Review

Polyunsaturated Fatty Acids: Biochemical, Nutritional and Epigenetic Properties

Paola Benatti, MC, Gianfranco Peluso, MD, Raffaella Nicolai, PhD, and Menotti Calvani, MD

Scientific Department, Sigma Tau S.p.A. Pomezia, Rome (P.B., R.N., M.C.), Department of Experimental Oncology, National Cancer Institute, Naples (G.P.), ITALY

Key words: polyunsaturated fatty acids, nutrition, carnitine, eicosanoid metabolism, gene expression, chronic diseases

Dietary polyunsaturated fatty acids (PUFA) have effects on diverse physiological processes impacting normal health and chronic diseases, such as the regulation of plasma lipid levels, cardiovascular and immune function, insulin action and neuronal development and visual function. Ingestion of PUFA will lead to their distribution to virtually every cell in the body with effects on membrane composition and function, eicosanoid synthesis, cellular signaling and regulation of gene expression. Cell specific lipid metabolism, as well as the expression of fatty acid-regulated transcription factors, likely play an important role in determining how cells respond to changes in PUFA composition. This review will focus on recent advances on the essentiality of these molecules and on their interplay in cell physiology, leading to new perspective in different therapeutic fields.

Key teaching points:

- During the last 100–150 years nutritional changes have been taking place in the food supply, with the result of increased intake of saturated- and *trans*-fatty acids and enormously of n-6 fatty acids, thus altering the equilibrium between n-6 and n-3 polyunsaturated fatty acids.
- Polyunsaturated fatty acids in the plasma membranes are converted into a number of important, very active, short-lived, hormone-like compounds referred to as "eicosanoids."
- Polyunsaturated fatty acids elicit changes in gene expression that precede changes in membrane composition by directly governing the activity of nuclear transcription factors.
- The therapeutic efficacy of polyunsaturated fatty acids has been demonstrated in cardiovascular disease, dyslipidemias and diabetes. Promising results has been obtained in inflammatory diseases, cancer and osteoporosis.
- The central nervous system is particularly rich in polyunsaturated fatty acids and the cerebral accretion of these fatty acids may have been decisive in the evolution of *Homo sapiens.*

INTRODUCTION

Dietary intake of lipids amounts to 25%–45% of total energy in most industrialized countries. From a biochemical point of view the fatty acids have attracted the greatest interest recently. Most of the dietary fatty acids are derived from meats, oils and dairy products, giving rise to a large intake of saturated as well as monounsaturated fatty acids and relatively modest

Address reprint requests to: Menotti Calvani, MD, Scientific Director of Sigma-tau S.p.A., Scientific Department, Sigma Tau S.p.A., via Pontina Km 30,400, 00040 - Pomezia, Rome, ITALY. E-mail: menotti.calvani@sigma-tau.it

The authors have a significant relationship as Senior Scientists (Paola Benatti and Raffaella Nicolai) and Scientific Director (Menotti Calvani) with Sigma-tau S.p.A. a pharmaceutical company which produces a commercial PUFA preparation. Professor Gianfranco Peluso is the Director of Experimental Oncology of National Cancer Institute. Nevertheless, no financial supports or benefits have been received by the authors or by any member of their immediate families for the writing of this paper.

Abbreviations: AA = arachidonic acid, BMD = bone mineral density, COX = cyclooxigenase, DHA = docosahexaenoic acid, DGLA = di-homo-gamma-linolenic acid, EFA = essential fatty acids, EGF = epithelial growth factor, EPA = eicosapentaenoic acid, HETE = hydroxyeicosatetraenoic acid, HNF = hepatic nuclear factor, HPETE = hydroperoxyeicosatetraenoic acid, IFN = interferon, IGF = insulin-like growth factor, IL = interleukin, LA = linoleic acid, LNA = alpha-linolenic acid, LOX = lipooxygenase, LT = leukotriene, LXR = liver X receptors, NF-kB = nuclear factor kB, NSAIDs = nonsteroidal antiinflammatory drugs, PBMC = peripheral blood mononuclear cells, PGE = prostaglandin, PGI = prostacyclin, PIF = proteolysis-inducing factor, PLA2 = phospholipase A_2, PPAR = peroxisome proliferator-activated receptor, PUFA = polyunsaturated fatty acids, RXR = retinoid X receptors, SREBP = sterol regulatory element-binding protein, TGF = transforming growth factor, TNF = tumor necrosis factor, TR = thyroid hormone receptor, TX = thromboxane.

Journal of the American College of Nutrition, Vol. 23, No. 4, 281–302 (2004)
Published by the American College of Nutrition

amount of polyenes. Saturated fats and cholesterol represent the most established risk factor in our diets for CVD, whereas monoenes and PUFA probably are the most important lipids that would provide beneficial effects if the dietary intake is increased.

Cardiovascular diseases, dyslipidemias, diabetes, osteoporosis, inflammatory diseases and cancer are all related to dietary factors. How is it possible that PUFA are involved in such different pathologies? First of all, PUFA metabolism is deeply involved in eicosanoid biosynthesis, essential for the maintenance of physiological homeostasis. Secondly, PUFA interact with nuclear receptor proteins that bind to certain regions of DNA and thereby are able to modulate transcription of regulatory genes. This newly determined mechanism of action will probably give much more understanding on how some of the metabolic effects of PUFA can be promoted.

These interactions provide a good basis for clinical use of these very interesting nutrients, both as dietary components and as future drugs with potentially beneficial effects and few side effects.

BACKGROUND

Chemistry

Chemically, PUFA belong to the class of simple lipids, as they are fatty acids with two or more double bonds in *cis* configuration.

There are two main families of PUFA: n-3 and n-6. These fatty acids family are not convertible and have very different biochemical roles.

Linoleic acid (n-6) (LA) and alpha-linolenic acid (n-3) (LNA) are two of the main representative compounds, known as dietary essential fatty acids (EFA) because they prevent deficiency symptoms and cannot be synthesized by humans.

Sources of PUFA

The predominant sources of n-3 fatty acids are vegetable oils and fish. Vegetable oils are the major sources of LNA. In particular, LNA is found in the chloroplast of green leafy vegetables, such as purslane and spinach, and in seeds of flax, linseed, walnuts and others [1]. Fish is the main source of eicosapentaenoic acid (C20:5n-3, EPA) and of docosahexaenoic acid (C22:6n-3, DHA) [2]. Vegetables are the main sources of n-6 fatty acids. The most important n-6 fatty acid, LA, is found in large amounts in western diets in corn oil, safflower oil, sunflower oil, and soybean oil [3]. A more detailed description of PUFA content in food is reported in Table 1.

Evolutionary Aspects of Diet

Studies of hunter-gatherer societies indicate that humans evolved on a diet that was low in saturated fat and the amount of n-3 and n-6 fatty acids was quite equal. Over the past 10,000 years with the development of agriculture, changes began to take place in the food supply. But it was especially during the last 100–150 years that nutritional changes have led to an increase in saturated fats from grain-fed cattle, an increase in *trans*-fatty acids from the hydrogenation of vegetable oils and an enormous increase in n-6 fatty acids (about 30 g/day) due to the production of oils from vegetable seeds such as corn, safflower and cotton.

