The omega-6/omega-3 fatty acid ratio: health implications

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Abstract: *Today, Western diets are characterized by a higher omega-6 and a lower 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. Their balance is an important determinant for brain development and in decreasing the risk for coronary heart disease (CHD), hypertension, cancer, diabetes, arthritis, and other autoimmune and possibly neurodegenerative diseases. Both omega-6 and omega-3 fatty acids influence gene expression. Because of single nucleotide polymorphisms (SNPs) in their metabolic pathways, blood levels of omega-6 and omega-3 fatty acids are determined by both endogenous metabolism and dietary intake making the need of balanced dietary intake essential for health and disease prevention. Whether an omega-6/omega-3 ratio of 3:1 to 4:1 could prevent the pathogenesis of many diseases induced by today's Western diets (AFSSA, 2010), a target of 1:1 to 2:1 appears to be consistent with studies on evolutionary aspects of diet, neurodevelopment, and genetics. A target of omega-6/omega-3 fatty acid ratio of 1:1 to 2:1 appears to be consistent with studies on evolutionary aspects of diet, neurodevelopment and genetics. A balanced ratio of omega-6/omega-3 fatty acids is important for health and in the prevention of CHD and possibly other chronic diseases.*

Key words: *omega-6/omega-3 fatty acid ratio, coronary heart disease, cancer, single nucleotide polymorphisms, cytokines, nutrigenetics/nutrigenomics*

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 (EFA) and in the antioxidant content of foods (Eaton and Konner, 1985; Simopoulos, 1991) *(figure 1)*. An absolute and relative change of omega-6/omega-3 fatty acids in the food supply of Western societies has occurred over the last 150 years. A balance existed between omega-6 and omega-3 fatty acids 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 eons of evolution, omega-3 fatty acids were present in all foods consumed: meat, wild plants, eggs, fish, nuts, and berries (Crawford, 1968; Simopoulos, 2002). However, rapid dietary changes over short periods of time as they have occurred over the past 100–150 years is a totally new phenomenon in human evolution *(figure 1*, *tables 1 and 2)*. These dietary changes are the result of agribusiness and modern agriculture that led to animal feeds consisting primarily of grains, instead of the animals grazing, and to the production of vegetable oils from seeds such as corn, sunflower, safflower, cottonseed, and soybean that are high in omega-6 fatty acids and poor in omega-3s.

Today, industrialized societies are characterized by 1) an 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, vitamins especially C, E and D, trace elements and calcium intake. The increase in trans fatty acids is detrimental to health as shown in *table 3*. 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 (Simopoulos, 1995) *(figure 2)*.

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 (Eaton and Konner, 1985). Humans today live in a nutritional environment that differs from that for which our genetic constitution was selected. Yet as shown in *figure 3*, it is the interaction of genes with various environmental factors that determines the phenotype throughout development. Nutrition is an environmental factor of major importance.

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 brain development, coronary heart disease (CHD), cancer, inflammatory bowel disease, rheumatoid arthritis, psoriasis, mental health, and neurodegenerative diseases (Simopoulos, 2002).

In this review, I discuss:

– the importance of the balance of omega-6 and omega-3 essential fatty acids in terms of their biological effects and the omega-6/omega-3 ratio;

– the balance of omega-6/omega-3 fatty acids is important for health: the evidence from gene transfer studies;

– omega-3 fatty acids and gene expression;

