev Nutr Diet. Basel: Karger, Vol. 80, 1997.
4. Simopoulos AP, Pavlou KN (Eds.). Nutrition and Fitness 1: Diet, Genes, Physical Activity and Health. World Rev Nutr Diet. Basel: Karger, Vol. 89, 2001.
5. Simopoulos AP. Genetic variation and dietary response: nutrigenetics/nutrigenomics. Asia Pacific J Clin Nutr 11(S6):S117–S128, 2002.
6. Simopoulos AP, Ordovas JM (Eds.). Nutrigenetics and Nutrigenomics. World Rev Nutr Diet. Basel: Karger, Vol. 93, 2004.
7. Eaton SB, Konner M. Paleolithic nutrition. A consideration of its nature and current implications. New Engl J Med 312:283–289, 1985.
8. Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 54:438–463, 1991.
9. Simopoulos AP. Genetic variation and evolutionary aspects of diet. In: Papas A, Ed. Antioxidants in Nutrition and Health. Boca Raton: CRC Press, pp65–88, 1999.
10. Simopoulos AP. Evolutionary aspects of omega-3 fatty acids in the food supply. Prostaglandins, Leukotr Essent Fatty Acids 60:421–429, 1999.
11. Simopoulos AP. New products from the agri-food industry: The return of n-3 fatty acids into the food supply. Lipids 34(suppl):S297–S301, 1999.
12. Eaton SB, Konner M, Shostak M. Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am J Med 84:739–749, 1988.
13. Eaton SB, Eaton SB III, Sinclair AJ, Cordain L, Mann NJ. Dietary intake of long-chain polyunsaturated fatty acids during the Paleolithic. World Rev Nutr Diet 83:12–23, 1998.
14. Leaf A, Weber PC. A new era for science in nutrition. Am J Clin Nutr 45:1048–1053, 1987.
15. Simopoulos AP. Overview of evolutionary aspects of w3 fatty acids in the diet. World Rev Nutr Diet 83:1–11, 1998.
16. Weber PC. Are we what we eat? Fatty acids in nutrition and in cell membranes: cell functions and disorders induced by dietary conditions. Svanoybukt, Norway: Svanoy Foundation (Report no. 4), pp9–18, 1989.
17. Simopoulos AP. Evolutionary aspects of diet: fatty acids, insulin resistance and obesity. In: VanItallie TB, Simopoulos AP, Sr. Eds. Obesity: New Directions in Assessment and Management. Philadelphia: Charles Press, pp241–261, 1995.
18. Simopoulos AP. Trans fatty acids. In: Spiller GA, Ed. Handbook of Lipids in Human Nutrition. Boca Raton: CRC Press, pp91–99, 1995.
19. Simopoulos AP. Omega-3 fatty acids in inflammation an autoimmune diseases. J Am Coll Nutr 21:495–505, 2002.
20. Shannon J, King IB, Moshofskky R, Lampe JW, Li Gao D, Ray RM, Thomas DB. Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China. Am J Clin Nutr 85:1090–1097, 2007.
21. Hughes-Fulford M, Tjandrawinata RR, Li CF, Sayyah S. Arachidonic acid, an omega-6 fatty acid, induces cytoplasmic phospholipase A2 in prostate carcinoma cells. Carcinogenesis 26:1520–1526, 2005.
22. Hedelin M, Chang ET, Wiklund F, Bellocco R, Klint A, Adolfsson J, Shahedi K, Xu J, Adami HO, Gronberg H, Balter KA. Association of frequent consumption of fatty fish with prostate cancer risk is modified by COX-2 polymorphism. Int J Cancer 120:398–405, 2007.
23. Hunter JE. Omega-3 fatty acids from vegetable oils. In: Galli C, Simopoulos AP, Eds. Biological Effects and Nutritional Essentiality. Series A: Life Sciences. New York: Plenum Press, Vol. 171:pp43–55, 1989.
24. Litin L, Sacks F. Trans-fatty-acid content of common foods. N Engl J Med 329:1969–1970, 1993.
25. Raper NR, Cronin FJ, Exler J. Omega-3 fatty acid content of the US food supply. J Am College Nutr 11:304, 1992.
26. Ledger HP. Body composition as a basis for a comparative study of some East African animals. Symp Zool Soc London 21:289–310, 1968.
