Review

Dietary unsaturated fatty acids: interactions and possible needs in relation to eicosanoid synthesis

John E. Kinsella, K. Shane Broughton, and Jay W. Whelan

Lipids Research Laboratory, Institute of Food Science, Cornell University, Ithaca, NY, USA

In addition to providing energy and essential fatty acids, dietary fatty acids can affect numerous biochemical and physiologic reactions related to secretory, cardiovascular, and immune functions. The major dietary unsaturated fatty acid, linoleic acid, affects tissue arachidonic acid and can influence eicosanoid-mediated reactions. Chronic, excess, or imbalanced eicosanoid synthesis may be conducive to excessive inflammation, thrombotic tendencies, atherosclerosis, and immune suppression. Dietary n-3 polyunsaturated fatty acids (PUFAs) may ameliorate eicosanoid-related phenomena by reducing tissue arachidonic acid and by inhibiting eicosanoid synthesis. This review summarizes information concerning the metabolism of unsaturated fatty acids, with emphasis on tissue arachidonic acid levels and eicosanoids, and discusses the need for data concerning the appropriate intake of dietary n-6 and n-3 PUFAs to modulate arachidonic acid and eicosanoid synthesis and to minimize possible adverse reactions.

Key words: Linoleic acid; linolenic acid; arachidonic acid; n-3 polyunsaturated fatty acids; eicosanoids; prostanoids; dietary requirements; health

Introduction

The interactions between dietary lipids, especially as they affect plasma cholesterol and lipoproteins, has received immense attention in relation to coronary heart disease. Thus, when dietary fat intake is high (40% of calories), exogenous and endogenous cholesterol and saturated fatty acids (SFAs) interact to increase plasma cholesterol and low-density lipoproteins (LDLs),[1,2] apparently by impairing hepatic receptor-mediated uptake of LDL particles.[3] Replacement of SFAs with polyunsaturated fatty acids (PUFAs) or oleic acid results in a reduction of plasma cholesterol.[2,4] Because lipid research in the United States has focused primarily on plasma cholesterol and lipoproteins, limited attention has been devoted to the effects of, and interactions between, different dietary fatty acids. Recent research concerning the differences between SFAs, the effects of monoenoic acids,[4,5] the numerous potential eicosanoid-mediated effects of dietary n-6 fatty acids, and the different biochemical and biologic effects of PUFAs of the n-6 and n-3 families[6-8] has cogently dramatized the need for more information concerning the interactions between and optimum intake of dietary unsaturated fatty acids.

Dietary fatty acids: metabolism and effects

The preponderance of dietary fats are triacylglycerols with widely differing fatty acid composition (*Table 1*). The amount and mix of component fatty acids and their location on the glyceride molecule influences their biologic impact. During digestion, pancreatic lipase releases the fatty acids from positions sn-1 and sn-3, and these are absorbed as free fatty acids. The fatty acid on sn-2 is facilely absorbed, mostly as the monoglyceride.[9] Dietary fats with SFAs on the sn-2 position tend to be more hypercholesteremic and atherogenic than fats with similar fatty acid composition in which the SFAs are mostly on the sn-1 and sn-3 positions, e.g., lard and cocobutter.[10] This hypercholesterolemic effect is apparently related to reduced absorption of the free SFAs, especially when insoluble saturated calcium salts are formed. In the case of fish oils, location of the n-3 PUFA on the sn-2 position facilitates their absorption.[11]

Address reprint requests to Dr. John E. Kinsella, Lipids Research Laboratory, Institute of Food Science, Cornell University, Ithaca, NY 14853, USA.

Table 1 The fatty acid composition (weight %) of fats and oils from different food sources

Fatty acid	Source											
	Milk	Beef	Pork	Chicken	Coconut	Olive	Palm	Soybean	Canola	Spinach	Salmon	Menhaden
Short- and medium-chain (C4-C10) Saturated	10	—	—	—	15	—	—	—	—	—	—	—
12:0	2	trace	—	—	48	—	—	—	—	—	—	—
14:0	10	3	1.2	0.7	16	—	—	—	—	—	—	—
16:0	28	28	22	23.0	9	12	42	10	5	13	19	19
18:0	10	2	13	6	2	2	4	4	2	1	2	4
Monounsaturated												
16:1	4	5	4	5	trace	trace	trace	trace	—	7	6	9
18:1	34	43	48	36	8	72	43	25	48	0	24	13
Other	—	—	—	—	—	—	—	—	2	—	5	2
Diunsaturated												
18:2 n-6	2.5	2	10	22	2	11	8	52	25	16	3	1
Triunsaturated												
18:3 n-3	trace	1	—	1	—	—	trace	7	15	60	13	1
Polyunsaturated												
20:4 n-6	trace	trace	—	trace	—	—	—	—	—	—	2	trace
20:5 n-3	—	—	—	—	—	—	—	—	—	—	12	15
22:6 n-3	—	—	—	—	—	—	—	—	—	—	9	9

On absorption, dietary fatty acids (greater than decanoic acid) are reacylated into triacylglycerol in the intestinal mucosa, which are assembled into chylomicrons and enter the blood stream via the lymphatic system.[9] The chylomicron triacylglycerols are hydrolyzed in the peripheral tissue and liver as a source of fatty acids. Following uptake by the liver, exogenous and endogenous fatty acids and cholesterol are incorporated into very low-density lipoproteins (VLDLs). The VLDLs are secreted into the circulation, where they are converted into LDLs via progressive lipolysis.[2] Both provide fatty acids for body tissues and organs.[2,4,5]

Most dietary fatty acids are oxidized for energy via tissue β-oxidation in mitochondria of tissues.[9] Because of limited permeability of mitochondria, the n-3 PUFAs of fish oils are primarily oxidized by an induced peroxisomal system.[12] The rates of oxidation of lauric, myristic, palmitic, stearic, oleic, α-linolenic, linoleic, γ-linolenic, dihomo-γ-linolenic, and arachidonic acids were studied in weanling rats by Leyton et al.[13] The longer the chain length of the SFA, the slower the rate of oxidation. Oleic acid (18:1) was oxidized very rapidly, as was lauric acid. Linoleic acid (18:2 n-6) was oxidized at a faster rate than any of its metabolites, while arachidonic acid (20:4 n-6) was oxidized at the slowest rate. The rate of oxidation of γ-linolenic acid (18:3 n-6) was almost as fast as that of lauric and oleic acids.[13]

Fatty acids of short and medium chain lengths (C4-C10) are readily absorbed into the portal system and are rapidly transported to liver and tissues where they provide a ready source of metabolizable energy.[14] These fatty acids do not depend on carnitine for oxidation. They circumvent the need for chylomicron formation, thereby saving energy and avoiding chylomicronemia and intestinal VLDL production.[14] These fatty acids are not lipogenic and have little effect on metabolism of other fatty acids or membrane lipid composition.[15] They have become popular as an energy source in enteral and parenteral foods.

Effects of saturated fatty acids

Various types of dietary fatty acids affect the metabolism lipoproteins.[2,16,17] SFAs, especially myristic and palmitic acid (and perhaps lauric acid when ingested in excess of energy needs, i.e., in high-fat diets) are hyperlipidemic and hypercholesteremic.[16,18-20] However, when consumed in a balanced diet in conjunction with an appropriate mixture of unsaturated fatty acids that matches energy needs, these fatty acids may be mostly oxidized and nonhyperlipidemic. Much of the data concerning the hyperlipidemic effects of SFAs are based on studies that have used a single fat source, usually approximately 40% of calories. The resultant data may be of questionable validity with respect to conventional diets. Nevertheless, epidemiologic data are generally consistent with the hyperlipidemic effects of dietary SFAs.[1,16-18,20]

Excessive dietary SFAs may alter membrane composition and lipoprotein metabolism, and may influence the metabolism of unsaturated fatty acids and the pattern of acylation of fatty acids into tissue phospholipid pools. Dietary SFAs are also conducive to platelet aggregation[21,22] and have been associated with arrhythmia.[23] Galli et al.[24] observed that higher amounts of arachidonic acid accumulated in the platelets from rabbits consuming butter compared with those fed corn oil. This indicates that the fatty acids of butter facilitate arachidonic acid synthesis and its disposition in platelets, whereas the high amounts of linoleic acid in corn oil reduce arachidonic acid and the tendency of platelets to aggregate.

Currently, SFAs contribute around 14% of calories to the American diet, with a significant amount being stearic acid. Because of its facile conversion to oleic acid, stearic acid may not be hypercholesterolemic.[25] However, its association with increased platelet aggregability,[21,22,26] which may increase thromboembolic tendencies, may be an undesirable feature of stearic acid.

Effects of monoenoic acids

Oleic acid is the principal monenoic fatty acid in the American diet, accounting for around 15% of dietary fat calories.[27] There is strong epidemiologic evidence that dietary oleic acid is associated with decreased risk of coronary disease.[20,28,29]

Dietary oleic acid is hypocholesterolemic.[4,18] Keys and colleagues[19,20] adduced epidemiologic evidence of an inverse association between regular olive oil consumption and the incidence of ischemic heart disease. Compared with SFAs, the hypolipidemic and long-term antithrombotic effects of oleic acid may relate to its rapid oxidation, its lack of a negative effect on hepatic LDL receptors, its inhibition of Δ6 desaturase, and its associated reduction of arachidonic acid synthesis. Oleic acid may also affect phospholipid species composition; i.e., it may decrease arachidonyl-containing phospholipid species in both cyclooxygenase and lipoxygenase substrate pools, thereby decreasing eicosanoid production. There is some evidence of each of these affects, but the mechanism(s) need to be established in the context of practical diets in which all the fatty acid components are well-defined in order to clarify possible interactions.

The other monoenoic fatty acids in the American diet are mostly trans isomers, e.g., elaidic acid from hydrogenated vegetable oils and dairy fats. Available evidence indicates that these acids are facilely oxidized, do not accumulate, and do not exert deleterious effects on membranes or on synthesis of eicosanoids unless ingested in abnormal amounts.[30-32]

Effects of dienoic acids

Linoleic acid, a diunsaturated fatty acid, is the predominant PUFA in the American diet. Linoleic acid is the major component of many common seed oils. With the development of oilseed processing technology and the promotion of linoleic acid as a hypocholesteremic agent, linoleic acid now accounts for around 7% of calories in the American diet.[27]

When linoleic acid replaces SFAs in high-fat diets (i.e., 40% of calories), it reduces plasma LDLs and cholesterol[18,19] by reducing VLDL triglyceride production and by improving LDL clearance via hepatic LDL receptors. A number of investigators have shown an inverse relationship between linoleic acid intake/tissue linoleic acid levels and the incidence of heart disease. The concentrations of linoleic acid in subcutaneous fat, which reflects dietary intake, have progressively increased in the United States (i.e., from 10 to 15 g/100 g fatty acid) since 1960, whereas they have remained unchanged in the United Kingdom at 7%. This is associated with a decrease in coronary heart disease in the United States, while coronary heart disease has increased in the United Kingdom.[33] Some investigators have asserted that tissue linoleic acid level is an independent predictor of myocardial infarction.[34] However, Keys et al.[20] noted differences in coronary heart disease mortality between Scottish and Greek men despite similar adipose concentrations of linoleic acid.

Dietary linoleic acid is facilely incorporated into phospholipids of membranes, but generally does not accumulate in phospholipids beyond a certain concentration.[35] Linoleic acid can be oxidized as an energy source[13] and/or stored in adipose tissue reserves. Linoleic acid performs many functions, both directly and indirectly, via further metabolism to arachidonic acid, as discussed below. It directly affects membrane fluidity and membrane receptor and enzyme functions, e.g., LDL receptor turnover, and causes a decrease in plasma cholesterol associated with buildup of cholesterol esters in the liver.[36]

Linoleic acid is less thrombogenic than SFAs, as assessed using the exteriorized loop method,[22] and it reduces platelet aggregability in humans.[28,29] Dietary linoleic acid may be antiarrhythmic,[23] presumably via eicosanoid-mediated mechanisms, as discussed below. Dietary linoleic acid can reduce blood pressure,[37] although other studies have generated conflicting results. Dietary linoleic acid reduced blood pressure in response to salt loading in rats on an essential fatty acid-deficient diet.[38]

Essential fatty acid deficiency

The overriding metabolic effects of linoleic acid derive from its further metabolism to arachidonic acid and thence to eicosanoids. In early research of fat-soluble vitamins, Burr and Burr[39] discovered that diets devoid of linoleic acid resulted in poor growth, scaly skin, alopecia, impaired energy metabolism, excess water loss, bleeding, etc. in experimental animals. This was termed essential fatty acid deficiency (EFAD)[40,41] and could be avoided by providing 1% to 2% of calories as linoleic acid. EFAD is characterized by reduced growth rates, keratosis, increased water loss via skin, increased susceptibility to bacterial infections, male and female sterility, decreased eicosanoid synthesis, reduced contraction of myocardial tissue, abnormal platelet aggregation, and impaired monocyte and macrophage function.[42] EFAD symptoms may reflect different functions of linoleic acid, i.e., as a membrane component, as an integral component of skin acylglucoceramides, and as precursor of arachidonic acid.[42-44]

Linoleic acid deficiency is rare in humans; however, its supply may be of concern in premature infants because its catabolism and utilization is rapid and reserves are limited. Unless linoleic acid is supplied to premature infants in parenteral or enteral diets, EFAD may occur.[45]

Essential fatty acids are important in membrane

structure as integral parts of phospholipids required for the integrity of intracellular and plasma membranes. In this regard, long-chain PUFAs with 20 and 22 carbons are apparently preferred.[46] The long-chain n-3 PUFA, eicosapentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid (DHA 22:6 n-3), are readily incorporated into membranes, as is eicosatrienoic acid (ETA, 20:3 n-9), when arachidonic acid is limiting.[40,43] Membranes appear to function normally with a wide range of arachidonic acid and different PUFA contents.[46,47]