doi: 10.1684/ocl.2010.0325

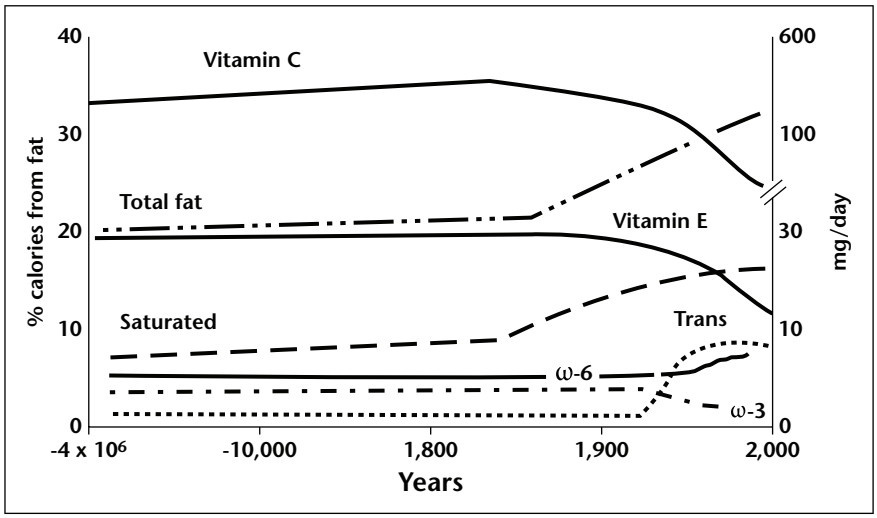

Figure 1. *Hypothetical scheme of fat, fatty acid (ω6, ω3, trans, and total) intake (as percent of calories from fat), and intake of vitamins E and C (mg/d). Data were extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative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

Table 1. *Omega-6:omega-3 ratios in various populations.*

Population	ω6/ω3
Paleolithic	0.79
Greece prior to 1960	1.00-2.00
Current Japan	4.00
Current India, rural	5-6.1
Current United Kingdom and northern Europe	15.00
Current United States	16.74
Current India, urban	38-50

– genetic variants, FADS1 and FADS2, in estimating nutritional requirements of omega-6 and omega-3 fatty acids;

– genetic variants in FADS1 and FADS2 and coronary heart disease risk;

– linoleic acid and arachidonic acid increase atherogenesis: evidence from diet-gene interactions: genetic variants in the 5-lipoxygenase (5-LO) and omega-6 and omega-3 fatty acid intake in the risk for cardiovascular disease;

– genetic variants in the 5-lipoxygenase activating protein (ALOX5AP) gene, omega-6 fatty acids, and breast cancer;

– genetic variants of cyclooxygenase-2 (COX-2) and the protective effect of long-chain omega-3 fatty acids in cancer of the prostate;

– the omega-6/omega-3 ratio for neurodevelopment; and finally;

– conclusions and recommendations.

Biological effects and the omega-6/omega-3 ratio

There are two classes of EFAs: 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 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 from 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 *(figure 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 a slow process. This conversion was shown by using deuterated ALA (Emken *et al.*, 1989). There is competition between omega-6 and omega-3

Table 2. *Estimated omega-3 and omega-6 fatty acid intake in the late paleolithic period (g/d)*[a,b].

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
A A + DTA/EPA +DPA + DHA	1.79
Total ω6/ω3	0.79[b]

LA, linoleic acid; ALA, linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetranoic acid; DPA, docosapenlaenoic acid; DHA, docosahexaenoic acid.

[a]Data from Eaton *et al.*

[b]Assuming an energy intake of 35:65 of animal:plant sources.

Table 3. *Adverse effects of trans fatty acids.*

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)