27. Crawford MA. Fatty acid ratios in free-living and domestic animals. Lancet i:1329–1333, 1968.
28. Crawford MA, Gale MM, Woodford MH. Linoleic acid and linolenic acid elongation products in muscle tissue of Syncerus caffer and other ruminant species. Biochem J 115:25–27, 1969.
29. Simopoulos AP, Norman HA, Gillaspy JE, Duke JA. Common purslane: a source of omega-3 fatty acids and antioxidants. J Am College Nutr 11:374–382, 1992.
30. Simopoulos AP, Norman HA, Gillaspy JE. Purslane in human nutrition and its potential for world agriculture. Basel: Karger, World Rev Nutr Diet 77:47–74, 1995.
31. Simopoulos AP, Salem N Jr. Purslane: a terrestrial source of omega-3 fatty acids. N Engl J Med 315:833, 1986.
32. Simopoulos AP, Gopalan C (Eds.). Plants in Human Health and Nutrition Policy. World Rev Nutr Diet. Basel: Karger, Vol. 91, pp47–74, 2003.
33. Zeghichi S, Kallithrka S, Simopoulos AP, Kypriotakis Z. Nutritional composition of selected wild plants in the diet of Crete. World Rev Nutr Diet 91:22–40, 2003.
34. Simopoulos AP. Omega-3 fatty acids in wild plants, seeds and nuts. Asian Pacific J Clin Nutr 11(S6):S163–S173, 2002.
35. Simopoulos AP. Omega-3 fatty acids and antioxidants in edible wild plants. Biological Research 37(2):263–277, 2004.
36. Simopoulos AP, Salem N Jr. n-3 fatty acids in eggs from range-fed Greek chickens. N Engl J Med 321:1412, 1989.
37. Simopoulos AP, Salem N Jr. Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. Am J Clin Nutr 55:411–414, 1992.

38. 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 51:1–2, 1990.
39. Cordain L, Martin C, Florant G, Watkins BA. The fatty acid composition of muscle, brain, marrow and adipose tissue in elk: evolutionary implications for human dietary requirements. World Rev Nutr Diet. Basel:Karger, Vol. 83, p. 225, 1998.
40. Sinclair AJ, Slattery WJ, O'Dea K. The analysis of polyunsaturated fatty acids in meat by capillary gas-liquid chromotography. J Food Sci Agric 33:771–776, 1982.
41. Sugano M, Hirahara F. Polyunsaturated fatty acids in the food chain in Japan. Am J Clin Nutr 71(suppl):189S–196S, 2000.
42. Pella D, Dubnov G, Singh RB, Sharma R. Effects of an Indo-Mediterranean diet on the omega-6/omega-3 ratio in patients at high risk of coronary artery disease: the Indian paradox. Basel: Karger, World Rev Nutr Diet 92:74–80, 2003.
43. Sanders TAB. Polyunsaturated fatty acids in the food chain in Europe. Am J Clin Nutr 71(suppl):S176–S178, 2000.
44. de Gomez Dumm INT, Brenner RR. Oxidative desaturation of alpha-linolenic, linoleic, and stearic acids by human liver microsomes. Lipids 10:315–317, 1975.
45. Emken EA, Adlof RO, Rakoff H, Rohwedder WK. Metabolism of deuterium-labeled linolenic, linoleic, oleic, stearic and palmitic acid in human subjects. In: Baillie TA, Jones JR, Eds. Synthesis and Application of Isotopically Labeled Compounds 1988. Amsterdam: Elsevier Science Publishers, 713–716, 1989.
46. Hague TA, Christoffersen BO. Effect of dietary fats on arachidonic acid and eicosapentaenoic acid biosynthesis and conversion to C22 fatty acids in isolated liver cells. Biochim Biophys Acta 796:205–217, 1984.
47. Hague TA, Christoffersen BO. Evidence for peroxisomal retroconversion of adrenic acid (22:4n6) and docosahexaenoic acid (22:6n3) in isolated liver cells. Biochim Biophys Acta 875:165–173, 1986.
48. 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 12:569–582, 1992.
49. Carlson SE, Rhodes PG, Ferguson MG. Docosahexaenoic acid status of preterm infants at birth and following feeding with human milk or formula. Am J Clin Nutr 44:798–804, 1986.
50. Singer P, Jaeger W, Voigt S, Theil H. Defective desaturation and elongation of n-6 and n-3 fatty acids in hypertensive patients. Prostaglandins Leukot Med 15:159–165, 1984.