Increases in meat consumption have led to increased amounts of arachidonic acid (C20:4n-6, AA), about 0.2–1.0 mg/day [4], whereas the amount of LNA is only 2.92 g/day [5], and amounts of EPA, DHA are 48 and 72 mg/day, respectively. Thus, a relative and absolute decrease in the amount of n-3 fatty acids has led to an imbalance and increase in the ratio of n-6/n-3.

Recommended Intakes of PUFA

The increased interest in the potential health benefits associated with the consumption of long-chain n-3 fatty acids has led to the sale of supplements and fortified foods containing these fatty acids.

In Europe, estimates of minimum requirements for essential fatty acids are often based on the *Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy*, in which the suggested minimum requirement for LA is approximately 1% of energy intake. The same report suggested that the minimum requirement for LNA was 0.2%–0.5% of energy intake [6,7].

In the United States, to date, no official dietary recommendations have been made; however, it is suggested that total PUFA intake should remain at 7% of energy and not exceed 10% of energy intake [8].

In Japan, despite the currently consumed dietary intake of fat as percent of energy bring approximately 26%, with a balanced intake of n-6 and n-3 PUFA, a gradual increased in the ratio, particularly in young persons, is observed. Hence, a more exhaustive nutritional education effort is necessary [9].

Metabolism of PUFA

Diet primarily contains EFA in the form of LA and LNA. Within the human organism these 18-carbon precursors can be elongated and desaturated to more highly unsaturated members of their family, principally AA and DHA. The liver is the primary site for EFA metabolism, although it does take place in other tissue as well [10].

The first part of the metabolic pathway leading to AA and EPA (C20:4 n-6 and C20:5 n-3) takes place in the endoplasmic reticulum and consists of sequential alternating elongation and desaturation steps catalyzed by fatty acid elongase, Δ^6- and Δ^5-desaturase. The Δ^6-desaturase seems to be the rate-limiting step of the pathway. The mechanism of the final conversion to

Table 1. PUFA Content of Selected Foods

Source	Linoleic Acid	Linolenic Acid	Arachidonic Acid
Cow's milk	89	61	trace
Emmental cheese	650	370	28
Feta cheese	330	260	
Gruyere cheese	1300	430	
Mother's milk	380	22	4.2
Mozzarella cheese	350	140	
Parmesan cheese	270	300	
Ricotta cheese	320	130	
Sheep milk	160	120	
Chicken egg (whole)	1350	70	70
Chicken egg (yolk)	3800	220	221
Butter fat	2300	1400	
Canola oil	19100	8600	
Coconut oil	1400		
Corn oil	50000	900	
Cotton seed oil	47800	1000	
Lard	8600	1000	1070
Linseed oil	13400	55300	
Margarine	17600	1900	
Olive oil	8000	950	
Peanut oil	23900	trace	
Safflower oil	74000	470	
Shark oil	270		5080
Soybean oil	53400	7600	
Sunflower oil	60200	500	
Bacon	6080	250	250
Beef (muscles only)	80		
Calf's kidney	61	61	
Chicken (breast)	980	2.7	112
Chicken (leg)	370	10	190
Ham cooked	1100	70	50
Ham raw	2480	160	130
Horse meat (average)	160	260	
Pork (muscles only)	110	25	
Turkey (breast)	180		50
Turkey (leg)	750		
Veal (muscles only)	197	9.1	53
Asparagus	70	6	
Beans	53	62	
Carrot	104.5	12.3	
Cauliflower	29	109	
Garlic	62	5.5	
Lettuce	52	71	
Peas	247	50	
Potato	32.12	22.75	
Purslane	89	405	
Soya bean	8650	1000	
Spinach	14	89	
Tomato	91	9	
Anchovy	50	30	10
Cod	4	2	3
Cray Fish	30	10	190
Eel			120
Herring	150	61.66	36.66
Mullet	60	26.66	210
Mussel	60	10	40
Oyster	10	40	10
Pike	27.57	47.33	50
Red fish (red perch)	100	45	240
Salmon	440	550	300
Sardine	100	50	10
Sole	47.5	10	23.33
Swordfish	40	230	90
Trout	74		30
Tuna	260	270	280
Maize	1630	40	
Pasta made with eggs	830	76	
Rice (unpolished)	780	30	
Almond	9860	260	
Apple	174	46	
Avocado	1970	trace	
Banana	34.5	24.5	
Brazil nut	24900	trace	
Cherry	47	46	
Coconut	680	trace	
Grape	111	36	
Grapefruit	42	12	
Macadamia nut	1300		
Olive	1120	130	
Peanut	13900	530	
Pear	108	36	
Pistachio	6500	270	
Plum	63	31	
Strawberry	132	112	
Walnut	34100	6800	590

Data are expressed as mg/100 g edible portion.

Sources: Souci SV, Fachmann W, Kraut H: Food Composition and Nutrition Tables 1989/1990. Wissenschaftliche Verlagsgesellschaft mbH Stuttgart. Simopoulos AP: Purslane: A terrestrial source of omega-3. N Engl J Med 315:833, 1986.

22:5n-6 and DHA is still not agreed upon by all lipid biochemists. Traditionally, it has been thought to occur via a Δ^4-desaturase [11], but no proof of the existence of this enzyme has ever been found [12]. Currently, most lipid biochemists are convinced that the last part occurs primarily via chain elongation and desaturation followed by a retro-conversion step of peroxisomal beta-oxidation, the so-called "Sprecher pathway" [13].

Infante and Huszagh [14,15] believe that the current evidence supports their proposal that the biosynthesis of 22:5n-6 and DHA occur via separate channeled carnitine-dependent mitochondrial pathways. According to their view, the outer mitochondrial membrane could well be the sole site for DHA, whereas 22:5 n-6, EPA and AA could be synthesized in the endoplasmic reticulum as well. They suggest that the mitochondria and microsomal system could be interregulated compensatory-redundant systems. The reaction starts with an 18:3n-3 carnitine complex which is transported across the outer mitochondrial membrane, followed by binding of 18:3n-3 to a

multifunctional enzyme complex. A series of desaturations and elongations follow; each of the three desaturation steps (Δ^6, Δ^5, Δ^4 n-3 desaturases) requires two molecules of the α-tocopherol metabolite as an electron withdrawing cofactor.

From several *in vivo* and *in vitro* studies with different animal species it is well known that LNA, LA and oleic acid (18:1n-9) compete for the same Δ^6-desaturase in the metabolic cascade. Dietary studies on rats and other animals have shown that LNA is a strong suppressor of n-6 fatty acid metabolism, whereas 10 times as much LA is required to give an equal suppression of n-3 metabolism [11]. *In vitro* studies on rat liver microsomes have confirmed that the n-6 and n-3 substrate competition occurs at several steps in the microsomal pathway [16]. These effects may not only be mediated by direct competitive mechanisms, but also very likely via regulation of the activity or abundance of desaturation and elongation enzymes at the level of expression of corresponding gene [17].

BIOLOGICAL AND FUNCTIONAL EFFECTS OF PUFA

Essentiality

Both LNA and LA are now regarded as nutritionally EFA. The classic symptoms of essential fatty acid deficiency (dermatitis, growth retardation and infertility) relate to the biological function of n-6 fatty acids [18]: LA is a structural component in the ceramides of the water barrier of the skin; AA is a precursor of eicosanoids; n-6 fatty acids possibly also play a role as second messengers in the process of signal transduction across cell membranes; LA deficiency may develop as a secondary condition in other disorders, such as protein energy malnutrition and fat malabsorption, as a consequence of total parenteral nutrition with inadequate LA intakes.