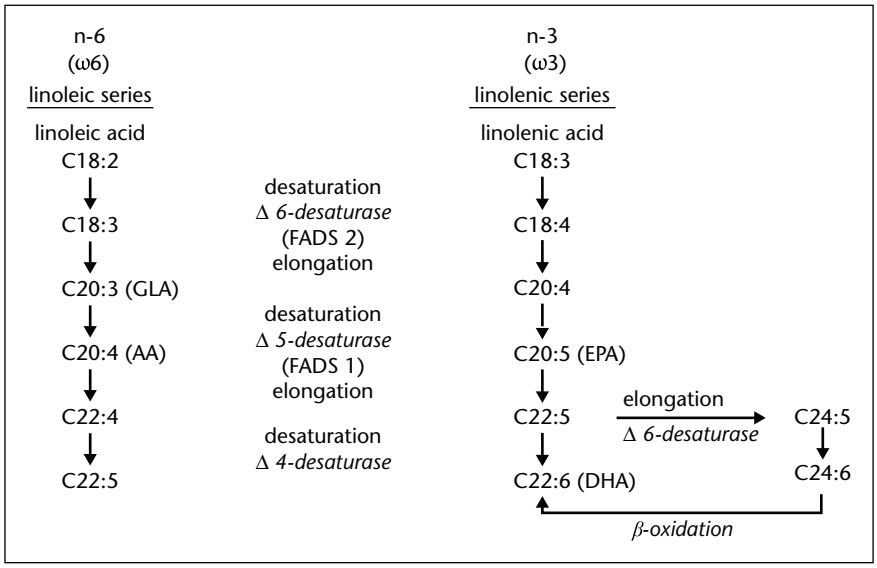

Figure 2. *The desaturation and elongation of ω3 and ω6 fatty acids.*

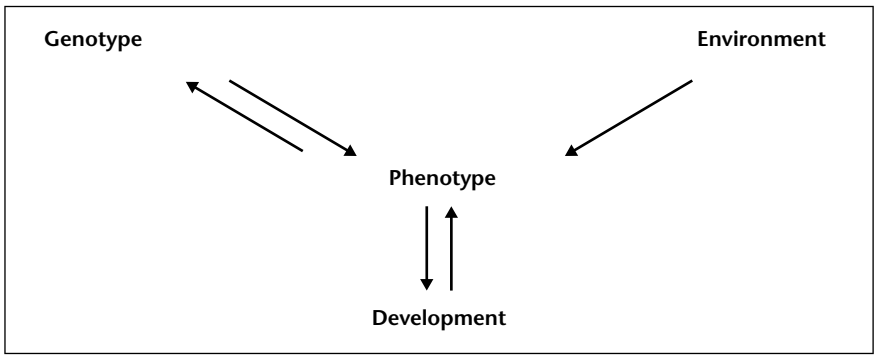

Figure 3. *Relationships between genes, environment, and development are dynamic.*

fatty acids for the desaturation enzymes. However, both D-5 and D-6 desaturases prefer omega-3 to omega-6 fatty acids. But, a high LA intake interferes with the desaturation and elongation of ALA (Emken *et al.*, 1989). Trans fatty acids interfere with the desaturation and elongation of both LA and ALA. D-6 desaturase is the limiting enzyme and there is some evidence that it decreases with age. Premature infants, hypertensive individuals, and some diabetics 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, dairy products, 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.

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. These two classes of EFA are not interconvertible, are metabolically and functionally distinct, and often have important opposing physiological functions *(table 4*, *figure 4)*. 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. 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, although recent studies indicate that polymorphisms in the FADS1 and FADS2 influence endogenous production of long-chain PUFA (see below). AA and EPA are the parent compounds for eicosanoid production.

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

Table 4. *Effects of ingestion of EPA and DHA from fish or fish oil.*

Decreased production of prostaglandin E2 (PGE2) metabolites
A decrease in thromboxane A2, a potent platelet aggregator and vasoconstrictor
A decrease in leukotriene B4 formation, an inducer of inflammation, and a powerful inducer of leukocyte chemotaxis and adherence
An increase in thromboxane A3, a weak platelet aggregator and weak vasoconstrictor
An increase in prostacyclin PGI3, leading to an overall increase in total prostacyclin by increasing PGI3 without a decrease in PGI2, both PGI2 and PGI3 are active vasodilators and inhibitors of platelet aggregation
An increase in leukotriene B5, a weak inducer of inflammation and a weak chemotactic agent