51. Honigmann G, Schimke E, Beitz J, Mest HJ, Schliack V. Influence of a diet rich in linolenic acid on lipids, thrombocyte aggregation and prostaglandins in type I (insulin-dependent) diabetes. Diabetologia 23: 175(abstract), 1982.
52. Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr 70(suppl):560S–569S, 1999.
53. Parthasarathy S, Khoo JC, Miller E, Barnett J, Witztum JL, Steinberg D. Low density lipoprotein rich in oleic acid is protected against oxidative modification: implications for dietary prevention of atherosclerosis. Proc Natl Acad Sci USA 87:3894–3898, 1990.
54. Reaven P, Parthasarathy S, Grasse BJ, Miller E, Almazan F, Mattson FH, Khoo JC, Steinberg D, Witztum JL. Feasibility of using an oleate-rich diet to reduce the susceptibility of low density lipoprotein to oxidative modification in humans. Am J Clin Nutr 54:701–706, 1991.
55. Reaven PD, Grasse BJ, Tribble DL. Effects of linoleate-enriched and oleate-enriched diets in combination with α-tocopherol on the susceptibility of LDL and LDL subfractions to oxidative modification in humans. Arterioscler Thromb 14:557–566, 1994.
56. Louheranta AM, Porkkala-Sarataho EK, Nyyssonen MK, Salonen RM, Salonen JT. Linoleic acid intake and susceptibility of very-low-density and low-density lipoproteins to oxidation in men. Am J Clin Nutr 63:698–703, 1996.
57. Regnstrom J, Nilsson J, Tornvall P, Landou C, Hamsten A. Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. Lancet 339:1183–1186, 1992.
58. Cleland LG, James MJ, Neumann MA, D'Angelo M, Gibson RA. Linoleate inhibits EPA incorporation from dietary fish-oil supplements in human subjects. Am J Clin Nutr 55:395–399, 1992.
59. Liou YA, King J, Zibrik D, Innis SM. Decreasing linoleic acid with constant α-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. J Nutr 137:945–952, 2007.
60. Zampelas A, Paschos G, Rallidis L, Yiannakouris N. Linoleic acid to alpha-linolenic acid ratio. From clinical trials to inflammatory markers of coronary artery disease. World Rev Nutr Diet 92:92–108, 2003.
61. Ambring A, Johansson M, Axelsen M, Gan L, Strandvik B, Friberg P. Mediterranean-inspired diet lowers the ratio of serum phospholipid n-6 to n-3 fatty acids, the number of leukocytes and platelets, and vascular endothelial growth factor in healthy subjects. Am J Clin Nutr 83:575–581, 2006.
62. Simopoulos AP, Visioli F (Eds.). Mediterranean Diets. World Rev Nutr Diet. Basel: Karger, Vol. 87, 2000.
63. Simopoulos AP, Visioli F (Eds.). More on Mediterranean Diets. World Rev Nutr Diet. Basel: Karger, Vol. 95, 2007.
64. Freese R, Mutanen M, Valsta LM, Salminen I. Comparison of the effects of two diets rich in monounsaturated fatty acids differing in their linoleic/alpha-linolenic acid ratio on platelet aggregation. Thromb Haemost 71:73–77, 1994.
65. Simopoulos AP, Cleland LG (Eds.). Omega-6/Omega-3 Essential Fatty Acid Ratio: The scientific evidence. World Rev Nutr Diet. Basel: Karger, Vol. 92, 2003.
66. Ferruci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, Martin A, Andres-Lacueva C, Senin U, Guralnik JM. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. J Clin Endocrinol Metab 91:439–446, 2006.
67. Kang ZB, Ge Y, Chen Z, Brown J, Lapasota M, Leaf A, Kang JX. Adenoviral transfer of Caenorhabditis elegans n-3 fatty acid desaturase optimizes fatty acid composition in mammalian heart cells. Proc Natl Acad Sci USA 98:4050–4054, 2001.
68. Ge Y-L, Chen Z, Kang ZB, Cluette-Brown J, Laposata M, Kang JX. Effects of adenoviral transfer of Caenorhabditis elegans n-3 fatty acid desaturase on the lipid profile and growth of human breast cancer cells. Anticancer Research 22:537–544, 2002.
69. Kang JX. The importance of omega-6/omega-3 fatty acid ratio in cell function. The gene transfer of omega-3 fatty acid desaturase. World Rev Nutr Diet 92:23–36, 2003.