Animals, particularly rats with EFAD, lose considerable amounts of water through the skin, which limits growth rates. In this regard, linoleic acid seems to be required as an integral component of the acylglucoceramides.[48] In the Thomasson assay, which is based on the reduction of skin water loss, linoleic acid has a basic essential fatty acid activity of 100, while α-linolenic acid, γ-homolinolenic acid, and arachidonic acid possess activities of 115, 102, and 139, respectively, and α-linolenic acid has an activity of 10. Linoleic acid at 1% of calories corrects excessive transepidermal water loss.[49] When rats are maintained in atmospheres of 90% relative humidity, growth rates appear almost normal, despite EFAD.[49]

Some arachidonic acid (i.e., the elongated desaturated product of linoleic acid), may be required for optimum membrane functions. Fragile membranes in erythrocytes and mitochondria are typical of EFAD. However, as pointed out by Hansen,[49] some cells are capable of growing in the absence of n-6 or n-3 PUFA and can survive with oleic acid. Furthermore, there is evidence that n-6 PUFAs are not absolutely required for the integrity, fluidity, and/or microviscosity of biologic membranes. Columbinic acid (5 trans 9,12,cis, cis 18:3 n-6) is as effective as linoleic acid in relieving EFAD symptoms, especially moisture loss, in the rat.[42,49] This fatty acid is not a precursor of the eicosanoids, indicating that synthesis of eicosanoids does not account for all essential fatty acid requirements and that arachidonic acid may not be essential to membrane structure. Both linoleic acid and columbinic acid are incorporated into epidermal acylglucoceramides, whereas very little arachidonic acid or ETA are incorporated.[48]

Brenner et al.[50] demonstrated that EFAD results in a progressive decrease in arachidonic acid, an increase in oleic acid, and a concomitant increase in its product, ETA (20:3 n-9). These changes reflect changes in enzymes, i.e., Δ9 desaturases increased 260%, Δ6 desaturase increased 160%, and Δ5 desaturase was unchanged in EFAD. Significantly, these changes in fatty acid composition of the membrane did not alter the temperature of phase transition as determined by electron spin resonance. The bulk of the microsomal lipids were in a highly fluid state even in EFAD,[50] indicating that the physical integrity of membranes can be maintained with different fatty acid components, including ETA.[46,49]

In humans, who are essential fatty acid-deficient, the skin is scaly and subject to rapid infection; surgical wounds heal very slowly.[41] This probably reflects the lack of arachidonic acid required for eicosanoid-mediated protective inflammatory and immune cell functions and for tissue proliferation.[51] In EFAD, lower amounts of eicosanoids are synthesized in response to normal stimuli (e.g., platelet adherence and aggregation is impaired[22]), because of limited thromboxane synthesis secondary to limiting supplies of arachidonic acid and possible inhibition by accumulated ETA. Likewise, immune functions are defective in EFAD because of impaired production of eicosanoids required for immune functions.[52] Eicosanoids (prostanoids, leukotrienes, and lipoxins) modulate many important physiologic functions (cardiovascular, pulmonary, immune, reproductive, and secretory), and the balanced production of low levels of different eicosanoids by different tissues is essential for normal physiologic homeostasis.[6,53,54]

The discovery of the multiple effects and functions of eicosanoids greatly clarified many of the functions of essential fatty acids and demonstrated why arachidonic acid was the most effective essential fatty acid for relieving several EFAD symptoms.[42,44] In this regard, knowledge of the factors controlling the enzymes involved in the synthesis of arachidonic acid from linoleic acid and its subsequent conversion to eicosanoids is essential in elucidating the metabolic interrelationships of dietary fatty acids and in establishing the minimum requirements for linoleic acid to ensure adequate arachidonic acid for eicosanoid-mediated functions. While the minimum requirement for dietary linoleic acid is not accurately known, 1% of calories as linoleic acid (i.e., 2 to 3 g/d) is adequate in preventing EFAD in growing babies.[41,43,44] The estimated excretion rate of prostanoid metabolites (approximately 100μg) represents only a minute portion of the total arachidonic acid in body pools.[46,55] Thus, pertinent questions are how much arachidonic acid is needed for membrane function and what is the minimum or optimum tissue concentration of arachidonic acid required for eicosanoid homeostasis? To clarify these questions, knowledge of factors controlling arachidonic acid synthesis and of agents which affect its further metabolism to eicosanoids is important.

Metabolism of unsaturated fatty acids: desaturation and elongation

In EFAD states, the conversion of stearic acid (18:0) to oleic acid (18:1 n-9) by Δ9 desaturase is enhanced, and the further conversion of 18:1 n-9 to 20:3 n-9, involving the rate-limiting Δ6 desaturase (*Figure 1*), is increased (*Table 2*). This pathway (which is normally inhibited by dietary linoleic acid and/or α-linolenic acid and other PUFAs) provides long-chain ETA to replace arachidonic acid and other 20 carbon PUFAs and to maintain membrane fluidity.[43,46,56] The desaturation/elongation pathway (*Figure 1*), by generating a polyenoic acid, may also be instrumental in the hypolipidemic effect of stearic acid in diets with low

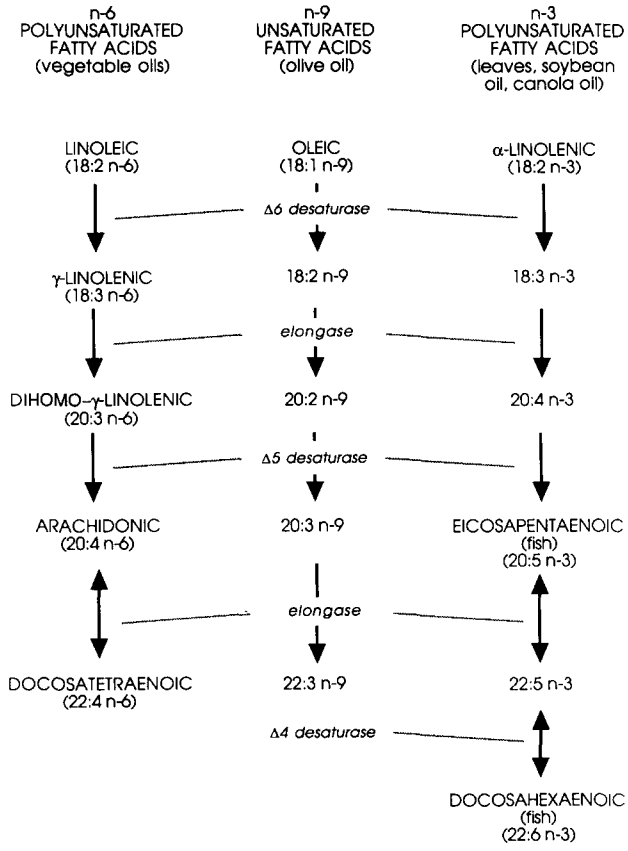

Figure 1 Outline of pathways of desaturation and elongation of dietary unsaturated fatty acids; the same enzymes are involved for each of the n-3, n-6, and n-9 fatty acid families

levels of PUFA. In subjects consuming predominantly domestic ruminant fats or olive oil, this pathway may be more active. This pathway may be suppressed in subjects consuming contemporary diets rich in linoleic acid.

Desaturases

Mammals possess a series of desaturases and elongases for metabolism of stearic, linoleic, and α-linolenic acids to long-chain PUFAs. Four desaturases, the Δ9, Δ6, Δ5, and Δ4 desaturases of the liver (particularly the rat liver), have been characterized.[50,57,58] These are involved in the desaturation of the three major families of unsaturated fatty acids: the n-9 oleic, n-6 linoleic, and n-3 linolenic acid families (*Figure 1*). These fatty acids compete with each other, especially at the rate-limiting Δ6 desaturase step.[56] Limited information is available concerning desaturases in other tissues, and remarkably little is known about the activities and properties of desaturases in human tissues, including the liver. This is a significant deficiency because of the physiologic importance of the products of this pathway. Sprecher[59,60] has noted that the fatty acid composition of tissue and membrane lipids is determined by the relative activities of these desaturases.

The fatty acid desaturase system involves three integral components, the desaturase, nicotinamide adenine dinucleotide (reduced) (NADH) cytochrome b_5 reductase, and cytochrome b_5, which are embedded in the microsomal membranes. Desaturases need an electron flux supplied mostly by NADH-cytochrome b_5 reductase and require the activated substrate, i.e., acyl-CoA. The regulatory steps in the desaturation of linoleic acid may be at the acyl-CoA formation step, the electron transfer from nicotinamide adenine dinucleotide (NAD) to the desaturase, or in the desaturase itself. However, Brenner et al.[50] concluded that neither the acyl thiokinase nor the electron transport system is limiting; therefore, regulation appears to exist at the enzyme desaturase step.[57]

Several factors (i.e., diet and hormonal status) affect desaturase activity.[57] Dietary protein, EFAD, and adenosine triphosphate levels act as activators, whereas fasting, glucose, glycerol, unsaturated fatty acids, and low-protein diets tend to depress Δ6 desaturase activity.[50,57,59] Insulin acts as an activator, whereas glucagon, epinephrine, glucocorticoids, and thyroxines depress activity. Diabetes is associated with reduced desaturase activity. The enzyme follows a circadian rhythm which may be related to the hormonal level in the blood, affecting tissue unsaturated fatty acid levels. Glucose refeeding after fasting induces Δ6 desaturase synthesis; however, a glucose-rich diet actually decreases enzymatic activity.[50,57] A high-protein diet increases Δ6 desaturase activity. The implications of this in Western diets warrants more examination. Blond et al.[61] reported that both Δ6 and Δ5 desaturase activity increased slightly with maturation of both obese and lean Zucker rats up to 12 weeks of age (i.e., in relatively young rats); however, the activity of the enzyme tends to decrease with the aging of rats.[62]

Leiken and Brenner[63] recently reported that the liver contains a cytosolic factor required for optimum Δ6 desaturase activity, which appears to prevent product inhibition of both Δ6 and Δ5 desaturase by γ-linolenic acid and arachidonic acid, respectively, as they are formed. Hence, the presence or absence of this factor could regulate the flow of products through this pathway; i.e., when it is low or absent, feedback

Table 2 Effects of essential fatty acid deficiency on fatty acids and desaturase activities of rat liver microsomes

		Period of deficiency (d)		
	Control	4	11	23
Fatty acid (weight %)				
18:1 n-9	5.6	9.7	12.8	17.4
18:2 n-6	12.8	10.1	4.1	5.1
20:3 n-9	—	—	1.0	5.0
20:4 n-6	24.2	12.0	9.9	12.5
Desaturases (relative activity %)				
Δ9	100	200	270	260
Δ6	100	105	160	120

18:1 n-9, oleic acid; 18:2 n-6, linoleic acid; 20:3 n-9, eicosatetraenoic acid; 20:4 n-6, arachidonic acid
From Brenner et al.[50]

inhibition would reduce product formation. Factors that influence the synthesis of this regulatory factor need to be identified.

The Δ5 desaturase is also influenced by diet and hormonal changes, but does not respond as rapidly as the Δ6 desaturase.[57] Evidence exists for coordination of the desaturase activities; however, the exact mechanisms of the coordinated regulation of these (Δ9, Δ6, Δ5, and Δ4 desaturases) and the regulation of PUFA synthesis has not been elucidated. There is a need for well-coordinated experimental studies to examine all of the factors that may affect these enzymes, using standardized controlled diets and animals of equivalent hormone status, gender, age, etc. There is a particular need for data on the regulation of desaturases in human tissues, which appear to have significantly lower activities than in rat tissue.[57]

The activity of the Δ6 desaturase is also affected by the amounts and types of dietary unsaturated fatty acids and the quantity of fat consumed.[41,43,44,56,57,62]

Linoleic acid and desaturase activities Dietary linoleic acid at low intake levels and in the absence of other unsaturated fatty acids is efficiently desaturated and elongated to arachidonic acid in the liver.[40,50,58-60]

In marginally essential fatty acid-deficient rats, increasing linoleic acid up to 0.75% of calories caused a rapid increase in tissue arachidonic acid levels. As linoleic acid intake was increased to 5%, arachidonic acid accumulation increased, but at a decreasing rate. This probably reflected the rate-limiting activity of the Δ6 desaturase, which is progressively inhibited by its products, unless they are bound by protein[63] or removed by transacylation.[56,57,60]

Other unsaturated fatty acids, trans isomers of linoleic acid, particularly α-linolenic acid, which are also substrates for Δ6 desaturase, are effective competitive inhibitors of this enzyme. The conversion of linoleic acid to arachidonic acid, especially at low levels of dietary intake, is inhibited by dietary α-linolenic acid.[56] Thus, at low levels of intake of dietary α-linolenic acid, the conversion of linoleic acid to arachidonic acid was inhibited even when available linoleic acid levels were increased.[40,56] With dietary linoleic acid at 0.3% of calories and increasing levels of α-linolenic acid (0.045%, 0.18%, 0.36%, and 0.7%), liver arachidonic acid levels decreased from 6.9 to 2.8, 20:3 n-9 from 3.3 to 2.2, and 22:5 n-6 from 1.2 to 0.1, while 22:6 n-3 increased from 1.9% to 7.0% of total fatty acids.[56] When linoleic acid was increased to 0.6% of calories while maintaining the n-3 to n-6 PUFA ratio, similar results were obtained, although 20:3 n-9 was greatly depressed, most linoleic acid accumulated, and more EPA, docosapentaenoic acid (DPA), and DHA were formed.[56] The animals were normal, indicating the marked plasticity in membrane composition and that 0.3% calories as linoleic acid may be quite adequate because oleic acid and α-linolenic acid (via desaturation/elongation) can provide the long-chain PUFAs needed for membranes.