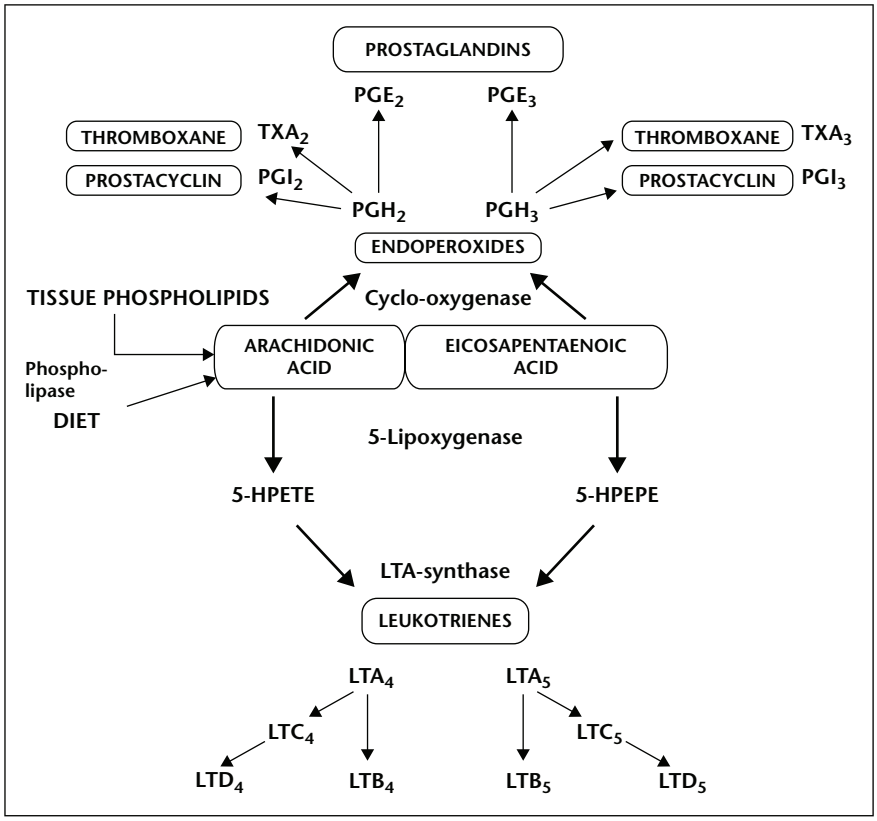

Figure 4. *Oxidative metabolism of arachidonic acid and eicosapentaenoic acid by the cyclooxygenase and 5-lipoxygenase pathways. 5-HPETE denotes 5-hydroperoxyeicosatetranoic acid and 5-HPEPE denotes 5-hydroxyeicosapentaenoic acid.*

omega-3 fatty acids, specifically EPA *(figure 4)*. 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 especially adipocytes leading to obesity. Thus, a diet high in omega-6 fatty acids, as is today's Western diet, shifts the physiological state to one that is prothrombotic and proaggregatory, with increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time *(tables 5 and 6)*. Bleeding time is decreased in groups of patients with hypercholesterolemia, hyperlipoproteinemia, myocardial infarction, 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.

As can be seen from *table 7* on Telomere length and function, the omega-6 and omega-3 fatty acids show additional opposing properties (Farzaneh-Far *et al.*, 2010).

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 (2004), which clearly show the ability of both normal rat cardiomyocytes and human breast cancer cells in culture to form all the omega-3s 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, 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 (Kang, 2003). 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 (Kang *et al.*, 2004). 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.

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 path-

Table 5. *Mechanisms of omega-6/omega-3 fatty acids.*

Linoleic acid inhibits eicosapentaenoic acid incorporation from dietary fish oil supplements in human subjects
Linoleic acid increases low-density lipoprotein oxidation and severity of coronary atherosclerosis
As the omega-6/omega-3 ratio decreases, so does the platelet aggregation
Plasma omega-6/omega-3 ratio and inflammatory markers (the omega-6/omega-3 ratio is a strong negative correlate of IL-10)
Omega-3 fatty acids downregulate the expression of genes involved in inflammation and obesity
A Lower omega-6/omega-3 ratio as part of a mediterranean diet decreases vascular endothelial growth factor
Decreasing linoleic acid with constant α-linolenic acid in dietary fat increases eicosapentaenoic acid in plasma phospholipids in healthy men

ways. 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 (Simopoulos, 1996). In the case of genes involved in inflammation, such as IL-1b, EPA and DHA suppress IL-1b mRNA whereas AA does not, and the same effect appears in studies on growth-related early response gene expression and growth factor (Simopoulos, 1996). 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 such as adipocyte glucose transporter-4, lymphocyte