70. Kang JX, Wang J, Wu L, Kang ZB. Fat-1 mice convert n-6 to n-3 fatty acids. Nature 427:504, 2004.
71. Kang JX. Balance of omega-6/omega-3 fatty acids is important for health: the evidence from gene transfer studies. World Rev Nutr Diet 95:93–102, 2004.
72. Lai L, Kang JX, Li R, Wang J, Witt WT, Yong HY, Hao Y, Was DM, Murphy CN, Rieke A, Samuel M, Linville ML, Korte SW, Evans RW, Starzl TE, Prather RS, Dai Y. Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nat Biotechnol 4:435–436, 2006.
73. Kang JX. Fat-1 transgenic mice: a new model for omega-3 research. Prostaglandins Leukot Essent Fatty Acids 77:263–267, 2007.
74. Simopoulos AP. The role of fatty acids in gene expression: health implications. Ann Nutr Metab 40:303–311, 1996.
75. Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, Lusis AJ, Mehrabian M. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. N Engl J Med 350:29–37, 2004.
76. de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin J-L, Monjaud I, Guidollet J, Touboul P, Delaye J. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 343:1454–1459, 1994.
77. Renaud S, de Lorgeril M, Delaye J, Guidollet J, Jacquard F, Mamelle

N, Martin JL, Monjaud I, Salen P, Toubol P. Cretan Mediterranean diet for prevention of coronary heart disease. Am J Clin Nutr 61(suppl):1360S–1367S, 1995.

78. de Lorgeril M, Salen P. Modified Cretan Mediterranean diet in the prevention of coronary heart disease and cancer. World Rev Nutr Diet 87:1–23, 2000.
79. de Lorgeril M, Salen P. Modified Cretan Mediterranean diet in the prevention of coronary heart disease and cancer. An update. World Rev Nutr Diet 97:1–32, 2007.
80. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet 354:447–455, 1999.
81. Li Y, Kang JX, Leaf A. Differential effects of various eicosanoids on the contraction of cultured neonatal rat cardiomyocytes. Prostaglandins 54:511–530, 1997.
82. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM. Effect of changes in fat, fish and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 2:757–761, 1989.
83. Singh RB, Rastogi SS, Verma R, Laxmi B, Singh R, Ghosh S, Niaz MA. Randomized controlled trial of cardioprotective diet in patients with recent acute myocardial infarction: results of one year follow up. BMJ 304:1015–1019, 1992.
84. Singh RB, Niaz MA, Sharma JP, Kumar R, Rastogi V, Moshiri M. Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival—4. Cardiovasc Drugs Ther 11: 485–491, 1997.
85. Leaf A, Albert CM, Josephson M, Steinhaus D, Kluger J, Kang JX, Cox B, Zhang H, Schoenfeld D, Fatty Acid Antiarrhymnia Trial Investigators. Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762–2768, 2005.
86. Raitt MH, Connor WE, Morris C, Kron J, Halperin B, Chugh SS, McClelland J, Cook J, MacMurdy K, Swenson R, Connor SL, Gerhard G, Kraemer DF, Oseran D, Marchant C, Calhoun D, Shnider R, McAnulty J. Fish oil supplementation and risk of ventricular tachycardia and ventricular fibrillation in patients with implantable defibrilators. A randomized controlled trial. JAMA 293:2884–2891, 2005.
87. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimoda K, Shirata K, for the Japan EPA lipid intervention study (JELIS) Investigation. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet 369:1090–1098, 2007.
88. Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, Kokubo Y, Tsugane S; JPHC Study Group: Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese. The Japan Public Health Center-Based (JPHC) Study Cohort I. Circulation 113:195–202, 2006.
89. Zhao G, Etherton TD, Martin KR, Gillies PJ, West SG, Kris-Etherton PM. Dietary α-linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. Am J Clin Nutr 85:385–391, 2007.
90. Paschos GK, Magkos F, Panagiotakos DB, Votteas V, Zampelas A. Dietary supplementation with flaxseed oil lowers blood pressure in dyslipidaemic patients. Eur J Clin Nutr. advance online publication 31 January 2007; doi: 10.1038/sj.ejcn.1602631:1–6, 2007.
91. Kew S, Banerjee T, Minihane AM, Finnegan YE, Muggli R, Albers R, Williams CM, Calder PC. Lack of effect of foods enriched with plant- or animal-derived n-3 fatty acids on human immune function. Am J Clin Nutr 77:1287–1295, 2003.