The data of Mohrhauer and Holman[56] indicate that as linoleic acid was increased, the level of α-linolenic acid required to reduce tissue arachidonic acid was increased. Hence, in the contemporary American diet which contains more than 20 g linoleic acid/d, large amounts of α-linolenic acid may be required to reduce tissue arachidonic acid levels. Dietary α-linolenic acid at 0.1% of calories was sufficient to inhibit the elongation of linoleic acid by 50%, whereas approximately 30 times the concentration of linoleic acid was required for a similar magnitude of inhibition of linolenic acid desaturation.[40] However, it should be emphasized that these studies were conducted at low dietary fatty acid intake, possibly representing appropriate conditions for studying enzyme activities and substrate interactions.

In EFAD, the presence of dietary α-linolenic acid (or EPA) inhibited the formation of 20:3 n-9 (ETA) which has been used as an index of EFAD.[41,43] However, since ETA can occur without overt signs of EFAD, it may be of limited consequence to cells if there is adequate arachidonic acid for eicosanoid synthesis. Likewise, the conversion of dietary α-linolenic acid to long-chain n-3 PUFA (EPA, DHA) via Δ6 desaturase is progressively inhibited by increasing dietary linoleic acid from 5% to 15% of calories. This may be more pronounced in human tissue, in which the activity of Δ6 desaturase is apparently low.[47,55,57]

Oleic acid and desaturase activity Oleic acid can inhibit Δ6 desaturase activity, but high intake levels are required to reduce arachidonic acid synthesis. This is also influenced by linoleic acid concentrations.[40] The high levels of oleic acid in the typical Mediterranean diet may cause reduction of linoleic acid desaturation which, together with ETA generation, may account for some of the beneficial effects of this diet possibly via eicosanoid modulation. More data are needed to ascertain this possibility. Lokesh et al.[64] observed that the arachidonic acid content of tissue was lower in mice consuming diets containing olive oil compared with lard (*Table 3*). This may indicate some inhibition of Δ6 desaturase by the high level (68%) of oleic acid in the olive oil, although effects on specificity of acyltransferase may also be involved.

N-3 polyunsaturated fatty acids and desaturase activity Kurata and Privett[65] showed that the level of fat (at 5%

Table 3 Effects of olive oil and lard on the arachidonic acid content of phosphatidylcholine of mouse lung and spleen

	Lung		Spleen	
	Lard	Olive oil	Lard	Olive oil
	(nmol/mg protein)			
Fatty acid				
18:1	13.0	17.1	3.7	5.0
18:2 n-6	4.2	3.4	1.3	0.9
20:4 n-6	4.9	2.9	2.7	1.7

Diets containing 10% fat from olive oil or lard and equivalent amounts of linoleic acid (approximately 14%) were fed for 14 days From Lokesh et al.[64,115]

and 20%) affected the composition of liver fatty acids. The composition of the fat (composed of coconut oil and corn oil plus menhaden oil [C-MO]), further modified the impact of intake levels (*Table 4*). Generally, as the level of coconut oil or corn oil plus menhaden oil was increased, the arachidonic acid content of the liver increased. The arachidonic acid increased from 11% to 21% in rats consuming 5% and 20% coconut oil for 33 weeks, whereas the corresponding levels were 8.6 and 11.6, respectively, in the rats receiving 5% and 20% corn/menhaden oil mix at a ratio of 3:7. Of note was the high level of ETA (20:3 n-9) in animals fed the 5% coconut oil, indicative of EFAD, although clinical symptoms were not apparent and arachidonic acid levels were much higher than in the corresponding animals fed 5% C-MO. The low ETA in the C-MO diet indicated that the n-3 PUFA in the menhaden oil effectively inhibited desaturation and elongation of oleic acid. The activities of the Δ6 desaturase were markedly reduced in microsomes from animals consuming menhaden oil, indicating the marked inhibitory action of the EPA and DHA components.

The n-3 PUFAs in dietary fish oils markedly depress both the Δ6 and Δ9 desaturase enzymes, particularly when linoleic acid levels in diets are limiting.[66] This may indicate that competition and inhibition by the n-3 PUFAs may affect the amount of linoleic acid required to maintain a certain tissue level of arachidonic acid. DeSchrijver and Privett[66] showed that the activity of Δ6 desaturase tended to be comparable when dietary linoleic acid was constant, regardless of whether hydrogenated coconut oil or menhaden oil was fed as the remaining fat source. However, in an EFAD diet of hydrogenated coconut oil, the Δ5 desaturase typically increased threefold (*Table 5*). On replacement of 10% of the coconut fat with 10% menhaden oil in these diets, the Δ6 desaturase was reduced fourfold, being only 50% of the activity observed in diets containing 5% safflower oil. It should be interesting to determine if the reduced activity of Δ6 desaturase reflected lack of enzyme and/or its actual inhibition by the n-3 PUFAs in the microsomal preparations.

Table 4 The effect of dietary fat type and level on liver fatty acids and Δ6 desaturase activity

	CO		C-MO	
	5	20	5	20
	(% of dietary calories)			
Linoleic acid intake	0.4	1.5	2.5	10.0
Fatty acids (weight %)				
Microsomal				
18:2 n-6	4.1	7.4	8.8	14.0
20:3 n-9	8.7	2.1	—	—
20:4 n-6	11.5	20.8	8.6	11.6
Δ6 Desaturase (nm/mg/min)	0.46	0.25	0.13	0.16

Rats fed 36 weeks on 5% and 20% weight coconut (CO) or corn-menhaden oil mix (3:7 C-MO)
From Kurata and Privett[65]

Table 5 The effects of different dietary fats on microsomal fatty acids and desaturase activities in rat liver microsomes

	Dietary treatment				
Parameter	A	B	C	D	E
Dietary linoleic acid (%)	25.8	26.1	26.5	0.1	0.8
Microsomes—fatty acids (weight %)					
18:2 n-6	13.2	17.7	17.9	2.5	3.1
20:3 n-9	—	—	—	14.0	0.7
20:4 n-6	27.9	17.2	15.3	6.9	11.2
ΣE20: n-3	2.8	8.4	7.1	1.2	13.5
Δ6 Desaturase (nm/m/mg)	0.17	0.12	0.13	0.3	0.07
Δ9 Desaturase (nm/m/mg)	0.36	0.27	0.20	0.79	0.13

Fats fed at 15% for 33 weeks were as follows: A, 5% safflower (SF) + 10% hydrogenated coconut oil (HCO); B, 5% SF + 5% HCO + 5% menhaden oil (MO); C, 5% SF + 10% MO; D, 15% HCO; E, 5% HCO + 10% MO; desaturase activity, nanomoles of product per milligram protein per minute; Σ20: n-3, total long chain n-3 fatty acids
From De Schrijver and Privett[66]

The activity of Δ9 desaturase behaved in a manner similar to the Δ6 desaturase in microsomes from rats on the different diets. Rats fed 15% hydrogenated coconut oil exhibited classic dermal symptoms of EFAD, whereas those fed 5% hydrogenated coconut oil plus 10% menhaden oil did not show deficiency symptoms. The n-3 PUFAs were potent inhibitors of the elongation of oleic acid to 20:3 n9, and the level of 20:3 n-9 in the microsomes from animals on these treatments was approximately 14% and 1%, respectively. This is interesting because the diets provided only 0.1% and 0.8% linoleic acid, respectively. This would indicate that both groups should have been essential fatty acid-deficient; however, the group fed the menhaden oil was not, indicating that the n-3 fatty acids were partly substituting for n-6 PUFAs or that the fish oil contained arachidonic acid. The respective arachidonic acid levels in these animals were 7% and 11% compared with 17% and 20% in microsomes from rats fed essential fatty acid-adequate diets.[66]

Shimp et al.[62] showed that increasing dietary trans, trans linolaidic acid (tt 18:2) depressed Δ6 desaturase activity. Increasing the tt 18:2 from 0% to 6.0% of calories in diets containing 1.1% calories from linoleic acid caused a progressive decrease in Δ6 desaturase from 1.4 to 0.6 nmol 18:2 converted/mg protein/min after 12 weeks on a diet providing 12% calories from fat. Chern et al.[67] observed that dietary tt 18:2 significantly depressed both arachidonic acid and ETA in hepatic tissues.

Thus, several factors can influence desaturase activities. In addition, in vivo, there is competition between desaturases and the acyl transferases, which determine the concentrations of available unesterified,

activated fatty acids for desaturation in tissues. Unfortunately, many of the studies that have examined the effects of dietary fatty acids on desaturase activity have used variable diets with differing fatty acid composition in terms of n-6 and n-3 PUFA families and oleic acid levels. Generally, mixtures of triglycerides are used as sources of the unsaturated fatty acids. Consequently, the results of these experiments may be misleading because recent data indicate that the amount and, perhaps, type of fatty acids and the level of cholesterol in the diet can affect desaturase activity.[36] Diets with limiting linoleic acid enhance Δ6 desaturase[50] and, in diets containing low amounts of linoleic acid and large amounts of SFAs, the conversion of linoleic acid to arachidonic acid occurs efficiently,[24] presumably because of lack of competition.

With increasing intake of linoleic acid, tissue concentrations of linoleyl-CoA and of its desaturated, elongated products (γ-linolenic acid and dihomo-γ-linolenic acid [DH-γ-linolenic acid]) may not only inhibit Δ6 desaturase but may also alter acyl-CoA transferase specificity. This would competitively displace arachidonic acid from normal "eicosanoid precursor pools," thereby affecting eicosanoid generation. Dietary n-3 PUFA may be more effective for altering arachidonic acid levels in phospholipid pools. Dietary oleic acid may also alter the patterns of acylation of arachidonic acid and eicosanoid production. More analytical data on the distribution of arachidonic acid in phospholipid species, especially in human tissues, as affected by dietary fatty acids, are needed to ascertain interactions in vivo.

In examining the relative inhibitory effects of different dietary unsaturated fatty acids on desaturases, it is important to control for each of these variables so that the activities of the enzymes observed experimentally reflect the interaction between the competing substrate fatty acids. Of course, the products of the desaturase, whether n-6 or n-3 PUFAs, may have different potencies as feedback inhibitors; hence, the rate at which these are removed (i.e., acylated) from the desaturase environment may be a factor in determining rates of desaturation. Because the activity of desaturases affects the pool sizes of arachidonic acid, more definitive and reliable data are needed to truly assess the interactions between dietary PUFAs in human tissues.

To circumvent some of the shortcomings of in vitro studies with microsomes, Voss and Sprecher[68] used hepatocytes to study the conversion of linoleic acid to arachidonic acid by the alternating series of position-specific desaturases and malonyl CoA-dependent chain elongation steps. The Δ6 desaturation step is rate-limiting. Liver lipids normally contain relatively large amounts of linoleic acid and arachidonic acid, but only low levels of the intermediate γ-linolenic acid (18:3 n-6) and DH-γ-linolenic acid (20:3 n-6). However, in vitro studies do not include all of the competing pathways of desaturation, elongation, and, particularly, competitive acylation. Voss and Sprecher[68] observed that most exogenous linoleic acid was directly incorporated into the triglycerides and phospholipids of hepatocytes. Significant quantities were desaturated and elongated to γ-linolenic acid and DH-γ-linolenic acid. Maximum conversion was attained at approximately 0.3 mM concentrations of linoleic acid. Added γ-linolenic acid (below 0.15 mM) was mostly incorporated into acyl lipids, but some was converted to DH-γ-linolenic acid and arachidonic acid. In these experiments, γ-linolenic acid was a better substrate for chain elongation than linoleic acid. The studies showed that when the concentration of intracellular γ-linolenic acid was high, it inhibited its own elongation to DH-γ-linolenic acid. Hence, if the Δ5 desaturase is impeded, it could result in the buildup of DH-γ-linolenic acid in tissue. This could affect the amount and type of eicosanoids synthesized.[69] These studies indicate that measuring individual reaction rates for either fatty acid or phospholipid biosynthesis cannot explain regulation of PUFA metabolism in the liver, and all competing reactions should be simultaneously monitored.[68]

Factors affecting arachidonic acid levels and eicosanoid synthesis

Because arachidonic acid is the immediate precursor of the various eicosanoids, the concentration of arachidonic acid in particular phospholipid pools of tissue may be a primary factor in regulating the amounts of eicosanoids synthesized in vivo. If arachidonic acid is the only long-chain PUFA in membranes, on its release, its further metabolism via cyclooxygenase and lipoxygenase may be unimpeded. In addition, excess or imbalanced eicosanoid production can perturb many physiologic functions and be conducive to or exacerbate diseased states.[6,7,54,55,70] Because excess production of eicosanoids can cause various pathophysiologic repercussions,[6,54,55,70] it is important to determine the relationships between dietary PUFAs, tissue arachidonic acid levels, and eicosanoid synthesis.