The understanding of the essentiality of the n-3 fatty acids lags behind. The n-3 fatty acids can in part substitute for the n-6 fatty acids, maybe as a sparing effect, in ameliorating some of the EFA deficiency symptoms, but are now considered also to have their own distinct role.

The biological functions of dietary n-3 fatty acids in the organism are [18]: to provide energy and carbon atoms; EPA and DHA serve as a precursor for "n-3 eicosanoids;" increasing evidence points to a specific role of DHA in membrane function, especially in retina and in neuronal tissues. Deficiencies of n-3 PUFA lead to a loss of DHA from brain and retina-rod outer segment phospholipids with a compensatory replacement by 22:5 n-6. This minor change in membrane phospholipid structure is sufficient to lead to memory loss, learning disabilities and impaired visual acuity.

Eicosanoid Metabolism

EFA in the plasma membranes serve as substrates for the enzyme cyclooxigenase (COX) and lipooxygenase (LOX) and are converted into a number of important, very active, short-lived, hormone-like compounds referred to as "eicosanoids."

In human beings, AA is the most abundant precursor; it is either derived from dietary LA or ingested as a dietary constituent. Arachidonate is esterified to the phospholipids of cell membranes or other complex lipids; since the concentration of free AA in the cell is very low, the biosynthesis of eicosanoids depends primarily upon its release from cellular stores by phospholipase A_2 (PLA_2).

Eicosanoids influence numerous metabolic activities including platelet aggregation, inflammation, hemorrhage, vasoconstriction and vasodilatation, blood pressure and immune functions.

In particular, AA is the substrate for the "series 2" prostaglandins (PGE), prostacyclins (PGI), thromboxanes (TX) and leukotrienes (LTB4, series B4 LT); EPA is the substrate for the "series 3" prostanoids and LTB5 (series B5 LT);

The PUFA-mediated immune response may be altered by changes in the production of immunologic mediators such as cytokines [19].

From the precursors AA and EPA, the synthesis of prostaglandins is accomplished by COX, a ubiquitous complex of microsomal enzymes (also called endoperoxide synthase or fatty acid cyclooxygenase). There are at least two isoforms of this enzyme: COX-1 and COX-2. The former is constitutively expressed in most cells (i.e., gastric mucosa, vasculature, glomeruli and collecting ducts of the kidney), but is more concentrated in the endoplasmic reticulum. In contrast, COX-2 is normally not present, but may be induced, both in the endoplasmic reticulum and over the surface of the nucleus, at sites of inflammation, by certain serum factors as inflammatory cytokines (IL-1β, IL-6, TNF-α) and growth factors (TGF-β, EGF), tumor promoters (phorbol esters), and cAMP [20].

The preferred substrates of COXs contain at least three double bonds in well-defined positions. These are DGLA, AA and EPA, which contain 3, 4 and 5 double bonds, respectively. Another major n-3 PUFA in fish oil, DHA, inhibits COX-1 [21]. Moreover, COX-2 appears much more accommodating than COX-1 in that it oxidizes 18 carbon PUFA with much higher efficiency than COX-1 [22].

More recently an "unorthodox" metabolic route for PUFA oxidation has been focused on. In particular, PUFA derived from membrane phospholipids can undergo autooxidation *in vivo*, generating a complex mixture of hydroperoxides, epoxides and cyclic peroxides [23].

Of particular medical interest are the isoprostanes and epoxides of AA and other PUFA. Isoprostanes of PUFA constitute a family of prostaglandin-related compounds, acting as autacoids: the urinary isoprostane index offers a method to estimate lipid peroxidation in various diseases. Epoxides of PUFA can be formed by autooxidation, by cytochrome P450 and possibly by the oxidative burst of inflammatory cells [23]. Epoxides of LNA are toxic, whereas epoxides of AA have a wide range of biological effects; in particular, the 5,6-epoxide

of AA is an excellent substrate of COX and thromboxane synthase, with vascular and renal effects [24,25].

The metabolites of EPA, DHA and AA have competitive functions: i.e., ingestion of EPA and DHA from fish or fish oil replaces AA from membrane phospholipids in practically all cells, especially those of platelets, erythrocytes, neutrophils, monocytes and liver cells.

Because of the increased amount of n-6 fatty acids in the Western diet, the eicosanoid metabolic products from AA are formed in larger quantities than those formed from n-3 fatty acids. The eicosanoids from AA are biologically active in small quantities and, if formed in large amounts, contribute to the formation of thrombi and atheromas, the development of allergic and inflammatory disorders, and cell proliferation.

Therefore, ingestion of EPA and DHA from fish or fish oil leads to a more physiologic state characterized by the production of prostanoids and leukotrienes that have antithrombotic, antichemotactic, antivasoconstrictive and anti-inflammatory properties [26,27].

Gene Expression: Epigenetic Properties of PUFA

Fuel metabolism in mammals is exquisitely regulated to ensure the generation of the energy needed for cellular functions. The search for the signaling pathway involved in the effects of specific nutrients on gene transcription is now under way, and several candidate proteins have been identified.

Dietary n-6 and n-3 PUFA have long been recognized as being able to exert experimentally unique influences on metabolic pathways and cellular growth [28]: the ingestion of very long-chain PUFA (e.g., EPA) enhances mitochondrial and peroxisomal fatty acid oxidation [29,30]; linoleate consumption suppresses the hyperproliferation of keratinocytes associated with essential fatty acid deficiency [31]; arachidonate promotes cellular growth in chemically induced mammary cancer [32] and stimulates *in vitro* the conversion of preadipocytes to adipocytes [33]; changes in mRNAs encoding several lipogenic enzymes can be detected within hours of feeding animals diets enriched in n-3 PUFA [34,35].

These effects are sustained so long as the n-3 PUFA remains in the diet. In these cases, the fatty acids act like a hormone to control the activity or abundance of key transcription factors. The discovery that some fatty acids can act as hormones that control the activity of transcription factors demonstrated that fatty acids are not merely passive energy-providing molecules but are also metabolic regulators. This finding opens novel perspectives for deeper understanding of energy metabolism and therapeutic interventions.

Through the application of molecular biology techniques it was discovered that PUFA elicit changes in gene expression that precede changes in membrane composition by directly governing the activity of nuclear transcription factors [17,36]. PUFA regulation of gene transcription occurred within a matter of minutes: such a time frame was too rapid to be explained simply by changes in membrane composition and altered hormone release or signaling, but is most consistent with a ligand-mediated event [17].

Peroxisome proliferator-activated receptor-α (PPAR-α) was the first transcription factor identified as a prospective fatty acid receptor [37]. PPAR-α plays a role in the regulation of an extensive network of genes involved in glucose and lipid metabolism.

The finding that PUFA are potent PPAR activators parallels the metabolic findings that in animal models n-6 and n-3 PUFA are potent inducers of fatty acid oxidation and potent suppressors of fatty acid and triacylglycerol synthesis [29,30,36].