92. Toborek M, Lee YW, Garrido R, Kaiser S, Hennig B. Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cells. Am J Clin Nutr 75:119–125, 2002.
93. Raheja BS, Sadikot SM, Phatak RB, Rao MB. Significance of the n-6/n-3 ratio for insulin action in diabetes. Ann NY Acad Sci 683:258–271, 1993.
94. James MJ, Cleland LG. Dietary n-3 fatty acids and therapy for rheumatoid arthritis. Semin Arthritis Rheum 27:85–97, 1997.
95. Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. Am J Clin Nutr 65:1011–1017, 1997.
96. Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. Am Rev Respir Dis 147:697–704, 1993.
97. Bartram H-P, Bostner A, Scheppach W, Reddy BS, Rao CV, Dusel G, Richter F, Richteer A, Kasper H. Effects of fish oil on rectal cell proliferation, mucosal fatty acids, and prostaglandin E_2 release in healthy subjects. Gastroenterology 105:1317–1322, 1993.
98. Bartram H-P, Gostner A, Reddy BS, Rao CV, Scheppach W, Dusal G, Richter A, Richter F, Kasper H. Missing anti-proliferative effect of fish oil on rectal epithelium in healthy volunteers consuming a high-fat diet: potential role of the n-3:n-6 fatty acid ratio. Eur J Cancer Prev 4:231–237, 1995.
99. Maillard V, Bougnoux P, Ferrari P, Jourdan M-L, Pinault M, Lavillonnieree FF, Body G, Le Floch O, Chajes V. N-3 and n-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. Int J Cancer 98:78–83, 2002.
100. Eisman JA. Genetics of osteoporosis. Endocr Rev 20:788–894, 1999.
101. Seeman H, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J, Jerums G. Reduced bone mass in daughters of women with osteoporosis. N Engl J Med 320:554–558, 1989.
102. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, Vogt TM. Risk factors for hip fracture in white women. Study of Osteoporosis Fracture Research Group. N Engl J Med 332:767–773, 1995.
103. Sakaguchi K, Morita I, Murota S. Eicosapentaenoic acid inhibits bone loss due to ovariectomy in rats. Prostaglandins Leukot Essent Fatty Acids 50:81–84, 1994.
104. Iwami-Morimoto Y, Yamaguchi K, Tanne K. Influence of dietary n-3 polyunsaturated fatty acid on experimental tooth movement in rats. Angle Orthod 69:365–371, 1999.
105. Li Y, Seifert MF, Ney DM, Grahn M, Grant AL, Allen KG, Watkins BA. Dietary conjugated linoleic acids alter serum IGF-1 and IGF binding protein concentrations and reduce bone formation in rats fed (n-6) or (n-3) fatty acids. J Bone Miner Res 14:1153–1162, 1999.
106. Das U. Interaction(s) between essential fatty acids, eicosanoids, cytokines, growth factors and free radicals: relevance to new therapeutic strategies in rheumatoid arthritis and other collagen vascular diseases. Prostaglandins Leukot Essent Fatty Acids 44: 201–210, 1991.
107. Weiss LA, Barret-Connor E, von Muhlen D. Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study. Am J Clin Nutr 81:934–938, 2005.
108. Hogstrom M, Nordstrom P, Nordstrom A. n-3 fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO_2 study. Am J Clin Nutr 85:803–807, 2007.
109. Sun D, Krishnan A, Zaman K, Lawrence R, Bhattacharya A, Fernandes G. Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. J Bone Miner Res 18: 1206–1216, 2003.
110. Watkins BA, Li Y, Lippman HE, Feng S. Modulatory effect of n-3 polyunsaturated fatty acids on osteoblast function and bone metabolism. Prostaglandins Leukot Essent Fatty Acids 68:387–398, 2003.
111. Hibbeln JR, Salem N Jr. Dietary polyunsaturated fatty acids and

depression: when cholesterol does not satisfy. Am J Clin Nutr 62:1–9, 1995.

112. Hibbeln JR, Umhau JC, Linnoila M George DT, Ragan PW, Shoaf SE, Vaughan MR, Rawlings R, Salem N Jr. A replication study of violent and nonviolent subjects: cerebrospinal fluid metabolites of serotonin and dopamine are predicted by plasma essential fatty acids. Biol Psychiatry 44:243–249, 1998.
113. Hibbeln JR, Linnoila M, Umhau JC, Rawlings R, George DT, Salem N Jr. Essential fatty acids predict metabolites of serotonin and dopamine in cerebrospinal fluid among healthy control subjects, and early- and late-onset alcoholics. Biol Psychiatry 44:235–242, 1998.