Tissue arachidonic acid levels and/or the presence of other PUFAs (i.e., γ-linolenic acid, DH-γ-linolenic acid, n-3 PUFA) that can compete for or inhibit enzymes involved in eicosanoid synthesis (i.e., cyclooxygenase and lipoxygenase) may be important in the physiologic regulation of eicosanoid generation (*Figure 2*). Because dietary linoleic acid is the precursor of tissue arachidonic acid, in vivo it should be possible to alter tissue arachidonic acid pools via manipulation of dietary fatty acids, which affect the Δ6 desaturase pathway. In addition, arachidonic acid in tissue can be changed by altering the acyl transferase pathway for esterification of arachidonic acid into phospholipid pools. Thus, dietary oleic acid, linoleic acid, trans trans linoleic acid, linolenic acid (α and γ), and long-chain n-3 PUFA (EPA and DHA) can all affect the flow of acyl substrates through the Δ6 pathway and can potentially alter tissue arachidonic acid concentrations (*Figure 2*). They can compete with arachidonic acid for acyltransferase and inhibit lipooxy-

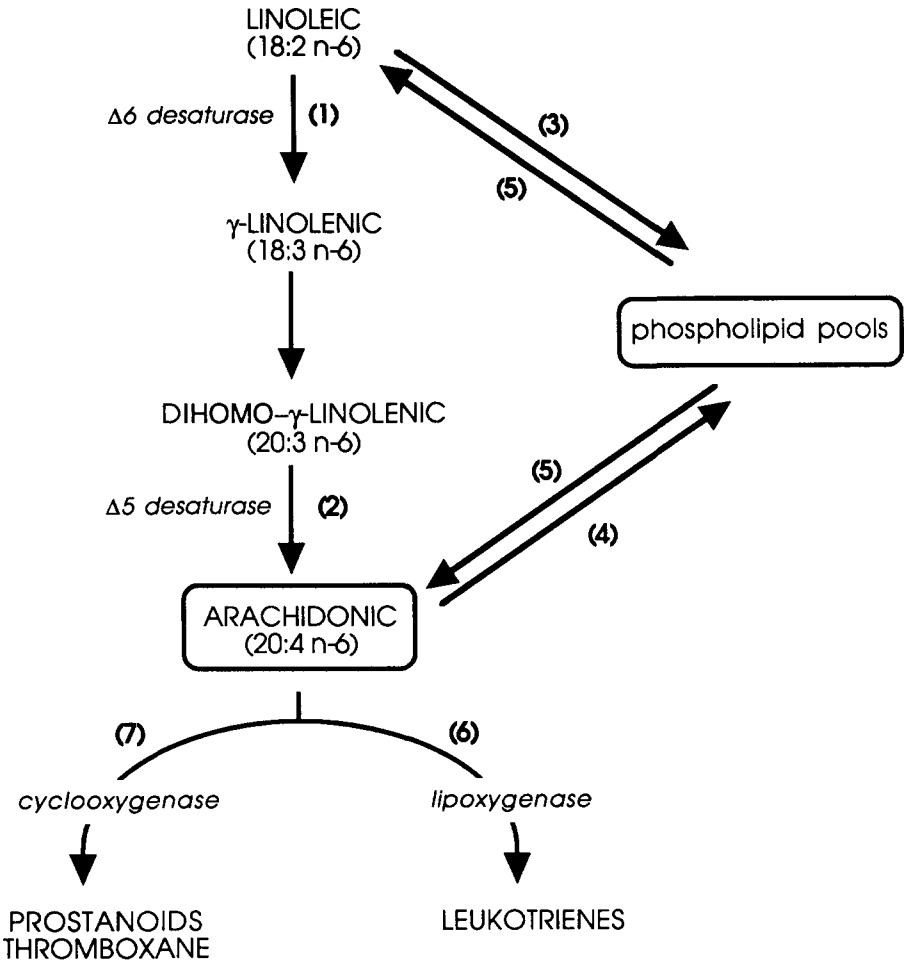

Figure 2 Dietary n-3 PUFAs may affect the metabolism of n-6 linoleic and arachidonic acid at several loci; they can compete for and inhibit desaturases (1,2), acyltransferases (3,4), and dioxygenases (6,7); they may also affect the specificity of phospholipases (5)

genase and cyclooxygenase with a net reduction in eicosanoid production.[68,69,71]

Effects of dietary linoleic acid The apparent minimum requirements for linoleic acid for growing subjects is around 1% to 2% of calories, with higher quantities recommended for pregnant/lactating women and neonates.[41,43,72] This is required to ensure an adequate supply of essential fatty acids for tissue proliferation and membrane integrity, and to provide precursor arachidonic acid for eicosanoids. The minimum requirement for adults is unknown, but is apparently very low.[46,55]. Because long-chain n-3 PUFA (EPA, DHA) and ETA (20:3 n-9) can apparently meet most of the membrane requirements, and since arachidonic acid utilization for eicosanoids is in the order of 100 μg/d,[7,90] the absolute need for arachidonic acid either directly or indirectly from linoleic acid may be very low.[46,55] Current consumption of linoleic acid in the United States is around 6% to 7% of calories (20 to 25 g/d). While in a high fat diet this amount of linoleic acid may be desirable for the reduction of elevated plasma cholesterol, there is reason to question how these high intakes might affect tissue arachidonic acid pools. There is concern that a high intake of linoleic acid, via conversion to arachidonic acid, may be associated with heightened or chronic inflammatory states and suppression of immune functions, and may be conducive to various pathophysiologies.[6,7,54,70,73]

Eicosanoid synthesis is impaired in essential fatty acid-deficient rats, even though arachidonic acid is present in tissue phospholipid pools.[74] At very low intakes of linoleic acid (in the absence of n-3 PUFA), the 20:3 n-9 which is synthesized may reduce eicosanoid formation, as shown by Lefkowith et al.[75] Arachidonic acid is required for platelet function as a precursor of the proaggregatory thromboxane. Essential fatty acid-deficient rats exhibit low platelet aggregability. However, supplementation with linoleic acid (3% of calories) relieved EFAD and increased platelet aggregability.[21] Further stepwise increases in dietary linoleic acid from 3% to 40% of calories actually decreased platelet aggregation, indicating an antithrombotic effect of linoleic acid, which led to the suggestion that linoleic acid intake be increased to >12% to minimize thrombotic events.[21,22] This may reflect decreased arachidonic acid synthesis and displacement of arachidonic acid from "eicosanoid" precursor pools by linoleic acid, γ-linolenic acid, and DH-γ-linolenic acid. It may also indicate some inhibition of

eicosanoid synthesis by these n-6 PUFAs and the formation of eicosanoids from DH-γ-linolenic acid.[69]

Increasing the intake of dietary linoleic acid (i.e., from 0.5% to 15% of calories) generally increases tissue arachidonic acid levels, but at a decreasing rate. Usually, with linoleic acid levels above 10% to 12% (depending on other dietary components), further accumulation of arachidonic acid declines.[22,24,35,76] Hamm et al.[35] showed that progressive increases in linoleic acid (from 0.4% and 12.1% of calories in a diet containing 20% calories as fat) resulted in a gradual increase in the linoleic acid content of the plasma membrane of liver from 5% to 16%, with concomitant but smaller increases in hepatic arachidonic acid levels from 12.9% to 20% (*Table 6*). Garg et al.[36] reported that linoleic acid at 20% by weight in diets decreased arachidonic acid levels in the rat liver, but increased plasma arachidonic acid levels; this observation warrants further study.

The current high intake of linoleic acid (megadose, in relation to requirements) may actually be reducing the total arachidonic acid pool, but it is not known how the "eicosanoid precursor pool" is affected. A high intake of linoleic acid increased linoleic acid in platelet phospholipid pools at the expense of arachidonic acid and reduced thromboxane synthesis.[24] Apparently, increasing linoleic acid causes inhibition of Δ6-desaturase by either precursor or product inhibition. In diets providing meats which contain small concentrations of arachidonic acid, dietary linoleic acid may displace arachidonic acid from eicosanoid precursor pools. Curiously, in ex vivo systems, exogenous arachidonic acid actually increased Δ6 desaturase activity by competing with linoleic acid for acylation, making more unesterified linoleic acid available for desaturation.[6] Hence, it cannot be assumed that increased intracellular levels of linoleic acid progressively increases arachidonic acid synthesis. The recent report of a regulatory binding protein suggests that product build-up causes inhibition of the desaturase.[63] This may result in acylation of greater amounts of linoleic acid and γ-linolenic acid and DH-γ-linolenic acid into tissue phospholipid pools at the expense of arachidonic acid.

Table 6 The effects of increasing dietary linoleic acid on fatty acid composition of liver membrane

	Linoleic acid levels					
	A	B	C	D	E	F
Dietary						
18:2 (% calories)	0.4	2.6	5.4	7.5	10.0	12.1
Liver membrane fatty acids						
18:2 n-6	5.0	9.1	11.3	13.6	16.0	15.6
20:4 n-6	12.9	18.8	19.9	18.3	20.0	19.3
n-3 PUFA	5.7	4.1	4.9	3.2	4.2	4.0

Fat, 10% of diet weight composed of butterfat (A)/corn oil (F) mixtures (B-E) to give increasing linoleic acid and decreasing saturated fatty acids, was fed for 4 weeks; 18:2 n-6, linoleic acid; 20:4 n-6, arachidonic acid; n-3 PUFA, n-3 PUFAs
From Hamm et al.[35]

Effects on eicosanoids Several investigators have reported relationships between dietary linoleic acid, tissue arachidonic acid, and eicosanoid synthesis. Galli et al.[24] fed diets containing approximately 25% of calories as either corn oil or butterfat to rabbits for 3 weeks and 3 months, respectively, and analyzed tissue phospholipids. The corn oil diet increased linoleic acid in phospholipids of liver and platelets, with minor changes in the aorta. Following consumption of corn oil, the arachidonic acid level in liver phospholipid classes were increased while arachidonic acid decreased in platelets, with negligible changes in aorta contents. On continued ingestion of these fats for 3 months, the most notable change was an increase in arachidonic acid levels in the phospholipid classes of platelets. The platelets from animals fed butterfat showed a marked decrease in threshold for aggregation in response to arachidonic acid, whereas there was no significant difference in response to collagen. The platelets from rabbits fed butterfat synthesized slightly less thromboxane than those fed corn oil, and Prostacyclin (PGI_2) production by the aorta of rabbits fed corn oil was significantly less than that produced in rabbits consuming butterfat. These studies indicated that different tissues showed different responses to dietary fatty acids. Thus, whereas arachidonic acid accumulation in the liver was higher with the corn oil diet, the accumulation of arachidonic acid in platelets and aorta phospholipids was generally higher in the animals fed the butterfat diet. This may reflect a lack of inhibition of linoleic acid desaturation and the selective acylation of arachidonic acid by these tissues.

Platelets from rabbits fed different dietary fats, composed of 8% safflower oil made up to 40% energy with olive, palm, linseed, fish, or sunflower seed oil, for 18 months showed little difference in arachidonic acid levels. However, thromboxane production in response to collagen stimulation differed with treatment; there was significant inhibition in animals consuming n-3 PUFA.[77] Gratoroli et al.[78] observed that increasing dietary linoleic acid from 1.3% to 10% caused a marked increase (from 10% to 36%) in linoleic acid of gastric mucosa, decreased arachidonic acid from 9.5% to 6.9%, and depressed Prostaglandin E_2 (PGE_2) synthesis from 327 to 250 ng/g mucosal tissue.

Dupont et al.[74] reported that dietary linoleic acid affected prostanoids synthesized during clotting of whole blood from female rats. As linoleic acid increased, from 0% to 2%, total prostanoid synthesis increased. As linoleic acid increased to 7%, total prostanoid synthesis decreased. It then increased slowly but linearly as linoleic acid increased to 27% of calories. The drop in prostanoids between intakes of 2% to 7% may have reflected effects of accumulating γ-linolenic acid and DH-γ-linolenic acid, which inhibited conversion of arachidonic acid to diene eicosanoids. Significantly, PGE_2 increased linearly over the whole range of linoleic acid intake (an observation that is relevant to immunosuppression by PGE_2).[79] The production of prostanoids PGE_2 and $PGF_{2\alpha}$ was correlated with tissue arachidonic acid

Table 7 Effects of increasing intake of linoleic acid on n-6 polyunsaturated fatty acids in rat platelet phospholipids

Platelet fatty acids	Linoleic acid in diet (% of total fatty acids)					
	1.0	2.0	5.0	7.5	12.5	17.5
18:2 n-6	5.6	5.1	5.4	5.9	6.5	7.0
20:3 n-9	3.3	2.1	1.4	1.1	1.1	1.0
20:4 n-6	14.8	16.4	17.3	18.2	18.6	18.3
22-n-6	5.2	5.9	6.8	7.4	7.7	7.7
ΣLCn-6	20.0	22.3	24.1	25.6	26.3	26.0

ΣLC, total long chain fatty acids of n-6 family; 22 n-6, PUFAs with four and five double bonds
From Zevenbergen and Haddeman[76]

levels. Dupont[80] suggested that more than 6% dietary linoleic acid is beneficial in controlling prostanoid production in a diet providing around 30% of energy from fat.

Zevenbergen and Haddeman[76] reported the effects of dietary linoleic acid on tissue fatty acids. Dietary fat (40% of calories) containing incremental amounts of linoleic acid (1.0%, 2.0%, 5.0%, 7.5%, 12.5%, and 17.7% of dietary fatty acids) at the expense of lauric and myristic acid were fed to rats for up to 14 weeks. Analyses of the fatty acids of aorta phospholipids revealed a progressive increase in arachidonic acid with incremental amounts of dietary linoleic acid. This was associated with a linear increase in prostacyclin synthesis by aorta. The long-chain n-6 PUFA pool of platelets also increased progressively with dietary intake of linoleic acid (*Table 7*). On stimulation with collagen (to obtain maximum aggregation), there was a progressive increase in the generation of 12-hydroxy eicosatetraenoic acid (12HETE), presumably by 12-lipoxygenase. Of note was the large increment that occurred between intakes of 0.4 energy % and 2.0 energy % linoleic acid. The former may represent a marginal EFAD state, as indicated by the 20:3 n-9 levels in the platelets. The production of 12-hydroxy hepatadecatetraenoic acid (HHT), which is generated during the synthesis of thromboxane, showed only a slight increase with dietary intake of linoleic acid although the platelet arachidonic acid was increased. Platelet aggregation in response to low and high concentrations of collagen was not significantly affected by dietary linoleic acid.

Quantitative relationships between eicosanoid formation and dietary intake of linoleic acid have been explored mostly in vitro, and data from such studies are inconclusive because in vitro eicosanoid formation is affected by many extrinsic factors. Various catabolites of eicosanoids are excreted in urine; hence, urinary excretion may provide a useful, but low, esimate of in vivo synthetic rates.[81] Humans excrete around 1 mg/d of prostanoid metabolites. However, the amounts synthesized and excreted may be sensitive to various physiologic and pathophysiologic states of the subject (vascular activity, reproductive phase, drugs, etc.) and, hence, may not be sensitive enough for monitoring the effects of dietary fatty acids. Nevertheless, many studies indicate that dietary linoleic acid does affect eicosanoids. When linoleic acid intake is deficient or low, eicosanoid synthesis is depressed.[52,82] Nugteren et al.[83] reported a positive relationship between linoleic acid intakes (0.6%, 11%, and 29% of calories) and excretion of prostanoid metabolites in rats. In some studies using rats, as linoleic acid intake is increased up to 1% to 2%, there is enhanced synthesis of eicosanoids followed by a decrease with up to 10% dietary linoleic acid. Synthesis again increases as linoleic acid intake is further increased to 30%.[80,84,85] Mathias and Dupont[85] reviewed the data on rat experiments, which showed that EFAD reduced and increasing dietary linoleic acid increased secretion of prostanoids in a somewhat erratic biphasic manner.