Numerous reports unequivocally established that the 5′ flanking regions of genes encoding carnitine palmitoyltransferase, acyl-CoA oxidase, mitochondrial hydroxymethylglutaryl-CoA synthase, fatty acyl-CoA synthetase and mitochondrial uncoupling proteins all contain DNA recognition sequences for PPAR [38–41]. Moreover, dietary studies revealed that, in animals fed with a diet rich in 20-carbon and 22-carbon PUFA, the expression of the aforementioned genes significantly increases and the induction of these genes is associated with higher rates of fat oxidation and reduced body fat deposition [39–41].

However, studies with the PPAR-α null mouse have shown that PPAR-α is not the sole transcription factor involved in mediating fatty acid effects on gene transcription.

PPAR-γ also binds 20:5n-3. Activated PPAR-γ induces lipoprotein lipase and fatty acid transporters and enhances adipocyte differentiation as well as inhibits the function of the transcription factor NF-kB and cytokines, and therefore COX-2 expression [37].

In rodent models, pharmacological activation of PPAR-α and PPAR-γ reduces lipid levels in muscle and adipose tissue and improves insulin sensitivity in these tissues [42,43]. Although n-3 PUFA are weak agonists of PPAR when compared with pharmacological agonists (e.g. thiazolidinediones), n-3 PUFA have significant effects on insulin sensitivity in various tissues, particularly skeletal muscle [44].

In addition to the PPAR family (PPAR-α, -β, -γ1, and -γ2) several other transcription factors have been identified as targets for fatty acid regulation, including hepatic nuclear factor-4α (HNF-4α), sterol regulatory element-binding protein (SREBP), liver X receptors (LXR-α and -β), retinoid X receptors (RXR-α), and NF-kB [17,45–48]. It is noteworthy to mention that in order to bind to DNA and activate transcription, PPAR requires the formation of heterodimers with RXR.

Recently, the liver X receptors (LXR-α and LXR-β) were identified as targets for fatty acid regulation [43,48]. LXRs bind oxysterols and regulate the expression of genes involved in hepatic bile acid synthesis [49]. PUFA antagonize oxysterol activation by LXR-α in HEK 293 and hepatoma cell lines by interfering with oxysterol binding.

LXRs also play a major role in lipogenesis through the regulation of transcription of the gene encoding the SREBP-1c

isoform [50], a transcription factor required for the insulin-mediated induction of hepatic fatty acid and triglyceride synthesis [51]. Diets supplemented with olive, corn, soybean or walnut oil at <20% of total calories suppress hepatic lipogenic gene expression by suppressing the transcription of many genes involved in *de novo* lipogenesis including fatty acid synthase, stearoyl-CoA desaturase-1, L-pyruvate kinase, and S14 protein [35,52–55]. One more piece evidence is that PUFA suppress the nuclear content of SREBP-1c [17]. The hierarchy for fatty acid regulation of mRNA SREBP-1c levels is 20:5n-3 = 20:4n-6 > 18:2n-6 > 18:1n-9.

These studies provided the first indication that fatty acid regulation of hepatic *de novo* lipogenesis and fatty acid oxidation was not mediated through a common factor, i.e. PPAR-α. Coupling this action with the PUFA-mediated induction of PPAR-α-regulated genes shifts hepatic metabolism away from lipid synthesis and storage toward lipid oxidation [52,55]. This mechanism prevents lipotoxicity associated with lipid overload.

Although PUFA regulation of the SREBP-1c isoform appears to be a key player in PUFA suppression of lipogenic genes, not all glycolytic and lipogenic genes that are suppressed by dietary PUFA contain recognition sites for SREBP-1c. An alternative explanation is that a second PUFA-regulated transcription factor may exist in the nucleus of liver cells.

One nuclear protein which may fulfill this role is hepatic nuclear factor-4 (HNF-4) [56]. Like PPARs, HNF-4 is also a member of the steroid receptor superfamily. HNF-4 appears to enhance the promoter activity of selected genes (i.e., fatty acid synthase). This enhancer activity is suppressed when PUFA esters bind to the ligand domain of the HNF-4. In addition, an HNF-4 recognition sequence is also a component of the PUFA-response region of the pyruvate kinase gene [57].

Recent evidence indicates that the activity or abundance of other nuclear receptors may be affected, including thyroid hormone receptors (TR-α, TR-β).

TRs play an important role in metabolism, growth and differentiation. Some data reported that PUFA inhibited binding of T3 to TR-α and TR-β [58]. Although several T3-regulated hepatic genes are suppressed by PUFA, transfection studies using primary hepatocytes have failed to show that TR or thyroid hormone response elements are major targets for PUFA regulation of these genes [28,34]. An exception to this is seen when n-3 PUFA activate PPAR-α, leading to a sequestration of RXR (as mentioned above PPAR requires the formation of heterodimers with RXR) and inhibition of gene transcription through the interference with T3 action at the thyroid hormone response elements [59].

FACTORS AFFECTING PUFA STATUS

Adequate supplies of EFAs are required throughout development and adult life in order to maintain normal functions (e.g., brain retinal function, reactivity of immune and inflammatory system, cardiovascular performance). As noted above, the truly essential fatty acids are LA and LNA, but it is clear that their long-chain PUFA derivatives (AA, EPA and DHA) are most important too. Unfortunately, various factors can interfere with the conversion of the parent EFAs to long-chain PUFA [60] acting at the level of desaturase.

Exercise Training

Regular exercise training *per se* influences the phospholipid fatty acid composition of muscle membranes. The effect, exerted by regular exercise training on the muscle membrane phospholipid fatty acid composition in humans, was examined by Helge *et al.* [61]. Subjects performed endurance training of the knee extensors of one leg for 4 weeks. The other leg served as a control. Before training, after 4 days, and after 4 weeks muscle biopsies were obtained from the *vastus lateralis* muscle. After 4 weeks, the phospholipid fatty acid contents of oleic acid 18:1(n-9) and DHA were significantly higher in the trained than in the untrained leg. The ratio between n-6 and n-3 fatty acids was significantly lower in the trained than in the untrained leg. Alterations in the activity of desaturase and elongase enzymes (estimated as product-to-precursor ratios of fatty acids in skeletal muscle phospholipids) could probably also influence fatty acid profile in skeletal muscle but it is not still exhaustively demonstrated. In this model, diet plays a minimal role, as the influence of dietary intake is similar on both legs.

Insulin Resistance

Several clinical studies show that insulin resistance is related to muscle phospholipid fatty acid composition [62–64]. Insulin resistance is characterized by specific changes of the composition of fatty acids in the serum lipids and in the skeletal muscle membranes. Impaired insulin sensitivity is associated with high proportions of palmitic acid (16:0) and low levels of LA in serum. In addition, there are apparent changes of the fatty acid desaturase activities, suggesting an increased activity of the Δ-9 and Δ-6 desaturases and a decreased activity of the Δ-5 desaturase.

Experimental studies have indicated that insulin activates the Δ-9 and Δ-6 desaturases. In experimental diabetes and in spontaneously diabetic rats, there are reduced activities of Δ-9, Δ-6, and Δ-5 liver microsomal desaturases, which are restored after insulin treatment [65,66].

Insulin-deficient patients with type 1 diabetes have high levels of LA and low levels of the metabolites including AA in their serum lipids, with an increase of AA and a normalization of the PUFA after insulin treatment [67].