114. Maes M, Smith R, Christophe A, Cosyns P, Desnyder R, Meltzer H. Fatty acid composition in major depression: decreased omega 3 fractions in cholesteryl esters and increased C20:4 omega 6/C20:5 omega 3 ratio in cholesteryl esters and phospholipids. J Affect Disord 38:35–46, 1996.
115. Maes M, Smith R, Christophe A, Vandoolaeghe E, Van Gastel A, Neels H, Demedts P, Wauters A, Meltzer HY. Lower serum high-density lipoprotein cholesterol (HDL-C) in major depression and in depressed men with serious suicidal attempts: relationship with immune-inflammatory markers. Acta Psychiatr Scand 95:212–221, 1997.
116. Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. Biol Psychiatry 43:315–319, 1998.
117. Locke CA, Stoll AL. Omega-3 fatty acids in major depression. World Rev Nutr Diet 89:173–185, 2001.
118. Stoll AL, Severus WE, Freeman MP, Rueter S, Zboyan HA, Diamond E, Cress KK, Marangell LB. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. Arch Gen Psychiatry 56:407–412, 1999.
119. Kiecolt-Glaser JK, Belury MA, Porter K, Beversdorf DQ, Lemeshow S, Glaser R. Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. Psychosom Med Mar 30; (Epub ahead of print), 2007.
120. Kunert KS, Tisdale AS, Stern ME, Smith JA, Gipson IK. Analysis of topical cyclosporine treatment of patients with dry eye syndrome: effect of conjunctival lymphocytes. Arch Ophthalmol 118:1489–1496, 2000.
121. Marsh P, Pflugfelder SC. Topical nonpreserved methylprednisolone therapy for keratoconjuntivitis sicca in Sjogren syndrome. Ophthalmology 106:811–816, 1999.
122. Solomon A, Dursun D, Liu Z, Xie Y, Macri A, Pflugfelder SC. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Opthalmol Vis Sci 42:2283–2292, 2001.
123. Miljanovic B, Trivedi KA, Dana MR, Gilbard JP, Buring JE, Schaumberg DA. Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. Am J Clin Nutr 82: 887–893, 2005.
124. Snow KK, Seddon JM. Do age-related macular degeneration and cardiovascular disease share common antecedents? Ophthalmic Epidemiol 6:125–143, 1999.
125. Seddon JM, Chen CA. Epidemiology of age-related macular degeneration (4th ed.). In: Ryan SJ, Ed. Retina, Vol. 2: Medical Retina. St. Louis, MO: CV Mosby, 2005.
126. Seddon JM, Hankinson S, Speizer F, Willett WC. A prospective study of cigarette smoking and age-related macular degeneration in women. JAMA 276:1141–1146, 1996.
127. Tomany SC, Wang JJ, van Leeuwen R, Klein R, Mitchell P, Vingerling JR, Klein BE, Smith W, De Jong PT. Risk factors for incident age-related macular degeneration: pooled findings from 3 continents. Ophthalmology 11:1280–1287, 2004.
128. The health consequences of smoking: a report of the Surgeon General, 2005. http://www.cdc.gov/tobacco/data_statistics/sgr/sgr_2004/index.htm. Accessed April 18, 2007.
129. Seddon JM, Rosner B, Sperduto RD, Yannuzzi L, Haller JA, Blair NP, Willett W. Dietary fat and risk for advanced age-related macular degeneration. Arch Ophthalmol 119:1191–1199, 2001.
130. Seddon JM, Cote J, Rosner B. Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake. Arch Ophthalmol 121:1728–1737, 2003.
131. Cho E, Hung S, Willett W, Rimm EB, Seddon JM, Colditz GA, Hankinson SE. Prospective study of dietary fat and the risk of age-related macular degeneration. Am J Clin Nutr 73:209–218, 2001.
132. Smith W, Mitchell P, Leeder SR. Dietary fat and fish intake and age-related maculopathy. Arch Ophthalmol 118:401–404, 2000.
133. Seddon J, Ajani U, Sperduto R. Dietary fat intake and age-related macular degeneration (ARVO abstract). Invest Ophthalmol Vis Sci 35:2003, 1994.
134. Seddon JM, George S, Rosner B. Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration. The US Twin Study of age-related macular degeneration. Arch Ophthalmol 124:995–1001, 2006.