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Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases

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Abstract

Anthropological and epidemiological studies and studies at the molecular level indicate 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 to 16.7/1. A high omega-6/omega-3 ratio, as is found in today's Western diets, promotes the pathogenesis of many diseases, including cardiovascular disease, cancer, osteoporosis, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 polyunsaturated fatty acids (PUFA) (a lower omega-6/omega-3 ratio), exert suppressive effects. Increased dietary intake of linoleic acid (LA) leads to oxidation of low-density lipoprotein (LDL), platelet aggregation, and interferes with the incorporation of EFA in cell membrane phospholipids. Both omega-6 and omega-3 fatty acids influence gene expression. Omega-3 fatty acids have anti-inflammatory effects, suppress interleukin 1β (IL-1β), tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6), whereas omega-6 fatty acids do not. Because inflammation is at the base of many chronic diseases, dietary intake of omega-3 fatty acids plays an important role in the manifestation of disease, particularly in persons with genetic variation, as for example in individuals with genetic variants at the 5-lipoxygenase (5-LO). Carotid intima media thickness (IMT) taken as a marker of the atherosclerotic burden is significantly increased, by 80%, in the variant group compared to carriers with the common allele, suggesting increased 5-LO promoter activity associated with the (variant) allele. Dietary arachidonic acid (AA) and LA increase the risk for cardiovascular disease in those with the variants, whereas dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) decrease the risk. A lower ratio of omega-6/omega-3 fatty acids is needed for the prevention and management of chronic diseases. Because of genetic variation, the optimal omega-6/omega-3 fatty acid ratio would vary with the disease under consideration.

Keywords: Evolutionary aspects of diet; Omega-6/omega-3 ratio; Genetic variation and nutrition

1. Introduction

A number of anthropological, nutritional and genetic studies indicate that human's overall diet, including energy intake and energy expenditure, has changed over the past 10,000 years with major changes occurring during the past 150 years in the type and amount of fat and in vitamins C and E intake [1–8].

Food technology and agribusiness provided the economic stimulus that dominated the changes in the food supply. From per capita quantities of foods available for consumption in the US national food supply in 1985, the amount of eicosapentaenoic acid (EPA) is reported to be about 50 mg per capita per day and the amount of docosahexaenoic acid (DHA) is 80 mg per capita per day. The two main sources are fish and poultry [9]. 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 15/1 to 16.7/1, instead of 1/1 as is the case with wild animals and presumably human beings [1–7]. An absolute and relative change of omega-6/omega-3 in the food supply of Western societies has occurred over the last 100 years. A

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; BMD, bone mineral density; CRP, C-reactive protein; DES, dry eye syndrome; DHA, docosahexaenoic acid; EFA, essential fatty acids; EPA, eicosapentaenoic acid; IL, interleukin; IMT, intima media thickness; IQ, intelligence quotient; LA, linoleic acid; LDL, low-density lipoprotein; LO, lipoxygenase; LTB, leukotriene; PDGF, platelet-derived growth factor; PGE, prostaglandin; PGI, prostacyclin; PUFA, polyunsaturated fatty acids; TNF, tumor necrosis factor; TXA, thromboxane; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

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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. Recent studies by Cordain [8] on wild animals confirm the original observations of Crawford [10]. However, rapid dietary changes over short periods of time as have occurred over the past 100–150 years is a totally new phenomenon in human evolution [11–15].

Genetically speaking, 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 (EFA) and in the antioxidant content of foods [1–7]. 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.

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

Mammalian cells cannot convert omega-6 to omega-3 fatty acids because they lack the converting enzyme, omega-3 desaturase. Linoleic acid (LA) and α-linolenic acid (ALA) 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. The balance of EFA is important for good health and normal development. 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 Ref. [4]). Whereas cellular proteins are genetically determined, the PUFA composition of cell membranes is to a great extent dependent on the dietary intake. Arachidonic acid (AA) and EPA are the parent compounds for eicosanoid production [4] (Table 1).

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 [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. 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 vasoconstriction and decreases in bleeding time. 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.

Table 1
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

Oxidative modification increases the atherogenicity of low-density lipoprotein (LDL). Oxidized LDL is taken up by scavenger receptors that do not recognize unmodified LDL leading to foam cell formation. The role of dietary fat and fatty acids in modifying fatty acid composition of LDL and its susceptibility to oxidation is of growing interest. Diets enriched with LA increase the LA content of LDL and its susceptibility to oxidation [16–21]. Reaven et al. [22] showed that a LA-enriched diet especially affects oxidation of small, dense LDL. Louheranta et al. [23] 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 [24].

Cleland et al. [25] showed that LA inhibits EPA incorporation from dietary fish oil supplements in human subjects. 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 4 weeks of fish oil 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.

Ambring et al. [26] 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 in healthy subjects. This is a very interesting and important study, because it clearly showed that

the plasma 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 C-reactive protein (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 after the Mediterranean diet, 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, which distinguished it from other Mediterranean diets, by being similar to the diet on which human beings evolved [7,27,28].

Freese et al. [29] compared the effects of two diets rich in monounsaturated fatty acids, differing in their LA/ALA ratio on platelet aggregation in human volunteers. Both diets were similar in saturated, monounsaturated and polyunsaturated fatty acids (PUFA). 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. 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 [30]. In the secondary prevention of cardiovascular disease, a ratio of 4/1 was associated with a 70% decrease in total mortality [31]. 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 [30]. 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.