A high ratio between AA and DGLA, as an indicator of Δ-5 desaturase activity, in the skeletal muscle phospholipids has been related to good insulin sensitivity. Moreover, *in vitro* studies, evidenced that an increased unsaturation and a decreased ratio of n-6 to n-3 fatty acids in the muscle membrane

are compatible with an increased membrane fluidity, findings that have been linked to the presence of an increased number of insulin receptors and an increased insulin binding [68,69].

Overall evaluation of the relationships between fatty acid composition of skeletal muscle phospholipids and muscle fiber type, and insulin sensitivity showed that lower proportions of PUFA and higher proportions of saturated fatty acids, particularly palmitic acid (16:0), in skeletal muscle phospholipids are associated with insulin resistance in both animals and humans [70–73], skeletal muscle characteristics are also influenced by environmental factors such as diet and physical activity in both animals and human [74–76], insulin resistance is associated with lower proportions of oxidative slow-twitch type I fibers and higher proportions of glycolytic fast-twitch type IIb fibers in human [77], long-term endurance training has been shown to modify in endurance runner, the muscle fiber distribution, with a shift away from the insulin-resistant type IIb fibers [78], a greater proportion of n-3 PUFA and a smaller proportion of palmitic acid (16:0) have been observed in rat membrane phospholipids of type I fibers compared with type IIb fibers [79].

Vitamins Deficiency

Vitamin B6 deficiency might be a crucial factor for Δ-6 desaturase activity, especially in aged people. In particular, using 20 month old rats fed a diet with a subnormal level of vitamin B6, a diminished Δ-6 desaturase activity for LA and also for LNA in vitamin B6-deficient animals, being approximately 63% and 81% respectively of the corresponding activity in control rats was observed. As a consequence, significant modifications in the relative molar content of microsomal fatty acids were observed. The content of AA and DHA decreased, LA content increased, and a decrease in the unsaturation index was observed in liver microsomes of B6-deficient rats. This may be particularly important in aging, where Δ-6 desaturase activity is already impaired [80].

Vitamin E addition in rat brain microsomal membrane suspension induced an increase by more than two-fold in Δ-6 desaturase activity measured at substrate saturation using LA. In contrast, this activity was reduced by 25% in the liver. This raises the question of the multiple roles of vitamin E in membranes, the control of membrane PUFA through synthesis and their protection against peroxidation [81].

Excessive Alcohol Consumption

PUFA play a major role in membrane structures that are modified during alcoholism as alcohol inhibits phospholipase activity. PUFA are also precursors of second messenger eicosanoids involved in the regulation of blood pressure. Therefore excessive alcohol consumption has been related to hypertension and to alterations in liver PUFA metabolism. For these reasons the effects of ethanol on PUFA biogenesis in hepatocytes of Wistar Kyoto (WKY) rats and Spontaneously Hypertensive Rats (SHR), and the effects of a diet enriched with n-3 PUFA which is known to modulate hypertension, was investigated. Results showed that ethanol strongly inhibits the synthesis of PUFA in hepatocytes from SHR, which can explain the deficit of prostaglandin precursors observed in cardiovascular diseases linked to ethanol intoxication. Diet supplemented with n-3 PUFA reinforces the inhibition of AA synthesis, likely by a substrate competition toward Δ-5 desaturation [82].

In chronic alcoholics it was demonstrated that peripheral blood mononuclear cells (PBMC) produced less PGE2, and neutrophils produced less LTB4 than controls. Reduced PGE2 production by PBMC of alcoholics was corrected by the addition of exogenous AA [83].

Stress

Gudbjarnason [84] showed that in rats repeated administration of epinephrine, a catecholamine increased during stress, can alter the fatty acid composition of cardiac phospholipids. In particular, an increase in AA and DHA content, a decrease in LA, and a decreased ratio of n-6 to n-3, have been induced by the catecholamine.

Drugs

Troglitazone downregulates Δ-6 desaturase gene expression in human skeletal muscle cell cultures associated with a change in the unsaturated fatty acid composition of the muscle cells [85]. Dexamethasone-induced reductions in piglet AA status is minimized by dietary PUFA supplement. Piglets treated with dexamethasone grew slower and had lower bone mineral content of whole body and lower proportions of AA [86].

PUFA IN CHRONIC DISEASES

Cardiovascular Diseases

Antiarrhythmic Effects. Epidemiological and interventional studies indicate that dietary n-3 PUFA reduce mortality due to coronary heart disease (CHD). They act at a low dose, since one or two meals with fatty fish per week is sufficient to provide protection when compared with no fish intake [87]. Numerous experimental studies have indicated that low concentrations of exogenous n-3 PUFA reduce the severity of cardiac arrhythmia. This effect is probably responsible for the protective action of n-3 PUFA on CHD mortality. Such studies should take into account the fact that only a low dose of n-3 PUFA (20 mg/kg/day) is necessary to afford protection [88]. Inhibition of myocardial thromboxane synthesis may play a role in this effect [89], as well as reduced cardiac responsiveness to α1-adrenergic stimulation [90].

Christensen *et al.* [91] indicate an antiarrhythmic effect of n-3 PUFA, in patients referred to coronary angiography, due to a favorable shift in vagal/sympathetic balance. This evidence is indirect but concordant with a large body of experimental and

clinical evidence that a shift in vagal/sympathetic balance in favor of vagal modulation of the heart decreases susceptibility to cardiac arrhythmia and sudden death. In addition, several cardiovascular drugs that increase survival also increase vagal modulation of the heart.

Although the overall body of evidence from epidemiological studies and clinical trials suggest that n-3 PUFA have an important antiarrhythmic effect in patients with CHD, the details of the antiarrhythmic action for n-3 PUFA remain to be elucidated (relative importance of cardiac ion channel, central or autonomic effects) [92,93].

Unfortunately, CHD is often announced by sudden cardiac death. Moreover, it has been demonstrated that, in healthy man followed for 17 years, base-line blood levels of long-chain n-3 fatty acids were inversely related to the risk of sudden death [94]. The epidemiological data suggest that the benefit of dietary fish is centered on a reduction in sudden cardiac death.

A case-control study in Seattle compared 334 victims of out-of-hospital primary cardiac arrest with 493 population-based controls [95]. Compared with no dietary intake of EPA, a diet containing >5.5 g of n-3 PUFA per month was associated with a 50% decrease in the risk of primary cardiac arrest. This study found a strong inverse association between red blood cell n-3 fatty acid composition at the time of the arrest and the risk of primary cardiac arrest among subjects with no history of clinically recognized cardiac disease.

The US Physician's Health Study investigated the effect of dietary fish on sudden cardiac death in 20,551 US male physicians who were free of cardiovascular disease at baseline and then followed for up to 11 years [96]. During follow-up, 133 sudden deaths (death within 1 hour) occurred. A dietary intake of >1 fish meal per week was associated with a 52% reduction in sudden death. Eating fish more often than once a week did not confer additional benefit. Eating fish at least once a week was also associated with a 30% reduction in total mortality but not with a decrease in total myocardial infarction or non-sudden death.

The Lyon Heart Study [97] and the Indian Heart Study [98] have both shown in clinical trials that diet can prevent fatal and nonfatal cardiovascular events in individuals with CHD. In both trials, saturated fats were replaced with monounsaturated fats and LNA present in canola oil. Vegetables and fruits were increased in the diets in these studies as well.