Adam et al.[86] reported that the levels of dietary linoleic acid affected excretion of eicosanoid metabolites. Thus, consumption of 0%, 4%, and 20% of calories as linoleic acid in a diet supplying 30% fat, human subjects excreted 123, 175, and 350 μg PG metabolites per 24 hours, indicating a relationship between linoleic acid and eicosanoid production, even though only a small amount of total pool was used. Friedman and Frolich[87] observed that reducing linoleic acid intake from 20% to 5% to 10% of calories actually increased eicosanoid formation in infants, suggesting that high linoleic acid intake caused displacement of arachidonic acid from the eicosanoid pools or that other products of linoleic acid reduced eicosanoid synthesis. Knapp and Fitzgerald[88] showed that the excretion of eicosanoids increased with the duration of ingestion of safflower oil (50 ml/d, rich in linoleic acid), presumably reflecting increased available arachidonic acid. Thus, the excretion rates before and after 4 weeks were 2.5 and 5.0 μg PGE_2, 75 and 110 ng PGI_2, and 245 and 243 ng thromboxane (TXA_2)/g creatinine, respectively.

The quantities of eicosanoids synthesized by tissue reflect presumably the availability of arachidonic acid. The levels of arachidonic acid in tissue phospholipid pools are affected by the elongation and desaturation of dietary linoleic acid and by the intake of arachidonic acid (around 100 mg/d in the contemporary diet). Because eicosanoid synthesis may reflect tissue arachidonic acid levels, there is a need to quantify the relationship between dietary n-6 PUFA and amounts of eicosanoids generated by tissues in normal and pathologic states. The possibility that dietary fatty acids can affect eicosanoid generation has been demonstrated.[6,8,69] Studies to determine the effects of conventional diets and diets rich in PUFA (being promoted to reduce coronary vascular disease) on eicosanoid production are of great interest, particularly in view of evidence that eicosanoids affect atherogenesis and thrombotic events.[69,70,89]

Ferretti et al.[90] monitored PGE_2 metabolite (PGE_2-M) excretion as affected by diet. Using a crossover design, 24 men aged 24 to 54 years were fed a regular diet, i.e., 41% energy from fat (P/S ratio, 0.59) and an experimental diet containing 19% energy from fat (P/S ratio, 1.3). The regular diet contained 600 mg choles-

Table 8 Relationship between lipid intake, fatty acid content of typical, regular, and experimental diets, and excretion of PGE_2 metabolite

Parameter	Experimental Diet	Regular Diet
Lipid		
g/d	67.2	144.7
en %	18.9	40.7
Total saturated		
g/d	18.1	51.6
en %	5.1	14.5
Oleic (18:1 n-9)		
g/d	26.6	49.7
en %	7.5	14.0
Linoleic (18:2 n-6)		
g/d	21.7	30.6
en %	6.1	8.6
α-Linolenic (18:3 n-3)		
g/d	2.0	1.9
en %	0.6	0.6
PGE_2 metabolite excretion/μg/24 h	11.53 ± 1.24	13.44 ± 1.63

en %, percent of calories from fat
From Ferretti et al.[90]

terol/3,200 calories, whereas the experimental diet contained 280 mg; both diets contained neutral detergent fiber, 13.9 and 35.5 g/d, respectively. Diets were consumed for 10 weeks and the groups were then switched for an additional 10 weeks. Urinary PGE-M excretion rates were lower in the low-fat diet group compared with the regular, high-fat diet group (*Table 8*). This study indicated that dietary fat intake and its composition affected endogenous levels of eicosanoids. Thus, the low-fat diet recommended for the prevention of heart disease, which contains less linoleic acid, was effective in reducing eicosanoid excretion compared with a typical American diet.

Adam et al.[86,91] also observed an increase in excretion of prostanoid metabolites from humans with increasing linoleic acid intake. However, the significance of the limited available data is uncertain. It is not known if human tissue show a biphasic response to dietary linoleic acid, as observed in rats,[80] at what level of intake of linoleic acid a reduction of eicosanoids may occur, or how this is affected by dietary fatty acid composition. The observations of Renaud et al.,[28,29] that at dietary P/S ratios in excess of 0.7, platelet activity increased and death rate from coronary heart disease was not decreased, are pertinent to these questions. The potential adverse effect of high linoleic acid intake on immune functions is also an important and relevant observation.[73,79,92a]

Significantly, the amounts of eicosanoids secreted represent only a small fraction of arachidonic acid available in body pools;[55] hence, it is difficult to demonstrate a direct relationship between dietary n-6 PUFA, tissue arachidonic acid, and amounts of eicosanoids synthesized in subjects fed contemporary diets. The dietary intake of preformed arachidonic acid varies depending on meat (especially organ meats), but, in normal diets, it may range from 100 to 200 mg/d. This apparently exceeds the amount normally converted to eicosanoids and, hence, dietary intake of arachidonic acid may be significant, especially in pathologic conditions. Conceivably, arachidonic acid is segregated into specific pools, i.e., a "structural" pool and an eicosanoid precursor pool.[55] It is possible that the eicosanoid pool is affected to a greater extent than realized by dietary PUFA. Evidence for this was shown by Lands[55] by the marked increase in eicosanoid generation in response to dietary arachidonic acid in rats; Seyberth et al.[92] observed a similar effect in humans. Slower changes occur in response to dietary linoleic acid, perhaps reflecting the rate of linoleic acid conversion to arachidonic acid, which appears to be slower in humans than in rats.[69,93] The modulatory and inhibitory effects of DH-γ-linolenic acid and its eicosanoid products may also affect metabolism.[69]

The concentrations of non-esterified arachidonic acid may range from 20 to >100 μM, exceeding the Km for cyclooxygenase.[94] However, most of these nonesterified PUFAs are apparently nonspecifically bound to proteins[47] and, hence, may not be available for eicosanoid formation. The total non-esterified PUFA pool increases with fasting (i.e., decreased insulin) and tends to be highest in humans in early morning, when many heart attacks occur. If arachidonic acid levels in the plasma pool are high, i.e., following activation of phospholipase,[53] or if competing n-3 PUFA are low, eicosanoid-mediated reactions could be precipitated (i.e., excess thromboxane could be generated, causing a thromboembolic event). This may be exacerbated when concurrent PGI_2 synthesis is inhibited (e.g., by nicotine from cigarettes or in an extensively atherosclerotic artery).[53,54,70]

Therefore, it should be recognized that it is the unesterified pool of arachidonic acid that really determines the rate and amount of eicosanoids produced in vivo. This is controlled by the relative activities of phospholipases and transacylases which normally are tightly regulated except in pathologic states or following injury.[6,53] To reduce the danger in such situations, the lower tissue concentration of arachidonic acid or a higher concentration of competitive PUFA (i.e., DH-γ-linolenic acid and especially n-3 PUFA) may be desirable.

The effects on N-3 polyunsaturated fatty acids on eicosanoids

It appears that using increasing amounts of dietary linoleic acid to reduce eicosanoid-related pathophysiologies (e.g., thrombotic tendencies) is not prudent strategy, notwithstanding its reduction of plasma lipids in subjects consuming high-fat diets. As knowledge of eicosanoid production and the modulatory functions of eicosanoids are elucidated, reduction or manipulation of the eicosanoid potential (i.e., arachidonic acid concentration, particularly tissue pools) may become a desirable end point. This may be achieved by control-

ling n-6 PUFAs, particularly linoleic acid intake, by regulating their metabolism to arachidonic acid and by modulating arachidonic acid conversion to eicosanoids. This may be accomplished by using the analogs of n-6 PUFA, namely, n-3 PUFA. However, extensive information is required to ascertain the appropriate dietary intake of these fatty acids for controlling tissue arachidonic acid levels. Different n-3 PUFA species may be used to modulate arachidonic acid metabolism in different pathways.

Linolenic Acid

The PUFA of the α-linolenic acid (18:3 n-3) family, especially (DHA 22:6 n-3), its major product in animal tissues, may be required for neural, visual, and possibly reproductive functions.[95,96] However, in the current context, the modulatory effects of α-linolenic acid and its products on linoleic acid, arachidonic acid, and eicosanoid metabolism are emphasized. Based on available data, dietary α-linolenic acid may be required at around 0.5% to 1% of calories, especially by pregnant females and by neonates, for the formation of particular membranes.[72,95-97] Additional amounts may modulate arachidonic acid metabolism and eicosanoid production.[6,93,98,99]

Dietary linolenic acid is desaturated and elongated by the same enzymes involved in linoleic acid metabolism, and there is mutual competition between these two PUFA families[58] (*Figure 1*). Competitive reactions indicate that desaturases have a higher affinity for the n-3 PUFA (i.e., α-linolenic acid over the n-6 (linoleic acid) substrate, and the relative rates of desaturation are α-linolenic acid > linolenic acid > oleic acid, 10:3:1.[57,58,64] Thus, dietary α-linolenic acid and linoleic acid are mutual inhibitors, and increasing dietary α-linolenic acid generally decreases linoleic acid conversion to arachidonic acid[91,93] while increasing its elongated n-3 PUFA products, EPA and DHA, which can also inhibit linoleic acid desaturation, arachidonic acid acylation, and enzymatic oxygenation, thereby decreasing eicosanoid synthesis.[6,7] Several trials with human subjects have corroborated this finding.[93,95,98] Sanders and Roshanai[100] showed that dietary α-linolenic acid, even at low intake levels in diets containing 40% calories as fat, increased platelet EPA without depressing linoleic acid or arachidonic acid. Adam et al.[93] fed 4% linoleic acid with 0%, 4%, 8%, 12%, and 16% α-linolenic acid to six women for 6 weeks in a diet containing 30% of calories from fat (mixtures of olive, linseed, and safflower oils). After six weeks, it was observed that α-linolenic acid had increased significantly in all tissues except platelets (*Table 9*). The EPA content of lipids increased by around 2%, reflecting limited desaturation and elongation. There was negligible change in esterified linoleic acid, and arachidonic acid levels were unchanged. The limited rates of desaturation and elongation of dietary α-linolenic acid observed in this study is also consistent with other reports.[26,28,29,86,93,95,96] This may reflect its rapid acylation, competition from linoleic acid, and/or limited Δ6 desaturase activity in humans. Urinary excretion of prostanoid PGE_2 and metabolites in subjects consuming 16% α-linolenic acid decreased by 52% and 85%, respectively.[93] Thus, α-linolenic acid reduces eicosanoids, which may be beneficial in the reduction of coronary heart disease following long-term ingestion of α-linolenic acid.[29] Laserre et al.[101] reported that dietary α-linolenic acid (linseed oil) increased EPA and DHA levels in serum lipids. In these studies, EPA accumulated to a greater extent than DHA, suggesting a slow Δ4 desaturase for DHA formation. The α-linolenic acid had limited effect on levels of arachidonic acid. Adam[91] concluded that in humans the conversion of linoleic acid to arachidonic acid occurs preferentially to the conversion of α-linolenic acid to EPA when intakes are controlled. Nevertheless, prolonged ingestion of α-linolenic acid reduces eicosanoids and may reduce thrombotic phenomena.[26,28]

Table 9 The effects of increasing linolenic acid at constant linoleic acid intake on platelet fatty acid composition and prostanoid secretion by six human female subjects

Parameter	Ratio of dietary linoleic acid to linolenic acid				
	4:0	4:4	4:8	4:12	4:16
Platelet fatty acids (weight %)					
18:2 n-6	4.5	4.7	4.5	6.1	6.3
18:3 n-3	0.8	0.8	1.2	1.4	1.5
20:4 n-6	33.6	34.9	33.1	35.5	35.4
20:5 n-3	0.5	0.5	0.5	0.7	0.8
Eicosanoid excretion					
PGE_2	254	202	190	69	39
$PGF_{2\alpha}$	450	397	471	174	121

Six women consumed a diet containing 30% calories from fat for 6 weeks and their fatty acid and urinary eicosanoid levels were analyzed; fatty acids same as in *Table 2*; 20:5 n-3, EPA
From Adam et al.[93]

In rats, increments of dietary α-linolenic acid (0, 0.5, 1.0, 2.0, and 4.0 weight %) fed with 4 weight % linoleic acid in diets containing 10% fat caused a progressive decrease in serum arachidonic acid (27% to 11%) and a corresponding decrease (almost threefold) in eicosanoid synthesis in clotted blood after 6 weeks.[102] Hwang et al.[103] fed rats diets of 10% fat containing 2% safflower oil and 0%, 0.4%, 0.8%, and 1.6% α-linolenic acid for 12 weeks. There was a progressive decrease in arachidonic acid in liver, plasma, and lung phospholipids, e.g., liver values were 29%, 22%, 18%, and 16%, respectively (*Table 10*). This was associated with a modest increase in EPA and a significant increase in DHA. The synthesis of thromboxane, PGE_2, and 12HETE were progressively and significantly decreased by α-linolenic acid at n-3:n-6 PUFA ratios of 0.28, 0.5, and 1.0. Increasing the ratio depressed arachidonic acid and eicosanoid synthesis by decreasing substrate arachidonic acid levels and increasing the concentrations of EPA, DPA, and DHA, which reduced eicosanoid synthesis by inhibiting cyclooxygenase and lipoxygenase.