Table 6. *Effects of Omega-3 Fatty Acids on Factors Involved in the Pathophysiology of Atherosclerosis and Inflammation.*

Factor	**Function**	**Effect of w-3 fatty acid**
Arachidonic acid	Eicosanoid precursor, aggregates platelets; stimulates white blooo 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 chemattractant; increase of intracellular Ca^{++}	↓
Fibrinogen	A member of the acute phase response; and a blood clotting factor	↓
Tissue pasminogen 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	↓

Table 7. *Effects of Omega-6 and Omega-3 Fatty Acids on Telomere Length.*

Telomeres are tandem repeat DNA sequences (TTAGGG)n that form a protective cap at the ends of eukaryotic chromosomes
During somatic cell division, DNA polymerase cannot fully replicate the 3′ end of linear DNA, resulting in an obligate and progressive loss of telomeric repeats. This process may eventually result in cellular senescence or apoptosis
Telomere length is emerging as a novel marker of biological age which integrates the cumulative life time burden of genetic factors and environmental stressors independent of chronological age
A strong association between short telomeres and cardiovascular morbidity and mortality has been documented in several populations
Increased dietary intake of marine omega-3 fatty acids is associated with prolonged survival in patients with Coronary Heart Disease
Telomeres may lengthen as well as shorten
In a cohort of patients with coronary artery disease, there was an inverse relationship between baseline blood levels of marine omega-3 fatty acids and the rate of telomere shortening over 5 years
Leukocyte telomere length is associated with diseases of aging and is a potential biomarker of chronic disease risk
Linoleic acid intake is inversely associated with leukocyte telomere length after multivariate adjustment in women

stearoyl-CoA desaturase 2 in the brain, peripheral monocytes (IL-1b 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.

Genetic variants, FADS1 and FADS2, in estimating nutritional requirements of omega-6 and omega-3 fatty acids

The levels of LC-PUFAs in plasma serum or red blood cell (RBC) membrane phospholipids depend on dietary intake and endogenous metabolism *(figure 2)*. There have been many indications for considerable interindividual variation in the capacity for endogenous formation of LC-PUFAs. For example, over 20 years ago, Koletzko *et al.* (1988) showed a rather close correlation of omega-6 and omega-3 fatty acid content in mature milk in human beings even though the main dietary sources were different. Thus, it appears that some women have a higher ability to synthesize and secrete milk LC-PUFAs of both the omega-6 and omega-3 series than others. Further, Guerra *et al.* (2007) showed that there was a tracking of plasma LC-PUFA levels in the absence of tracking of dietary intake patterns, suggesting that there is interindividual variation in the ability to endogenously synthesize LC-PUFAs among children, which persists over time and could most likely be due to genetically determined differences in metabolic turnover. Changes in PUFA conversion have been shown with stable isotope studies (Emken *et al.*, 1989). The FADS1 and FADS2 gene cluster involved in the metabolic pathway of LA and ALA, as well as the enzymes involved in the production of eicosanoids, 5-LO and cyclooxygenase (COX) from the AA and EPA, are polymorphic. Recent studies on their polymorphisms indicate that the minor alleles of the genetic variants in FADS1 and FADS2 are associated with higher LA and lower AA levels in RBC membrane and plasma phospholipids that may influence the estimation of dietary requirements (Koletzko *et al.*, 2008; Schaeffer *et al.*, 2006), particularly during pregnancy and lactation as well as the infant's IQ. The investigated single nucleotide polymorphisms (SNPs) in this cluster explained 28% of the variance of AA and up to 12% of its precursor fatty acids. The frequency of the minor alleles was about 26%. It can be concluded that the genetic variants indicate a difference in the conversion of omega-6 and omega-3 fatty acids catalyzed by the delta-5 and delta-6 desaturases, which suggests that individuals may require different amounts of dietary PUFAs or LC-PUFAs to achieve comparable biological effects. Furthermore, studies addressing the biological effects of PUFAs and LC-PUFAs should include genotyping for FADS1 and FADS2 polymorphisms, whereas an increase in the activity of the desaturase increases the AA-to-LA ratio and the risk for CHD (Martinelli *et al.*, 2008). Furthermore, genetic variants in the 5-LO and cyclooxygenase-2 (COX-2) genes have been associated with increased risk for CHD (Dwyer *et al.*, 2004) and cancer (Fradet *et al.*, 2009).