In addition, fish and fish oil have been shown to reduce all-cause mortality and cardiovascular death in patients who had myocardial infarction [99,100]. Recently a randomized clinical trial, the *GISSI-Prevenzione Trial* [100] included 11,324 persons from 172 participating Italian centers, randomized <3 months after myocardial infarction, to receive an approximately 900 mg capsule of n-3 PUFA (EPA:DHA of 1:2) or a 300 mg capsule of synthetic α-tocopherol (vitamin E), or placebo. Follow up averaged 3.5 years. There was a 20% decrease in all deaths, a 30% decrease in cardiovascular deaths, and a 45% decrease in sudden cardiac deaths. No significant benefit was found for vitamin E *versus* placebo treatment. The investigators concluded that n-3 PUFA supplementation significantly reduced death, particularly sudden death, but not reinfarction or stroke. An antiarrhythmic action of n-3 PUFA was supported by these findings.

Given the safety and low cost of implementing a recommendation for a modest amount of fish in the diet, adequate dietary fish intake has a significant role to play in the primary and secondary prevention of out-of-hospital sudden cardiac death.

At high doses, dietary n-3 PUFA have several beneficial properties in human [87]: act favorably on blood characteristics by reducing platelet aggregation and blood viscosity, are hypotriglyceridemic, exhibit antithrombotic and fibrinolytic activities, exhibit antiinflammatory action, reduce ischemia/reperfusion-induced cellular damage. This effect is apparently due to the incorporation of EPA in membrane phospholipids.

Hypolipidemic Effects. The hypolipidemic effects of n-3 fatty acids are similar to those of n-6 fatty acids, provided that they replace saturated fats in the diet. An added benefit is shown by n-3 PUFA which in hypertriglyceridemic patients, consistently lower serum triacylglycerol concentrations, whereas the n-6 fatty acids do not and may even increase them [101].

Another important consideration is the finding that during chronic fish-oil feeding, post-prandial triacylglycerol concentrations decrease. Furthermore, Nestel [102] reported that consumption of high amounts of fish oil blunted the expected rise in plasma cholesterol concentrations in humans due to saturated fatty acid replacement of PUFA. Studies in humans have shown that fish oils reduce the rate of hepatic secretion of very low-density lipoprotein and triacylglycerol and in normolipidemic subjects, n-3 fatty acids prevent and rapidly reverse carbohydrate-induced hypertriglyceridemia [103].

Antithrombotic Effects. The antithrombotic effects of fish oil are due to decreases in platelet aggregation, a decrease in TXA_2, increases in PGI2 and PGI3 production, decrease in whole blood viscosity and an increase in bleeding time [104].

Because of the increased amount of n-6 fatty acids in the Western diet, the eicosanoid metabolic products from AA, specifically PGE, TX, LT, are formed in larger quantity than those formed from n-3 fatty acids, specifically EPA. The eicosanoids formed from AA are biologically active in small quantities and if they are formed in large amounts, they contribute to the formation of thrombi and atheromas, the development of allergic and inflammatory disorders, and cell proliferation. Thus a diet rich in n-6 fatty acids shifts the physiologic state to one that is prothrombotic and proaggregatory, with increases in blood viscosity, vasospasm and vasoconstriction and decreases in bleeding time.

A recent randomized controlled trial showed the n-3 PUFA therapeutic effects on 188 stroke patients awaiting carotid endarterectomy. The patients, divided into three experimental groups, were treated over an average period of 42 days with

fish oil, sunflower oil or a placebo, six times a day. Fish oil patients received 1.4 g/daily of n-3 PUFA. Results demonstrated that n-3 PUFA helped to make scars harmless and stabilize the health in stroke patients who are at high risk of atherosclerotic plaques rupturing or forming clots. It was demonstrated that the proportions of EPA and DHA were higher in carotid plaque fractions in patients receiving fish oil compared with other groups. Fewer plaques from patients being treated with fish oil had thin fibrous caps and signs of inflammation and more plaques had thick fibrous caps and no signs of inflammation, compared with plaques in other groups. The number of macrophages in plaques from patients receiving fish oil was lower than in the other two groups. This finding suggests that within a short time, a modest level of dietary n-3 PUFA supplementation has a role in establishment of plaque stability, thus reducing the risk of neurological events in patients with advanced carotid atherosclerosis [105].

Prevention of Restenosis. Restenosis is a condition caused mainly by platelet aggregation, proliferation of smooth muscle cells, and coronary vasospasm.

The effect of n-3 supplementation on the incidence of restenosis after coronary angioplasty has been addressed in several clinical studies. Results suggest that patients undergoing coronary bypass surgery should be encouraged to consume high amounts of n-3 fatty acids [106]. Moreover, it appears that the longest the length of time that n-3 fatty acids were taken prior to surgery, the best the results obtained so far [107].

Hypotensive Effects. Evidence from laboratory investigations, observational studies, and clinical trials indicates that supplementation of diet with high doses of n-3 PUFA can reduce blood pressure [108]. However, large quantities (e.g., 3 g/day) are needed to see a minimal effect in non-hypertensive individuals and only very modest effects in hypertensive individuals. The most effective n-3 PUFA is DHA rather than EPA. It takes large amount of EFA to have a hypotensive effect; eating more fish or flaxseed is unlikely to be beneficial, so the only way to get clinically significant doses is to take EFA in supplement form.

Diabetes

Type 2 diabetes is a multigenic, multifactorial disorder, characterized by hyperglycemia in the presence of insulin resistance, hypertriglyceridemia and the development of vascular complications.

In 1993, Borkman *et al.* [109] showed that hyperinsulinemia and insulin resistance are inversely associated with the amount of 20- and 22-carbon fatty acids in muscle cell membrane phospholipids in patients with coronary heart disease and in normal volunteers. Such decreases in 20- and 22-carbon fatty acid concentrations could occur as a result of low dietary intake of 20- and 22-carbon fatty acids, high dietary intake of *trans* fatty acids, which interfere with the desaturation and elongation of LA and LNA and thus lower AA, EPA, and DHA concentrations, genetic defects of Δ^5 and Δ^6 desaturase, genetic defects that interfere with the transport or binding of 20- and 22-carbon fatty acids, such as intestinal fatty acid binding protein, high dietary intake of LA, which leads to decreased production of AA and interferes with the desaturation and elongation of LNA to EPA and DHA, increased catabolism of AA, which reduces the number of available 20- and 22-carbon fatty acids, an increase in 20- and 22-carbon PUFA (ie, AA, EPA, and DHA), leads to increases in membrane fluidity, the number of insulin receptors, and insulin action [110–112].

Moreover, maternal fasting insulin levels and triglyceride levels are significant predictors of the PUFA composition of the child's muscle membrane. The less unsaturated muscle membranes in children whose mothers have higher fasting insulin and triglyceride levels may reflect a genetic reluctance to incorporate PUFA into membranes, thus predisposing them to insulin resistance syndrome [113].

About 23 studies have been conducted on the effects of n-3 fatty acids in patients with type 2 diabetes [114]. In most studies, fish oil consumption lowered serum triacylglycerol concentrations significantly, but in some studies, plasma glucose concentrations rose. In many of these studies, however, the number of subjects was small and the dose of n-3 fatty acids was >3 g/day and controls were lacking.