Garg et al.[36] have shown that feeding safflower oil (linoleic acid) or 50:50 linseed oil (50% α-linolenic

Table 10 Comparison of relative effects of approximately equivalent amounts of α-linolenic acid or n-3 polyunsaturated fatty acids of fish oil on liver phospholipid fatty acids and platelet eicosanoid production in rats

	n-3 Fatty acids (% of total dietary fatty acids)					
	α-linolenic acids (weight %)			n-3 fatty acids (fish oil) (weight %)		
Parameter	7	12	23	9	16	28
Liver phospholipids						
20:4 n-6	22.0	18.8	16.4	13.5	10.2	8.8
PUFA (total n-3)	8.7	9.3	12.0	14.3	17.6	19.1
Platelet eicosanoids (ng/ml)						
TXB_2	120	88	64	50	40	24
PGE_2	7.0	4.5	2.8	1.8	1.5	0.8

Rats fed 10% fat containing 2% safflower oil and the added fatty acids for 12 weeks; fish oil, menhaden oil containing n-3 PUFAs
From Hwang et al.[103]

acid) plus safflower oil (20% of calories) resulted in differing effects in rats. The dietary safflower oil increased hepatic cholesterol ester and decreased plasma cholesterol when compared with a diet containing tallow. In contrast, the α-linolenic acid from linseed oil reduced both plasma and hepatic cholesterol levels. The linoleic acid and α-linolenic acid diet decreased liver arachidonic acid levels by 30%. Concurrently, there was a marked increase in arachidonic acid levels of plasma lipids in the animals consuming linoleic acid but not in animals consuming linseed oil. The investigators concluded that dietary α-linolenic acid is more potent than linoleic acid in lowering plasma cholesterol and does not result in its accumulation in liver tissue. However, dietary α-linolenic acid was relatively less effective in reducing arachidonic acid and cholesterol levels than EPA or DHA of fish oils.

Thus, at low intakes of linoleic acid, dietary α-linolenic acid can effectively modulate arachidonic acid content of tissue and eicosanoid synthesis. The contemporary American diet provides 20 to 25 g/d linoleic acid and only about 1 g α-linolenic acid, suggesting limited inhibition of linoleic acid desaturation and elongation to arachidonic acid and negligible conversion of α-linolenic acid to EPA or DHA. Nevertheless, studies indicate the α-linolenic acid can exert beneficial effects and suggest that it should be a component of normal diets.[72,95]

In addition to α-linolenic acid, dietary γ-linolenic acid can alter tissue arachidonic acid and 20:3 n-6 levels and the types and patterns of eicosanoids. These interactions are discussed elsewhere.[69,104]

Long-chain n-3 polyunsaturated fatty acids

The efficacy of dietary α-linolenic acid reflects its effects on the desaturases (i.e., inhibition of linoleic acid conversion to arachidonic acid); its conversion to EPA, DPA, and DHA, products that inhibit desaturase and compete for acyltransferases, thereby reducing the levels of n-6 PUFAs in phospholipid pools. Both α-linolenic acid and γ-linolenic acid, especially EPA and DHA, are transacylated in vitro, indicating that both exogenous and endogenous PUFAs can compete with and displace arachidonic acid from phospholipid pools.[6,46] Thus, both synthesis and acylation of arachidonic acid may be reduced by dietary PUFA. Furthermore, the n-3 PUFAs, especially EPA, DHA, and perhaps, DPA, are competitive inhibitors of both cyclooxygenase and lipoxygenase and thereby reduce conversion of free arachidonic acid to eicosanoids.[6,55,71,82,89]

Dietary α-linolenic acid, ingested over a prolonged period as part of a normal diet, can increase long-chain n-3 PUFA (EPA and DHA) and reduce arachidonic acid in tissues. This apparently is effective in reducing platelet aggregability.[26,28,29,93,96,105] The data of Adam et al.[93] suggest that dietary EPA is approximately four to five times as effective as α-linolenic acid in altering plasma tissue fatty acids in short-term studies. The long-chain n-3 PUFAs of fish oils and seafoods are more efficacious than α-linolenic acid in reducing tissue arachidonic acid and competitively reducing eicosanoid synthesis.[6,8,71,89,93,103,106,107] Croft et al.[108] showed that fish oil was equivalent to an essential fatty acid-deficient diet in reducing serum arachidonic acid, but more potent in depressing thromboxane synthesis and eicosanoid production in rats. At constant linoleic acid intake (2% safflower), Hwang et al.,[103] observed that equivalent amounts of fish oil (*Table 10*) were more effective than α-linolenic acid in reducing tissue arachidonic acid (twice as effective), increasing tissue EPA, DPA, and DHA (twice as effective), and decreasing eicosanoid production by collagen-aggregated platelets (three times as effective). Significant quantities of lipoxygenase products of EPA were synthesized in animals fed high levels of fish oils.

The numerous studies that consistently showed the efficacy of dietary n-3 PUFA in reducing eicosanoids and ameliorating physiologic pathologies have been reviewed and summarized.[6-8,70,89,106,107,109] The effects of dietary n-3 PUFA may vary with the tissues examined, e.g., platelets tend to exclude DHA, whereas cardiac tissue avidly acylates DHA from fish oils.[31,110,111] In this regard, fish oils containing different amounts of EPA or DHA may actually exert different effects. Thus Bruckner et al.[31] showed that refined shark liver oil, rich in DHA, was less effective than EPA-rich oil in suppressing TXA_2 synthesis and platelet aggregation, and only slightly depressed antiaggregatory prostacyclin PGI_2 synthesis by aorta tissue. Croft et al.[108] showed that in rats, MaxEPA (fish oil enriched in EPA) was more effective in decreasing arachidonic acid, increasing EPA of plasma and tissue phospholipids, whereas shark liver oil rich in DHA increased serum DHA but had little effect on liver or renal phospholipid fatty acids. The synthesis of TXA_2 in whole blood and PGI_2 in vascular tissue was diminished 65% and 36%, respectively, in animals fed MaxEPA, whereas these were depressed by less than 10% in animals fed shark liver oil. These data indicate that

the selectivity of acyltransferases vary with tissue and that platelet arachidonic acid and TXA_2 are differentially affected by different n-3 PUFA components of fish oils.

The rate of incorporation of dietary n-3 PUFA from fish oil into various tissues has been documented.[106-111] Generally, in tissue phospholipids, marked changes in fatty acids occur within 2 to 4 days of ingesting fish oil, with the rate depending on the dose and the concomitant intake of linoleic acid.[17,109] The residual effects following cessation of consumption vary in duration.[106] Generally, the hypolipidemic effects change rapidly, whereas effects on platelet functions occur more gradually.[109]

There have been numerous studies demonstrating the effects of varying doses of n-3 PUFA from fish oils and seafood on the fatty acid composition of plasma components, platelets, leucocytes, and erythrocytes, and the subsequent effects on eicosanoid synthesis and certain functions in humans.[6,51,71,112] These studies show that dietary fish oils containing EPA and DHA are more effective (and act more rapidly) than oils containing α-linolenic acid in replacing tissue arachidonic acid with EPA and DHA and in suppressing eicosanoids.[6,8,17,89,106,109]

With regard to the effects of different fatty acids of fish oils and seafoods, the occurrence of significant amounts of arachidonic acid in fish (e.g., trout[113]), especially fish from waters in temperate and tropical regions,[114] should be noted because dietary arachidonic acid could enhance prothrombotic tendencies.

Effects of polyunsaturated fatty acids on macrophages

The n-3 PUFA of fish oils are effective in displacing arachidonic acid of platelets and other tissues and in decreasing prostanoids.[6,22,71,89,106] This has been mostly attributed to the EPA component. However, their concomitant effects on other tissues; whether they also affect lipoxygenase products, and whether DHA (a major component of many fish oils) can exert specific effects, need to be further studied. In this regard, the macrophage is a useful model cell that possesses both cyclooxygenase and lipoxygenase activities, and performs many important functions that are affected by eicosanoids.[79]

Macrophages isolated from the peritoneum of mice consuming a balanced diet containing 2% safflower oil and 10% menhaden oil for 2 weeks contained elevated levels of EPA, DPA, and, particularly, DHA in all phospholipid and neutral lipid classes. There was a significant decrease in arachidonic acid and a slight increase in linoleic acid in these cells. Macrophages isolated from mice fed the diet containing menhaden oil synthesized significantly less (50%) prostanoids and thromboxane (50%) following stimulation using zymosan. Stimulation of macrophages with phorbol myristate showed that the consumption of menhaden oil reduced prostanoid PGE_2 and 6-keto-$PGE_{1\alpha}$ synthesis threefold.[112] These data confirmed other data showing that consumption of fish oils resulted in a reduction in the synthesis of eicosanoids by macrophages in rats and mice.[92]

Table 11 The relative concentration of unsaturated fatty acids, amount of arachidonic acid released on zymosan stimulation, and eicosanoid synthesis by macrophages from the peritoneum of mice consuming a diet with differing ratios of dietary polyunsaturated fatty acids for 14 days

	Ratio of docosahexaenoic acid to linoleic acid			
Fatty acid	0	0.08	0.16	0.81
Fatty acid	(nanomoles/mouse)			
18:0	5.9	10.0	7.5	6.9
18:1 n-9	3.5	5.5	4.5	4.3
18:2 n-6	2.2	4.5	3.8	3.7
20:4 n-6	8.6	7.8	4.3	2.7
20:5 n-3	—	—	0.1	0.5
22:4 n-6	2.2	2.0	1.0	0.5
22:5 n-3	—	0.4	0.6	0.5
22:6 n-3	0.4	3.8	4.4	5.4
Arachidonic acid Released	2.8	2.9	2.4	0.5
Eicosanoid production	(ng/mouse)			
LTE_4	7.30	36.0	22.0	20.0
PGE_2	5.0	2.4	2.3	2.2
6-keto $PGF_{1\alpha}$	46.0	18.3	6.0	5.0

Diets (10% fat) containing 4% linoleic acid and 0%, 0.4%, 0.8%, and 4% DHA were fed to mice for 14 days and, following zymosan stimulation, the eicosanoids in peritoneal exudate were analyzed From Lokesh et al.[116]

When fed at 25% of total dietary fat, dietary α-linolenic acid, EPA, or DHA were effective in displacing arachidonic acid, increasing n-3 PUFA in peritoneal macrophages, and reducing the synthesis of both prostanoids and leukotrienes in vivo following injection of zymosan. The quantities of leukotriene C_4 (LTC_4), leukotriene B_4 (LTB_4), and 12HETE synthesized by macrophages from animals consuming n-3 PUFAs were significantly lower than those synthesized by animals fed safflower oil.[115] Experiments were conducted to determine the ratio of n-3 to n-6 PUFAs required to reduce eicosanoid synthesis. Diets containing 4% weight linoleic acid were supplemented with 0.4%, 0.8%, and 4% DHA in diets containing 10% total fat. Increasing dietary DHA progressively increased the concentration of DHA in the macrophage membranes with a concomitant decrease in arachidonic acid (*Table 11*). On stimulation, there was a marked reduction in eicosanoid synthesis at a low ratio of n-3 to n-6 PUFAs. The amount of arachidonic acid released from the macrophages on stimulation was not significantly decreased at the lower levels of dietary DHA. Nevertheless, eicosanoid synthesis was depressed, indicating inhibition of the cyclooxygenase and lipoxygenase by free DHA concurrently released.[116] Thus, modest levels of dietary DHA can effectively depress eicosanoid synthesis in the macrophage model, and further studies are needed to test this observation with α-linolenic acid and EPA.

Effects of n-3 polyunsaturated fatty acids on membrane and enzymes

In addition to altering arachidonic acid levels and eicosanoid synthesis, n-3 PUFAs can also alter membrane-associated functions, e.g., membrane-bound enzymes.[110,111,117,118] Flier et al.[117] observed that menhaden oil changed the PUFA composition of hepatic membranes and doubled adenyl cyclase activity. Generally, in these studies, the DHA of dietary fish oil is selectively incorporated into membranes, and this n-3 PUFA may have a specific effect on some membrane-related functions.[119,120]

Conclusions

The available data demonstrate the differences between the metabolism of n-6 and n-3 PUFAs, the interactions between the different n-3 and n-6 PUFA species, and the importance of the dietary ratio of dietary n-6 to n-3 PUFA in affecting eicosanoid production.[116] While dietary n-3 PUFAs tend to displace arachidonic acid from phospholipid pools, different species of PUFAs affect these pools differently; this may vary between tissues and the level of supplementation with n-3 PUFA. The data generally indicate that the body possesses various mechanisms for maintaining the fatty acid composition of tissues within certain boundaries despite differences in dietary fatty acids. This is plausible in terms of maintaining physical properties of membranes. However, where a particular membrane PUFA, e.g., arachidonic acid, can be facilely converted to bioactive regulators (i.e., eicosanoids) on release from membranes, there is potential danger of metabolic perturbations accompanying their uncontrolled release. Conceivably, this could occur and result in pathophysiologic states,[55] especially when arachidonic acid is the major long-chain PUFA in membranes. Thus, to ameliorate the repercussions of imbalances or perturbations, it appears prudent to have other PUFAs to dilute and modulate arachidonic acid metabolism.

During evolution, the human diet was apparently lower in fat and contained more n-3 PUFAs (α-linolenic acid from leaves and EPA and DHA from wild animals, fish, etc.) and less n-6 PUFA, especially linoleic acid. Thus, higher levels of EPA and DHA may have accumulated in tissue phospholipid pools. With industrialization, animal fats and seed oils (rich in n-6 and low in n-3 PUFAs) became the major dietary fats. The human body may have adjusted to the increased ingestion of these by synthesizing more long-chain n-6 PUFA (e.g., arachidonic acid) instead of n-3 PUFA. While this ensures membrane fluidity, it also may enhance the potential for eicosanoid generation. In view of the pervasive and potent effects of eicosanoids,[53,54] this may predispose the subject to a hyperresponsive, proinflammatory state as reflected in many contemporary eicosanoid-related pathophysiologies (arthritis, atherogenesis, thrombosis, perturbation of immune homeostasis).[6,70,79,90] Thus, dietary n-3 PUFAs (α-linolenic acid, EPA, DHA) may be important in regulating arachidonic acid levels in tissue and in modulating conversion of arachidonic acid to eicosanoids. In this context, the relevant question is not how much linoleic acid is needed, but how little arachidonic acid is adequate and how can its metabolism be regulated by an appropriate combination of dietary fatty acids?