Genetic variants in FADS1 and FADS2 and coronary heart disease risk

In a recent genome-wide association study (GWAS) to identify genetic contributors of plasma omega-6 and omega-3 fatty acid concentrations in 1075 participants in the InCHIANTI Study on aging, Tanaka *et al.* (2009) noted that the strongest evidence was in the region of chromosome 11 that encodes FADS1, FADS2, and FADS3. The SNP with the most significant association was rs 174537 near FADS1 in the analysis of AA (AA; $P = 5.95 \times 10^{-46}$). Minor allele homozygotes had lower AA compared with the major allele homozygotes, and rs 174537 accounted for 18.6% of the additive variance in AA concentrations. Participants carrying the allele associated with higher AA, EDA ($P = 6.78 \times 10^{-9}$), and EPA ($P = 1.07 \times 10^{-9}$) also had higher LDL and total cholesterol levels. These results show that SNPs of genes encoding enzymes in the metabolism of PUFAs contribute to plasma concentrations of fatty acids. Desaturase activity is assayed in vitro or in animals by measurement of the rate of conversion of radio labeled precursor fatty acids to their respective products, but ethical and practical reasons prevent this possibility in humans. Instead, a product-to-precursor ratio (*e.g.*, AA/LA or EPA/ALA) as a surrogate measure to estimate desaturase activity is well established. Martinelli *et al.* (2008) analyzed RBC membrane fatty acids, genotyped 13-SNPs in the FADS region, estimated the ratio of RBC-AA to RBC-LA, and C-reactive protein (CRP) in an ongoing case-control study with or without angiographic evidence of coronary artery disease (CAD). Both AA/LA and the ratio of EPA to ALA were higher in participants with CAD than in those without CAD, but in a multiple logistic regression model, only a higher AA/LA resulted as an independent risk factor for CAD (odds ratio: 2.55; 95% confidence interval: 1.61, 4.05 for higher) compared with lower ratio tertile; P for trend < 0.001. Concentrations of high-sensitivity C-reactive protein (hs-CRP) increased progressively across tertiles of AA/LA.

Graded increases in hs-CRP concentrations and CAD risk were related to the carriership of FADS haplotypes, including the alleles associated with a higher ratio.

Studies by Kark *et al.* (2003) and Baylin and Campos (2004) have shown that higher amounts of AA in adipose tissue are associated with higher risk of acute myocardial infarction. In populations eating a Western diet rich in omega-6 PUFA, a high desaturase activity may promote an increased bioavailability of AA with prevailing synthesis of AA-derived proinflammatory eicosanoids leading to atherosclerosis and vascular damage *(figure 4*, *table 4)*. On the other hand, high desaturase activity in subjects on a diet rich in omega-3 fatty acids or receiving EPA and DHA supplementation could result in an opposite situation with a preferential synthesis of anti-inflammatory eicosanoids.

Linoleic acid and arachidonic acid increase atherogenesis: evidence from diet-gene interactions: genetic variants in the 5-lipoxygenase 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-LO gene promoter could relate to atherosclerosis in humans, and that this effect could interact with the dietary intake of competing 5-LO substrates (Dwyer *et al.*, 2004). The study consisted of 470 healthy middle-aged women and men from the Los Angeles Atherosclerosis study, randomly sampled. The investigators determined 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 (Simopoulos and Ordovas, 2004).