The largest and longest reported placebo-controlled trial of the effect of n-3 fatty acids (6 g EPA and DHA/day for 6 months) on type 2 diabetes showed convincingly that n-3 fatty acid intake, along with oral therapy for diabetes, can lower triacylglycerol concentrations, with no adverse effects on glycemic control [115].

It is also known that the concentration of serum leptin (a hormone expressed and secreted in proportion to adipose mass) in patients with type 1 diabetes mellitus is influenced by the type of fat in the diet [116]. In particular it has been found that n-3 fatty acids decreased leptin gene expression both *in vivo* and *in vitro*. The direct effects of PUFA on leptin promoter activity indicate a specific regulatory action of fatty acids on leptin expression [117].

Anti-inflammatory Effects

Eicosanoids derived from AA and EPA have very similar molecular structures but markedly different biologic effects. For example, the EPA-derived eicosanoids are in general much less potent inducers of inflammation than the AA-derived eicosanoids. Consequently, a predominance of n-6 fatty acids will result in a proinflammatory status with production of prostaglandins of the 2 series and leukotrienes of the 4 series. As the relative amount of n-3 fatty acids increases, more prostaglandins of the 3 series and leukotrienes of the 5 series are produced. These eicosanoids are considered to be less inflammatory [118]. A reduction in the amount of the more inflammatory products from AA (PGE2 and LTB4), has been

implicated as an underlying mechanism for the anti-inflammatory effects of fish oil [119].

The immune response also may be altered by changes in the production of immunologic mediators such as cytokines [19].

The effect of PUFA on the immune response may differ with age. In healthy geriatric dogs, dietary supplementation of n-3 fatty acids, changes the cell-mediated immunity as demonstrated by the delayed-type hypersensitivity (DTH) skin test [120]. Meydani *et al.* [119] studied the effect of dietary n-3 fatty acids on cytokine production and lymphocyte proliferation in young and older women and found the changes to be more dramatic in older women.

Arthritis

Supplementation with n-3 fatty acids can modulate the expression and activity of degradative and inflammatory factors that cause cartilage destruction during arthritis. *In vitro* study on bovine articular cartilage, demonstrated that incorporation of n-3 fatty acids into chondrocyte membranes results in a dose-dependent reduction in the expression and activity of proteoglycan degrading enzymes, and in the expression of inflammation-inducible cytokines (IL-1α and TNF-α) and COX-2, but not the constitutively expressed COX-1 [121].

These findings provide evidence that n-3 fatty acid supplementation can specifically affect regulatory mechanisms involved in chondrocyte gene transcription and thus further advocate a beneficial role for dietary fish oil supplementation in alleviation of several of the physiological parameters that cause and propagate arthritic disease.

Psoriasis

Altered AA metabolism plays a role in the pathogenesis of cutaneous scaly disorders. Abnormally high levels of AA and its lipooxygenase products LTB4 and 12-hydroxyeicosatetraenoic acid (12-HETE) are described in the lesions (plaques) of patients with psoriasis. Intravenous n-3-fatty acid administration causes reduction of psoriasis, which may be related to changes in inflammatory eicosanoid generation [122].

Ulcerative Colitis

LTB4 and PGE2, both products of AA metabolism, are increased in patients with ulcerative colitis. In ulcerative colitis LTB4 is an important mediator of inflammation and has the ability to recruit additional neutrophils from the blood stream into the mucosa, exacerbating the disease process by further increases of LTB4 [123]. Four months of diet supplementation with fish oil in patients with ulcerative colitis resulted in reductions in rectal dialysate LTB4 levels, improvements in histologic findings, weight gain and a reduction in the dose of prednisone administered [123].

Cancer

Although the role of individual fatty acids in human cancer risk has hitherto been poorly investigated, some recent epidemiological and experimental data linked a high dietary intake of n-6 PUFA, especially in association with a low intake of n-3 PUFA, to increased risk for cancer of the breast, colon, and possibly prostate. n-6 PUFA enhance tumorigenesis and metastasis in experimental animals by several mechanisms, whereas n-3 PUFA can inhibit the growth of initiated cancer cells.

PUFA Effects on Cell Proliferation and Signal Transduction. Fat may regulate cellular functions by affecting the expression or activity of genes in the signal transduction pathway related to the control of cell growth and apoptosis.

High intake of n-6 PUFA experimentally induces various physiological and metabolic effects [124,125]: increased ornithine decarboxylase activity in colon mucosa, resulting in enhanced epithelial polyamine levels and increased colon crypt cell proliferation; enhanced activities of protein kinases (i.e., protein kinase C) in rodent mammary gland and the increased number of estrogen receptor binding sites; increased prostaglandin concentrations; prostaglandins, thromboxanes, leukotrienes and hydroxy and hydroxyperoxy fatty acids are involved in tumor initiation and promotion, cell proliferation, tissue invasion and metastatic spread. Tumor cells produce larger amounts of eicosanoids than their normal cell counterparts and eicosanoids ultimately derived from linoleic acid have been linked to increased growth and metastasis. The finding that oleic acid and omega-3 PUFA, specifically EPA, block the desaturase reaction, the first step from linoleic acid to eicosanoids, may partially explain their inhibitory effects on tumorigenesis.

Experimental Data. Fay *et al.* [126] conducted a meta-analysis of data on mammary tumor incidence extracted from 97 reports of experiments involving over 12,800 mice and rats to study the effects of saturated and monounsaturated fats and n-6 PUFA and n-3 PUFA. The results indicated that n-6 PUFA have a strong tumor-enhancing effect, whereas the n-3 PUFA have a small, statistically non-significant protective effect; monounsaturated fats had no significant effect.

Hilakivi-Clarke [127] tested the hypothesis that consumption of a high fat diet during gestation increases the incidence of carcinogen-induced mammary tumors in rats. They demonstrated that consumption of a high n-6 PUFA diet during gestation increased the risk of developing carcinogen-induced mammary tumors, possibly by increasing the concentration of circulating estrogens. These data raise the possibility that human breast cancer might be prevented by dietary manipulation during pregnancy, which has not been addressed in epidemiological studies.

Hilakivi-Clarke [128] also showed that feeding pregnant rats a high fat diet would increase both circulating 17-α-estradiol concentrations in the dams and the risk of developing

carcinogen-induced mammary tumors among their female offspring.

Several studies subsequently showed that diets containing corn oil, with high levels of n-6 PUFA, enhance breast and colon tumorigenesis in rodents, whereas fish oil, which is rich in the n-3 PUFA, reduces carcinogenesis [124].

Clinical Data. In a population study, an inverse relationship was found between a high consumption of fat from fish, rich in n-3 PUFA, and the development of colorectal cancer. Thus, data on mortality from colorectal cancer in 24 European countries between 1984 and 1987 were correlated with consumption of fish and fish oil currently and 10–23 years earlier [129]. The study showed an inverse association between death from colorectal cancer and current fish intake, a weaker correlation with fish consumption 10 years earlier and none with consumption 23 years earlier among males; the association was not significant for females.

In a follow-up study [130], mortality from colorectal cancer correlated with the consumption of animal but not vegetable fat and an inverse correlation was observed with fish and fish oil consumption when expressed as a proportion of the total or animal fat (in countries with an animal fat consumption of 85 g/day). This correlation was significant for both males and females and for intakes currently or 10 or 23 years before death from cancer. The evidence from these two studies indicates that fish oil provides protection against the promotional effects of animal fat in colorectal carcinogenesis.