Based on the probable dietary fatty acid composition of which mankind's ancestors evolved[121,122] and on the subtle interactions between substrates and enzymes metabolizing dietary n-6 (i.e., linoleic acid) and n-3 PUFA (i.e., α-linolenic acid), it would seem that fat intake should be matched with the energy needs and that intakes of individual fatty acids should be compatible with the homeostatic mechanisms regulating PUFA metabolism, especially the synthesis, acylation, release, and oxygenation of arachidonic acid. Because of the contemporary preoccupation with dietary strategies for the reduction of plasma lipids, much of the recent knowledge and implications of PUFA metabolism has not been incorporated into dietary recommendations. Thus, increased consumption of linoleic acid has been advocated to reduce the hyerlipidemic effects of high fat/high SFA diets without much consideration for other possible adverse effects of high intakes of linoleic acid as the predominant dietary PUFA.

From a metabolic point of view, dietary fatty acids are required for energy and to provide essential fatty acids. At low fat intakes, essential fatty acid needs apparently are met with 1 to 2-g linoleic acid and 0.5 to 1.0 g/d of linolenic acid.[41,43,44,49,72,97] Additional fatty acids are used for energy needs or stored in adipose tissue. Linoleic acid intake of 0.5 g/d should provide all the arachidonic acid needed for eicosanoid synthesis which may, under nondisturbed conditions, amount to < 100 mg/d.[53] However, data for optimum intake by humans are needed. The current intake of linoleic acid (20 to 25 g/d) reflects its promotion to ameliorate the hyperlipidemic effects of excess SFAs. Conceivably, as fat intake is reduced, the need for corrective amounts of linoleic acid may be reduced. Furthermore, in view of the modulatory actions of n-3 PUFA (i.e., α-linolenic acid or the more potent EPA and DHA), a relative increase in n-3 PUFA may be desirable for both hypolipidemic effects and, more imporantly, for modulating arachidonic acid synthesis and eicosanoid production.[6,107]

Based on the low metabolic requirements for linoleic acid, the intake of fat should ideally be based on energy needs. Dietary fats rich in oleic and stearic acids which are hypolipidemic appear to be the most appropriate sources in meeting this need.[4,5,25] Intakes of n-6 PUFA above 1% of calories should be accompanied by n-3 PUFA to modulate linoleic acid and arachidonic acid metabolism and to provide n-3 PUFA so that arachidonic acid is not the only PUFA in membrane lipid pools. This may be protective in circumstances in which perturbation of the membrane causes release of PUFAs. In this regard, the long-chain n-3

PUFAs are much more effective in depressing eicosanoids than α-linolenic acid.[86,91,103] This reflects their greater efficacy in depressing Δ6 desaturase, competing for acyl transferases, and inhibiting both cyclooxygenase and lipoxygenase of various tissue. Although the activity of desaturase enzymes in humans may be low,[69,93] consistent ingestion of α-linolenic acid over long periods may represent a practical and effective source of n-3 PUFA.[26,29] Consumption of a balanced mixture of linoleic acid and α-linolenic acid would perhaps allow the endogenous enzymes (desaturases, elongases) to regulate the conversions of these two parent n-6 and n-3 fatty acids to their long-chain PUFA products. Furthermore, because of the lower tendency of α-linolenic acid toward autoxidation, it should be a more practical and acceptable source of n-3 PUFA in a wide range of food products. While it may be difficult to attain the optimum fat and balanced fatty acid intake from the current food supply, diligent selection and increased use of foods and edible fats containing oleic acid and n-3 PUFA appears to be prudent.

Because of the potential adverse effects of excess eicosanoids, more extensive and reliable data demonstrating the relationships between dietary unsaturated fatty acids (amounts, types, and ratios), tissue arachidonic acid levels, and eicosanoid synthesis are needed. This information is important in outlining rational dietary habits to realize the potential beneficial effects of the various PUFAs, not only with regard to plasma lipids but also eicosanoid-modulated physiologic functions.

Acknowledgments

Supported by funds from the National Seagrant Program.

References

1 Anonymous (1989). *Diet and Health Report*. National Academy of Sciences. National Academy Press, Washington, DC

2 Grundy, S. M. (1986). Cholesterol and coronary heart disease: a new era. *JAMA* **256,** 2849

3 Spady, D. K., Dietschy, J. M. (1988). Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in hamsters. *J. Clin. Invest.* **81,** 300

4 Grundy, S. M. (1987). Monounsaturated fatty acids, plasma cholesterol, and coronary heart disease. *Am. J. Clin. Nutr.* **45,** 1168

5 Grundy, S. M. (1986). Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. *N. Engl. J. Med.* **314,** 745

6 Lands, W. E. M (1986). *Fish and Human Health*. Academic Press, Orlando, FL

7 Lands, W. E. M. (1986). Renewed questions about polyunsaturated fatty acids. *Nutr. Rev.* **44,** 189

8 Lands, W. E. M. (1987). *Polyunsaturated Fatty Acids and Eicosanoids*. American Oil Chemical Society, Champaign, IL

9 Mead, J., Alfin-Slater, R., Howton, D., Popjack, G. (1986) *Lipids: Chemistry, Biochemistry and Nutrition,* Plenum Press, New York

10 Kritchevsky, D. (1988). Effects of triglycerides structure on lipid metabolism. *Nutr. Rev.* **46,** 177

11 Nelson, G., Ackman, R. (1988). Absorption and transport of fat in mammals with emphasis on n-3 PUFA. *Lipids* **23,** 1005

12 Nilsson, A., Prydz, K., Rortvrit, T., Christiansen, E. (1987). Studies on the interrelated stimulation of microsomal omega-oxidation and peroxisomal β oxidation in rat liver with a partially hydrogenated fish oil diet. *Biochim. Biophys. Acta* **920,** 114

13 Leyton, J., Drury, P. J., Crawford, M. A. (1987). Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *Br. J. Nutr.* **57,** 383

14 Babayan, V. K. (1987). Medium chain triglycerides and structured lipids. *Lipids* **22,** 417

15 Periago, J., Pita, M. L., Sanchez, M., Suarez, M. (1989). Changes in lipid composition of microsomes and acyl desaturase induced by medium chain triglyceride feeding. *Lipids* **24,** 383

16 McNamara, D. J. (1987). Effects of fat modified diets on cholesterol and lipoprotein metabolism. *Ann. Rev. Nutr.* **7,** 273

17 Harris, W. S. (1989). Fish oils and plasma lipid lipoprotein metabolism in humans: a critical review. *J. Lipid Res.* **30,** 785

18 Hegsted, D. M., McGandy, R. M., Myers, M. L., Stare, F. J. (1965). Quantitative effects of dietary fat on serum cholesterol in man. *Am. J. Clin. Nutr.* **17,** 281

19 Keys, A., Anderson, J. T., Grande, F. (1965). Serum cholesterol response to changes in the diet. IV: particular saturated fatty acids in the diet. *Metabolism* **14,** 776–787

20 Keys, A., Menotti, A., Karvonen, M., Aravanis, C., (1986). The diet and 15 year death rate in the seven countries study. *Am. J. Epidemiol.* **124,** 903–915

21 Hornstra, G. (1974). Dietary fats and arterial thrombosis. *Hemostasis* **2,** 21

22 Hornstra, G. (1982). Dietary Fats, Prostanoids and Arterial Thrombosis. Martinus-Nijhoff, Holland, Hague

23 Riemersma, R. A., Sargent, C. A., Saman, S. (1988). Dietary fatty acids and ischaemic arrhythmias. *Lancet* **II,** 285

24 Galli, C., Agradi, E., Petroni, A., Tremoli, E., (1981). Differential effects of dietary fatty acids on the accumulation of arachidonic acid and its metabolic conversion through the cyclooxygenase and lipoxygenase in platelets and vascular tissue. *Lipids* **16,** 165

25 Bonanome, A., Grundy, S. M. (1988). Effects of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N. Engl. J. Med.* **318,** 1244

26 Renaud, S., Morozain, R., Godsey, F. (1986). Nutrients, platelet function and composition in nine groups of French and British farmers. *Atherosclerosis* **60,** 47

27 Anonymous (1988). *Designing Foods: Animal Product Portions in the Marketplace*. Board on Agriculture, National Academy of Sciences, National Research Council, National Academy Press, Washington, DC

28 Renaud, S., Martin, J. C., Thevenon, C. (1987). Long-term effects of dietary linoleic and linolenic acids on platelet functions and lipemia in man and woman. In *Polyunsaturated Fatty Acids and Eicosanoids* (W. Lands, ed.), p. 56, American Oil Chemical Society, Champaign, IL

29 Renaud, S., Lorgeril, M. (1989). Dietary lipids and ischemic heart disease. *J. Intern. Med.* **225,**39

30 Kinsella, J. E., Bruckner, G., Mai, J., Shimp, J. (1981). Metabolism of trans fatty acids with emphasis on the effects of trans, trans octadecadieonate on lipid composition, essential fatty acids, and prostaglandins: an overview. *Am. J. Clin. Nutr.* **34,** 2307

31 Bruckner, G. G., Lokesh, B., German, G., Kinsella, J. E., (1984). Biosynthesis of prostanoids, tissue fatty acid composition and thrombotic parameters in rats fed diets enriched with docosahexaenoic (22:6 n-3) or eicosapentaenoic (20:5 n-3) acids. *Thromb. Res.* **34,** 479

32 Senti, F. (1985). Health aspects of trans fatty acid: metabolism. *FASEB Rep.*

33 Katan, M. B., Beynen, A. C. (1981). Linoleic acid consumption and coronary heart-disease in USA and UK. *Lancet* **II,** 371