Genetic variants in the 5-lipoxygenase activating protein gene, omega-6 fatty acids, and breast cancer

A number of epidemiological studies and animal experiments suggest that omega-6 fatty acids increase the risk of cancer and omega-3s decrease. However, not all studies have produced consistent results. The 5-LO pathway has been implicated in carcinogenesis and tumor progression in many types of cancer: lung, colon, prostate, kidney, and bladder. Earlier epidemiological studies on dietary fat intake and breast cancer did not find positive association between omega-6 and breast cancer risk. Those studies, however, did not take into account genetic predisposition related to omega-6 fatty acid metabolism. Wang *et al.* (2008) determined genetic variants in the 5-LO gene (ALOX5) and ALOX5AP in combination with dietary LA intake in a population-based multiethnic case-control study on breast cancer in Latin, African-American, and white women in the San Francisco area. The authors did not find significant main effects of ALOX5 and ALOX5AP genotypes on breast cancer risk that were consistent across race or ethnicity. A significant interaction was found between the ALOX5AP-4900, A > G polymorphisms and dietary LA intake ($P = 0.03$). Among women consuming a diet high in LA (top quartile of intake, 17.4 g/d), carrying the AA genotype was associated with higher breast cancer risk, compared with genotype AG or GG. Among women consuming ≤ 17.4 g/d of LA, ALOX5AP-4900 genotype was not associated with breast cancer risk. These findings indicate that studies on dietary fat intake and cancer should take into consideration type of fat and genetic variants. Furthermore, in the USA, 17.4 g/d is the intake that a significant portion of the population ingests. It is unfortunate that the American Heart Association recommended an LA intake up to 10% of calories, which is 22 g/d on a 2000 cal/d, thus putting a significant number of women at risk.

Genetic variants of cyclooxygenase-2 and the protective effect of long-chain omega-3 fatty acids in cancer of the prostate

Prostate cancer is one of the most common cancers in men. Increasing evidence points to chronic inflammation as one of the factors leading to cancer. Inflammation may result from bacterial or viral infections, intraprostatic urine reflux, or diet. Dietary components that are potent anti-inflammatory agents are the omega-3 PUFAs. Studies have shown that genetic variants at the COX-2 gene modify prostate inflammation through the COX-2 enzymatic pathway. COX-2 is a key enzyme in fatty acid metabolism and inflammation. In a case-control study of 466 men diagnosed with aggressive prostate cancer and 478 age- and ethnicity-matched controls, Fradet *et al.* (2009) genotyped nine COX-2 tag SNPs. Dietary history was assessed with a semi-quantitatively food frequency questionnaire. Increasing omega-3 intake was associated with a decreased risk of aggressive prostate cancer (P trend ≤ 0.0001), and this inverse association was even stronger among men with genetic

variants rs 4648310 (+8897 A/G) flanking the 3′ region of COX-2 (P interaction = 0.02). The patients with the lowest intake of omega-3s and the genetic variant had the most aggressive tumor, whereas the omega-3 PUFAs were protective and this effect was modified by the genetic variant. This gene by diet (omega-3s) interaction clearly shows that the main dietary effect was modified by the genetic variant, whereas men with the variant genotype AG or GG and low intake of omega-3s had much higher risk than men with the variant genotype and high intake of omega-3s.

Another study (Hedelin *et al.*, 2007) of Swedish men found that frequent consumption of fatty fish (rich in omega-3s) was inversely associated with prostate cancer risk, and this effect was modified by rs 5275 (+6364 A > G) SNP in COX-2 where only men carrying the variant allele maintained a strong inverse association between fatty fish intake and prostate cancer, suggesting that the protective effect of omega-3s on prostate cancer may be modified by COX-2 variants.

The interaction between dietary factors and genetic variants could explain the differences noted in association studies. Considering that a low omega-3 intake in the presence of certain genetic variants leads to a more aggressive disease, an increase in omega-3 intake and a decrease in omega-6 leading to a balanced omega-6/omega-3 ratio, as it was during evolution, when our genes were programmed to respond to a balanced ratio, is the recommendation most appropriate to improve public health.