The dietary ratio of omega-6/omega-3 fatty acids and bone mineral density (BMD) in older adults was studied in the Rancho Bernardo Study by Weiss et al. [32]. 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/day 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 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.

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 [33,34]. Increased concentration of inflammatory cytokines, such as interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor-α (TNFα) have been found in tear film in patients with DES [35]. Miljanovic et al. [36] 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. 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.

Ferruci et al. [37] studied the relationship of plasma PUFA to circulating inflammatory markers in 1123 persons aged 20–98 years in a community-based sample. The total omega-3 fatty acids were independently associated with lower levels of pro-inflammatory markers (IL-6, IL-1ra, TNFα, CRP), and higher anti-inflammatory markers (soluble IL-6r, IL-10, 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."

Aihlaud and his collaborators have studied the effect of the omega-6/omega-3 ratio in animals and humans relative to perinatal growth and adipose tissue development. In a recent review, Massiera et al. [38] summarized experimental evidence, which supports PUFA of the omega-6 series as being potent promoters of both adipogenesis in vitro and adipose tissue development in vivo during the gestation/lactation period. In rodent studies, the authors raise the issue of the high content of LA during pregnancy and lactation, and infant formulas and infant foods, in relation to the epidemic of childhood obesity. During the pregnancy–lactation period, mother mice were fed either a high fat diet rich in LA—the precursor of AA—the "LO diet", or the same isocaloric diet enriched in LA and ALA (LO/LL diet). Body weight and adipocyte size at 8 weeks of age were higher with the LO diet than with the LO/LL diet. In contrast, prostacyclin receptor-deficient mice fed either diet were similar in this respect, indicating that the prostacyclin signaling contributes to adipose tissue development.

Further support for the need to balance the omega-6/omega-3 EFA comes from the studies of Kang et al. [39,40] 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*. 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 [41]. More recently, Kang, et al. showed that transgenic mice 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 [42]. 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 [43,44].

3. 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 [45].

In the case of enzymes involved in carbohydrate and lipid metabolism, both omega-3 and omega-6 fatty acids appear to suppress the genes that encode for several enzymes, whereas saturated, trans and monounsaturated fatty acids fail to suppress. DHA appears more potent in its effect than other PUFA. Omega-6 and omega-3 fatty acids and monounsaturated fatty acids induce acyl-CoA oxidase, the enzyme involved in beta-oxidation, but here again, DHA appears to be more potent.

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 [45]. 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 stearoyl-CoA desaturase 2 in the brain, peripheral monocytes (IL-1β, and VCAM-1) and platelets (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. Because of their coordinate or opposing effects, both classes of PUFA are needed in the proper amounts for normal growth and development [4]. Although so far the studies in infants have concentrated on the effects of PUFA on retinal and brain phospholipid composition and intelligence quotient (IQ), motor development is very much dependent on intermediary metabolism and on overall normal metabolism, both of which are influenced by fatty acid biosynthesis and carbohydrate metabolism.

The amounts of PUFA found in breast milk in mothers fed diets consistent with our evolution, should serve as a guide to determine omega-6 and omega-3 fatty acid requirements during pregnancy, lactation and infant feeding [4]. Of interest is the fact that saturated, monounsaturated and trans fatty acids do not exert any suppressive action on lipogenic or glycolytic gene expression, which is consistent with their high content in human milk serving primarily as sources of energy. Because nutrients influence gene expression, and many chronic diseases begin in utero or in infancy, proper dietary intake of PUFA, even prior to pregnancy may be essential, as shown for folate deficiency in the development of neural tube defects.

4. 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 (5-LO). 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 [46]. 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 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-mediated 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 to the prevention and treatment of cardiovascular disease according to genotype, particularly in the populations of non-European descent.

5. Conclusion

- Evidence from studies on the evolutionary aspects of diet, modern day hunter-gatherers, and traditional diets indicate that human beings evolved on a diet in which the ratio of omega-6/omega-3 EFA was about 1, whereas in the Western diets the ratio is 15/1 to 16.7/1.
- Many of the chronic conditions—cardiovascular disease, diabetes, cancer, obesity, autoimmune diseases, rheumatoid arthritis, asthma and depression—are associated with increased production of thromboxane A_2 (TXA_2), leukotriene B_4 (LTB_4), IL-1β, IL-6, TNF and CRP. All these factors increase by increases in omega-6 fatty acid intake and decrease by increases in omega-3 fatty acid intake. Furthermore, the balance of omega-6 and omega-3 fatty acids is very important for homeostasis and normal development.
- The ratio of omega-6 to omega-3 EFA is an important determinant of health, because both omega-6 and omega-3 fatty acids influence gene expression. Since many chronic diseases begin in utero or early in infancy, proper dietary intake of PUFA even prior to pregnancy may be important, as shown for folate deficiency in the development of neural tube defects. Recent studies on diet–gene interaction further suggest that dietary omega-6 fatty acids promote, whereas marine omega-3 fatty acids EPA and DHA inhibit leukotriene-mediated inflammation that leads to atherosclerosis.
- Because chronic diseases are multigenic and multifactorial, 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.

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