34 Riemersma, R. A., Wood, D. A., Butler, S., Puska, P.,

Fidanza, F.,(1986). Linoleic acid in plasma and heart disease. *Br. Med. J.* **292,** 1423
35 Hamm, M. W., Sekowski, A., Ephrat, R. (1988). Dietary fat ratios and liver plasma membrane lipid composition. *Lipids* **23,** 829
36 Garg, M. L., Sevokova, E., Wierzbicki, A., Thompson, A. B., Clandinin MT (1988). Differential effects of dietary linoleic and linolenic on lipid metabolism in rat tissue. *Lipids* **23,** 847
37 Puska, P., Iacono, J. M., Nissinen, A. (1983). Controlled randomized trial of the effect of dietary fat on blood pressure. *Lancet* **I,** 1–5
38 Mills, D. E., Ward, R. P., Mah, M., Dezette, L. (1989). Dietary n-6 and n-3 fatty acids and salt induced hypertension in the borderline hypertensive rat. *Lipids* **24,** 17
39 Burr, G. O., Burr, M.M. (1930). On the nature and role of the fatty acids essential to nutrition. *J. Biol. Chem.* **86,** 1587
40 Holman, R. T. (1964). Nutritional and metabolic interrelationships between fatty acids. *Fed. Proc.* **23,** 1062
41 Holman, R. T. (1975). Deficiency of essential fatty acids in humans. In *The Essential Fatty Acid* (W. W. Hawkin, ed.), p. 45, Nutrition Society of Canada, Miles Labs, Rexdale, Ontario, Canada
42 Vergrossen, A. E., TenHoor, F., Hornstra, G., Houtsmuller, U. M. (1975). Physiological functions of essential fatty acids. In *The Essential Fatty Acids* (W. Hawkins, ed.), p. 5, Nutrition Society of Canada, Miles Sym, Rexdale, Ontario, Canada
43 Wertz, T. W., Downing, D. T. (1983). Acyglucosylceramides of pig epidermis. Structure determination. *J. Lipid Res.*, **24,** 753, 759
44 Kunau, W. H., Holman, R. T. (1977). *Polyunsaturated Fatty Acids*. American Oil Chemical Society, Champaign, IL
45 Holman, R. T. (1986). Essential fatty acids. Prostaglandins and leukotrienes. *Prog. Lipid Res.* **25,** 19–47
46 Farrell, P. M., Gutcher, G. R., Palta, M., DeMeets, D. (1988). Essential fatty acid deficiency in premature infants. *Am. J. Clin. Nutr.* **48,** 220
47 Lands, W. E. M. (1989). N-3 fatty acids as precursors for active metabolic substances: dissonance between expected and observed events. *J. Internal Med.* **225,** 11–20
48 Salem, N. (1989). Omega-3 fatty acids. Molecular and biochemical aspects. In *New Protective Roles for Selected Nutrients* (G. Spiller, J. Scala), p. 109, Alan Liss, New York
49 Hansen, H. (1989). *Linolenic Acid: Essential Fatty Acid and Eicosanoid Precursor* (Ph. D. Thesis), University of Copenhagen, Copenhagen, Denmark
50 Brenner, R. R., Garda, H., DeGomes, H., Dumm, I., and Pezzano, H. (1982). Early effects of EFA deficiency on the structure and enzymatic activity of rat liver microsomes. *Progr. Lipid Res.* **20,** 315
51 Kinsella, J. E., Lokesh, B., Broughton, S., Whelan, J. (1990). Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. *Nutrition* **5,** 101
52 Lefkowith, J. B., Schreiner, G. (1987). Essential fatty acid deficiency depletes rat glomeruli of resident macrophages and inhibits angiotensin II-induced eicosanoid synthesis. *J. Clin. Invest.* **80,** 947
53 Curtis-Prior, P. B. (1988). *Prostaglandins: Biology and Chemistry of Prostaglandins and Related Compounds*, Churchill Livingstone, London
54 Willis, A. L. (1987). *Handbook of Eicosanoids and Related Lipids*, CRC Press, Boca Raton, FL
55 Lands, W. E. M. (1986). The fate of polyunsaturated fatty acids, In *Health Effects of Polyunsaturated Fatty Acids in Seafoods* (A. Simopoulos, R. Kifer, R. Martin, eds.), p. 40, Academic Press, New York
56 Mohrhauer H, Holman R. T. (1963). Effect of linolenic acid upon the metabolism of linoleic acid. *J. Nutr.* **81,** 67–74
57 Brenner, R. R. (1989). Factors influencing fatty acid chain elongation and desaturation, In *The Role of Fats in Human Nutrition*, 2nd ed. (A. J. Vergroesen, M. Crawford, eds.), p. 4, Academic Press
58 Brenner, R. R., Peluffo, R. O. (1966). Effect of saturated and unsaturated fatty acids on the desaturation in vitro of palmitic, stearic, oleic, linoleic and linolenic acid. *J. Biol. Chem.* **241,** 5213
59 Sprecher, H. (1987). Comparison of omega-3 and omega-6 fatty acid metabolism, In *Health Effects of Polyunsaturated Fatty Acids in Seafoods* (A. Simopoulos, R. R. Kifer, R. E. Martin, eds.), Academic Press, Orlando, FL, p. 353
60 Sprecher, H. (1989). Interactions between metabolism of n-3 and n-6 fatty acids. *J. Internal Med.* **225,** 5–11
61 Blond, J. B., Henchrich, C., Bezard, J. (1989). Delta 6 and delta 5 desaturase activities in livers from obese zucker rats of different ages. *Lipids* **24,** 389
62 Shimp, J. L., Bruckner, G., Kinsella, J. E. (1982). Effects of dietary trinoelaidin on fatty acids and acyl desaturase in rat liver. *J. Natur.* **112,** 722
63 Leikin, A. I., Brenner, R. R. (1989). Microsomal Δ5 desaturation of eicosa-8,11,14-trienoic acid is activated by a cytosolic fraction. *Lipids* **24,** 101
64 Lokesh, B., Hsieh, W., Kinsella J. E. (1988). Olive oil enriched diets decreases arachidonic acid without affecting prostaglandin synthesis in mouse lung and spleen. *Nutr. Res.* **8,** 499–507.
65 Kurata, N. N., Privett, O. S. (1980). The effect of dietary fatty acid composition on the biosynthesis of unsaturated fatty acids in liver microsomes. *Lipids* **15,** 512–518
66 De Schrijver, R., Privett, O. S. (1982). Effects of dietary long chain fatty acids on the biosynthesis of unsaturated fatty acids in the rat. *J. Nutr.* **112,** 619–626
67 Chern, J. C., Bruckner, G., Kinsella, J. E. (1984). Effects of dietary trilinoelaidate on liver mitochondrial phospholipids and endogenous phospholipase activity. *Nutr. Res.* **4,** 79–92
68 Voss, A. C., Sprecher, H. (1988). Regulation of metabolism of linoleic to arachidonic acid in rat hepatocytes. *Lipids* **23,** 660
69 Willis, A. M., Smith, D. L. (1989). Dihomo-γ-linolenic acid γ-linolenic acids in health and disease, in *New Perspective Roles for Selected Nutrients* (G. A. Spiller, J. Scala, eds.), pp. 39–108, Alan Liss Inc., New York
70 Kinsella, J. E., Lokesh, B., Stone, R. (1990). Dietary w-3 PUFA and amelioration of cardiovascular disease: possible mechanisms. *Am. J. Clin. Nutr.* **50,** (in press)
71 Kinsella, J. E. (1987). *Seafoods and Fish Oils in Human Health and Disease*, Marcel Dekker, New York
72 Crawford, M. A., Hassam, A. G., Stevens, P. A. (1981). Essential fatty acid requirements in pregnancy and lactation with reference to brain development. *Progr. Lipid Res.* **20,** 31
73 Kinsella, J. E., Lokesh, B. (1990). Dietary lipids, eicosanoids and immune system. *Crit. Care Med.* **18,** (in press).
74 Dupont J., Mathias, M., Connally, P. T. (1980). Effects of dietary essential fatty acid concentration upon prostanoid synthesis in rats. *J. Nutr.* **110,** 1695
75 Lefkowith, J. B., Jakschik, B., Stahl, P., Needleman, P. (1987). Metabolic and functional alterations in macrophages induced by essential fatty acid deficiency. *J. Biol. Chem.* **262,** 6668
76 Zevenbergen, J. C., Haddeman, E. (1989). Lack of effect of trans fatty acids on eicosanoid biosynthesis with adequate intake of linoleic acid. *Lipids* **24,** 555
77 Houweligan, A. C., Hennissen, A., Seyberth, H., Hornstra, G. (1987). Effect of dietary fat type on prostaglandin E production in vivo. *Adv. Prostaglandin Thromboxane Leukotriene Res.* **17,** 871
78 Grataroli, R., Leonardi, J., Charbonnier, M., LaFonte, R., LaFont, H., Nalbone, G. (1988). Effects of dietary corn oil and salmon oil on lipids and prostaglandin E2 in gastric mucosa. *Lipids* **23,** 666
79 Goodwin, T. S. (1985). *Prostaglandins and Immunity*, Martinus Nijhoff, Boston
80 Dupont, J. (1987). Essential fatty acids and prostaglandins. *Prev. Med.* **16,** 485
81 Knapp, H. R., Reilly, I. A., Alessandrini, P., Fitzgerald, G. A. (1986). In vivo indexes of platelet and vascular function

during fish oil administration in patients with atherosclerosis. *N. Engl. J. Med.* **314,** 937
82 Needleman, P., Turk, J., Lefkowith, J. B. (1986). Arachidonic acid metabolism. *Ann. Rev. Biochem.* **55,** 69
83 Nugteren, D. H., vanEvert, W. C., Soeting, W. J., Spuy, J. H. (1980). The effect of different amounts of linoleic acid in the diet on the excretion of urinary prostaglandin metabolites in the rat. *Adv. Prostaglandin Thromboxane Res.* **8,** 1793–1796
84 Mathias, M. M., Maudlin, R.E. (1986). Urinary excretion of prostanoids by rats and men fed varying amounts of linoleate. *Prog. Lipid Res.* **25,** 157
85 Mathias, M., Dupont, J. (1989). Effects of dietary fat on eicosanoid production in normal tissues, In *Carcinogenesis and Dietary Fat* (S. Abraham, ed.), Martinus-Nijhoff, Boston, p. 100
86 Adam, O., Wolfram, G., Zollner, N. (1982). Prostaglandin formation in man during intake of different amounts of linoleic acid in diets. *Ann. Nutr. Metab.* **26,** 315
87 Friedman, Z., Frolich, J. C. (1979). Essential fatty acids and the major urinary metabolites of the E prostaglandins in thriving neonates and in infants receiving parenteral fat emulsions. *Pediatr. Res.* **13,** 932
88 Knapp, H., Fitzgerald, G. (1989). The antihypertensive effect of fish oil. *N. Engl. J. Med.* **320,** 1037
89 Leaf, A., Weber, P. C. (1988). Cardiovascular effects of w-3 fatty acids. *N. Engl. J. Med.* **318,** 549
90 Ferretti, A., Judd, T. J., Taylor, P. R., Schatzkin, A., Brown, C. (1989). Modulating influence of dietary lipid intake on the prostaglandin system in adult men. *Lipids* **24,** 419–422
91 Adam, O. (1985). Metabolism of essential fatty acids and prostaglandin biosynthesis in man. *Klin. Wochenschr.* **63,** 731
92 Seyberth, H. W., Oelz, O., Kennedy, T., Oates, J. (1984). Increased arachidonate in lipids after administration to man effects on prostaglandin biosynthesis. *Clin. Pharmacol.* **18,** 521
92a Johnston, P. V. (1988). Lipids in immunity, In *Nutrition and Immunology* (R. Chandra, ed.), p. 230, Alan Liss, New York
93 Adam, O., Wolfram, G., Zollner, N. (1986). Effect of alpha-linolenic acid in the human diet on linoleic acid metabolism and prostaglandin biosynthesis. *J. Lipid Res.* **27,** 421
94 Lands, W. E. M., Kulmac, R. J. (1986). The regulation of the biosynthesis of prostaglandins and leukotrienes. *Prog. Lipid Res.* **25,** 105
95 Budowski, P. (1988). w-3 Fatty acids in health and disease. *World Rev. Nutr. Diet.* **57,** 214
96 Neuringer, M., Anderson, G., Connor, W. (1988). The essentiality of w-3 PUFA for development and function of retina and brain. *Ann. Rev. Nutr.* **8,** 517
97 Bjerve, K. S., Fischer, S., Waimmer, F., Egeland, T. (1989). α Linolenic acid and long chain w-3 fatty acid supplementation in patients with w-3 acid supplementation in patients with w-3 fatty acid deficiency. *Am. J. Clin. Nutr.* **49,** 290
98 Dyerberg, J. (1986). Linolenate derived polyunsaturated fatty acids and prevention of atherosclerosis. *Nutr. Rev.* **44,** 125
99 Kinsella, J. E. (1989). Requirements for polyunsaturated fatty acids, In *Fish Oils. MIT Conference Proceedings* (R. Lees, M. Karel, eds.), Marcel Dekker, New York (in press)
100 Sanders, T. A. B., Roshanai, F. (1983). The influence of different types of w-3 PUFA acids on blood lipids and platelet function in healthy volunteers. *Clin. Sci.* **64,** 91
101 Lassere, M., Mondy, F., Speilman, D., Jacotot, B. (1985). Effects of different dietary intake of essential fatty acids on serum lipids in human adults. *Lipids* **20,** 227
102 Hwang, D., Carroll, A. E. (1980). Decreased formation of prostaglandins by dietary linolenate in rats. *Am. J. Clin. Nutr.* **33,** 590
103 Hwang, D., Boudrea, M., Chanmugam, P. (1988). Dietary linolenic acid and longer chain n-3 fatty acids. Effects on arachidonic acid metabolism in rats. *J. Nutr.* **118,** 427
104 Takashi, R., Nasser, B. A., Huang, Y., Begur, M., Horrobin, D. F. (1987). Effects of different ratios of dietary w-6 and w-3 fatty acids on fatty acids, prostaglandins and platelet aggregation in the rat. *Throm. Res.* **47,** 135
105 Zollner, N. (1986). Dietary linolenic acid in man: an overview. *Prog. Lipid Res.* **25,** 177
106 Van Schacky, C. (1987). Prophylaxis of atherosclerosis with marine w-3 fatty acids. *J. Int. Med.* **107,** 890
107 Kinsella, J. E. (1988). Food lipids and fatty acids. *Food Technol.* **42,** 124
108 Croft, K. D., Beillin, L., Legge, F., Vandougen, R. (1987). Effects of diets enriched in eicosapentaenoic or docosahexaenoic acids on prostanoid metabolism in the rat. *Lipids* **22,** 647
109 Herold, P., Kinsella, J. E. (1986). Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trials. *Am. J. Clin. Nutr.* **43,** 566
110 Swanson, J. E., Kinsella, J. E. (1986). Dietary n-3 polyunsaturated fatty acids: modification of rat cardiac lipids and fatty acid composition. *J. Nutr.* **116,** 514–523
111 Swanson, J., Black, M., Kinsella, J. E. (1988). Dietary menhaden oil: effects of phospholipid fatty acid composition of mouse heart and brain. *Br. J. Nutr.* **59,** 533
112 Lokesh, B., Hsieh, H., Kinsella, J. E. (1987). Peritoneal macrophages enriched with dietary n-3 polyunsaturated fatty acids secrete decreased levels of prostaglandins. *J. Nutr.* **116,** 2547–2552
113 Kinsella, J. E., Shimp, J. L., Mai, J., Weihrauch, J. (1977). Fatty acid content and composition of freshwater finfish. *J. Am. Oil Chem. Soc.* **54,** 424–429
114 Sinclair, A. J., O'Dea, K. (1987). The significance of arachidonic acid in the human diet, In *Polyunsaturated Fatty Acids and Eicosanoids* (W. E. M. Lands, ed.), Academic Press, Orlando, FL, p. 361
115 Lokesh, B. R., German, B., Kinsella, J. E. (1988). Differential effects of docosahexaenoic acid and eicosapentaenoic acid on suppression of lipoxygenase pathway in peritoneal macrophages. *Biochim. Biophys. Acta.* **958,** 99–107
116 Lokesh, B. R., Black, J. M., Kinsella, J. E. (1989). The suppression of eicosanoid synthesis by peritoneal macrophages is influenced by the ratio of dietary docosahexaenoic acid to linoleic acid. *Lipids* **24,** 589–593
117 Flier, J., Lokesh, B. R., and Kinsella, J. E. (1985). Increased 5′nucleotidase activity in plasma membranes from rat liver following ingestion of fish oil. *Nutr. Res.* **5,** 277–283
118 Zuniga, M. E., Lokesh, B. R., Kinsella, J. E. (1989). Disparate effects of n-3 polyunsaturated fatty acids on activity of 5′-nucleotidase of liver plasma membrane. *J. Nutr.* **119,** 152–160
119 Swanson, J., Lokesh, B., Kinsella, J. E. (1989). Ca.Mg ATPase of mouse cardiac sacroplasmic reticulum is affected by membrane n-6 and n-3 fatty acid content. *J. Nutr.* **119,** 364
120 Salem, N., Kimn, H. Y., Jergey, J. A. (1986). Docosahexaenoic acid: membrane functions and metabolism, In *Health Effects of Polyunsaturated Fatty Acids in Seafoods* (A. P. Simopoulos, R. R. Kifer, R. E. Martin, eds.), pp. 263–318, Academic Press, Inc., Orlando, FL
121 Leaf, A. (1988). The aging process: lessons from observations in man. *Nutr. Rev.* **46,** 40
122 Eaton, S. B., Konner, M. (1985). Paleolithic nutrition: a consideration of its nature and current implications. *N. Engl. J. Med.* **312,** 283