The omega-6/omega-3 ratio in neurodevelopment

Studies in rodents, chickens, primates, and visual and cognitive trials in human infants have shown that both AA and DHA are essential for brain development and function. AA and DHA have been shown to be independent determinants of brain growth and evolution (Simopoulos and Bazan, 2009). Moreover, the competition that exists between omega-6/omega-3 fatty acids applies to their balance being critical for brain development and structural integrity (Budowski and Crawford, 1985). DHA is essential for vision, brain neurons, and cell signaling. While DHA is clearly concentrated in the signaling systems of the brain, EPA is more likely to be involved in vascular blood flow and eicosanoid production where it can down-regulate the AA metabolites to maintain homeostasis.

The brain contains little parent EFA (LA and ALA) and typically has AA, docosatetraenoic acid, and DHA as the principal long-chain fatty acids. Although the size of the brain differs between mammalian species, the profile of AA and DHA does not vary suggesting a high degree of evolutionary conservation of the neural lipid profile. DHA is rapidly and selectively incorporated in the (sn)-2 position of neural phospholipid membranes, and is concentrated in the photoreceptor and selectively at synaptic signaling sites. It is the most unsaturated of the cell membrane fatty acids in the brain. The proportions of omega-6 and omega-3 in the diet are a determinant of biochemical efficiency, which is important in providing the optimal conditions for neurodevelopment. Therefore, approaching the ideal ratio of 2:1 or 1:1 could be of relevance to both neurodevelopment and the prevention of early neurodegeneration (Crawford *et al.*, 2003; Lukiw and Bazan, 2008). Because the enzymes involved in the metabolism of the LA and ALA are shared, there is competition between them, and the omega-6 and omega-3 fatty acids also regulate each other. The balance between LA and ALA and their (PUFA) metabolites in the diet is vital. In humans, the brain is the most outstanding organ in biological development: it follows that the priority is brain growth and development, and in the brain, the balance between omega-6 and omega-3 PUFA metabolites is close to 1:1. This ratio (between 2:1 and 1:1) should be the target for human nutrition. In Western diets, the omega-6/omega-3 ratio has increased to between 10:1 and 20:1. This high omega-6 proportion is largely made up by LA, is far from optimal, and is highly inappropriate for normal growth and development (Massiera *et al.*, 2010).

Conclusions and recommendations

Excessive amounts of omega-6 PUFA and a very high omega-6 to 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 and interfere with normal brain development.

In the Lyon Heart Study a ratio of 4:1 LA:ALA decreased total mortality by 70% in patients with one episode of myocardial infarction (de Lorgeril M *et al.*, 1994). Whether an omega-6/omega-3 ratio of 3:1 to 4:1 could prevent the pathogenesis of many diseases induced by today's Western diets (AFSSA, 2010), a target of 1:1 to 2:1 appears to be consistent with studies on evolutionary aspects of diet, neurodevelopment, and genetics.

Diets must be balanced in the omega-6 and omega-3 fatty acids to be consistent with the evolutionary understanding of the human diet. This balance can best be accomplished by decreasing the intake of oils rich in omega-6 fatty acids (corn oil, sunflower, safflower, cottonseed, and soybean) and increasing the intake of oils rich in omega-3s (canola, flaxseed, perilla, and chia) and olive oil which is particularly low in omega-6 fatty acids.

The ratio of omega-6/omega-3 fatty acids in the brain is between 1:1 and 2:1 which is in agreement with the data from the evolutionary aspects of diet, genetics, and the studies with the fat-1 animal model. Therefore, a ratio of 1:1 to 2:1 omega-6/omega-3 fatty acids should be the target ratio for health. Because chronic diseases are multigenic and multifactorial, it is quite possible that the therapeutic dose of the omega-3 fatty acids will depend on the degree of severity of diseases resulting from the genetic predisposition and the endogenous metabolism of LA and ALA.

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