In prospective studies, the protective effects of fish consumption are seen only in areas where fish consumption is high. For instance, the incidence of colorectal cancer among black fishermen on the west coast of South Africa is six times lower than that in white urban dwellers, and their consumption of fish was significantly higher (110 *versus* 30 g/day) [131]. A positive correlation was found between plasma fatty acid concentration and dietary intake, the fishermen having higher levels of circulating n-3 PUFA. In a study in Norway, intake of fish in general had no protective effect against colon cancer, but the relative risk of men and women who ate five or more fish meals per week was lower than that of people who ate fish less frequently [132]. Taken together, these results indicate that various types of fat may have opposite effects on the risk for cancer of the breast and colon that closely resemble the corresponding effects in experimental animals.

A high fat diet has been associated with the occurrence of the aggressive, metastatic phenotype of prostate cancer, although further research is required to establish the roles of the various classes of fatty acids. n-3 PUFA may retard the progression of prostate cancer [124].

There is now substantial experimental evidence that n-6 fatty acids enhance the risk for cancer of the breast and colon and for metastasis, whereas relatively high intakes of n-3 PUFA and n-9 monounsaturated fatty acids (olive oil) reduce cancer risk by mechanisms that may involve modification of the biosynthesis of eicosanoids. In future studies on breast cancer, measurements of fat intake early in life, circulating hormone levels and lipid peroxidation-related DNA modifications should be included. Another shortcoming of studies on the dietary habits of middle-aged women is the fact that the risk for breast cancer may be imprinted early in life or even *in utero* by a high n-6 PUFA diet and estrogenic stimuli, resulting in early onset of puberty and later risk for breast cancer, as convincingly demonstrated in rodents. Such risk modifiers acting early in life or during pregnancy should be taken into consideration.

Relationship between a High Dietary Fat Intake and Increased Cancer Risk. This relationship has been controversial for a long time, partly because of the lack of consensus on the mechanisms of action of dietary fat in mammalian cells.

Dietary fats, specifically n-6 and n-3 PUFA, affect a variety of steps in the multistage carcinogenesis process, adding further weight to a causal effect. The effects may be direct or indirect and include [133]: peroxidation of conjugated double bonds in PUFAs, leading to persistent oxidative stress and generation of reactive lipid peroxidation products (malondialdehyde, 4-hydroxyalkenals), which can induce DNA damage; conversion of essential fatty acid to eicosanoids, short-lived hormone-like lipids derived primarily from dietary linoleic acid; interaction of fatty acids with signal transduction pathways leading to altered gene expression; in the case of breast cancer, effects on unbound estrogenic hormone concentrations; effects on membrane (lipid)-bound enzymes such as cytochrome P450 (CYP) that regulate xenobiotic and estrogen metabolism; structural and functional changes in cell membranes resulting in alterations in hormone and growth factor receptors.

In initiated or preneoplastic cells, PLA_2, COX-2 and LOX are often constitutively overexpressed. This leads to increased release of AA and faster AA oxygenation, resulting in higher levels of n-6 eicosanoids, accompanied by generation of reactive oxygen species (ROS). These can cause DNA damage and trigger lipid peroxidation of PUFA in a self-perpetuating process, leading to various forms of exocyclic DNA base and protein modifications. In rapidly dividing cells, the resulting genetic changes and disrupted signaling pathways may drive premalignant cells to genetic instability and malignancy. n-3 PUFA inhibit AA metabolism and COX activity, thus blocking the formation of n-6 eicosanoids from diet-derived LA, which have been linked to tumor growth and metastasis [133].

Effects of PUFA in Cancer Cachexia. Patients with chronic diseases such as acquired immune deficiency syndrome (AIDS) or cancer (particularly those with tumors of the pancreas, stomach, colon, and lung) often experience a life-threatening muscle wasting syndrome known as cachexia. Cachexia is characterized by a dramatic loss of triglycerides from adipose tissue and proteins from skeletal muscle. Cachexia is associated with reduced survival time irrespective of tumor mass or the presence of metastases, and it also interferes with cancer therapy.

Knowledge of the molecular pathways leading to cachexia is required if an effective treatment is to be developed. Factors involved in activating protein catabolism in skeletal muscle

comprise: 1) the ubiquitin-dependent proteolytic pathway (proteasome) that breaks down most skeletal muscle proteins in a variety of wasting conditions [134]; 2) NF-kB which has been identified as an inhibitor of skeletal muscle cell differentiation and a mediator of cytokine-induced muscle wasting in mice; cytokines such as TNF-α together with IFN-γ activate NF-kB, this leads to decreased expression of MyoD, a transcription factor that is essential for skeletal muscle differentiation and for repair of damaged tissue, and it may be particularly important for the replenishing of wasted muscle [135]; 3) activated NF-kB also acts as a repressor of proteasome subunit expression and hence suppresses protein degradation, an activity that is antagonized by glucocorticoids [136]; 4) a proteolysis-inducing factor (PIF) has been observed in serum samples from cachectic mice and cancer patients; PIF is a sulfated glycoprotein produced by tumors that induces protein catabolism in isolated muscle cells [137] and appears to activate the ubiquitin-proteasome pathway directly, possibly through an intermediate molecule, 15-hydroxyeicosatetraenoic acid (15-HETE) but it is not known whether this is a direct or indirect effect.

An approach to cachexia could be to block NF-kB activity since it has been shown to inhibit cachexia in an animal model [138]; another approach could be to block signaling pathways induced by PIF that lead to proteasome activation [137].

A full knowledge of the mechanism of the beneficial effect of PUFA on cancer cachexia will provide vital information for the development of new agents. Of particular interest is recent progress with EPA. The mechanism by which EPA attenuates skeletal muscle protein catabolism in cancer cachexia was investigated by Whitehouse *et al.* [139]. Soleus muscles from mice bearing a cachexia-inducing tumor (MAC16) showed an increased protein degradation *in vitro*, as measured by tyrosine release, when compared with muscles from nontumor-bearing animals. After incubation under conditions that modify different proteolytic systems, lysosomal, calcium-dependent, and ATP-dependent proteolysis, elevated protein catabolism was found. Results showed that EPA induces an attenuation of the up-regulation of proteasome expression in cachectic mice, and this was correlated with an increase in myosin expression, confirming retention of contractile proteins. EPA also inhibited growth of the MAC16 tumor in a dose-dependent manner, and this correlated with suppression of the expression of the 20S proteasome α-subunits in tumor cells, suggesting that this may be the mechanism of tumor growth inhibition. Thus EPA antagonizes loss of skeletal muscle proteins in cancer cachexia by down-regulation of proteasome expression. This polyunsaturated fatty acid is effective in the attenuation of cachexia not only in murine models, but also in cancer patients. EPA inhibits 15-HETE production in response to PIF and prevents muscle wasting in cancer patients [137].

Moreover, in patients with unresectable pancreatic cancer, EPA treatment resulted in preservation of lean body mass [140]. In these patients, EPA was effective in attenuating the development of weight loss and when combined with nutritional supplementation resulted in significant weight gain. This weight gain is attributable to the accumulation of lean body mass with no change in adipose tissue or body water. Energy expenditure was decreased and food intake increased.

A double-blind, placebo-controlled randomized clinical trial currently underway in Europe was designed to determine the effects of EPA treatment in patients with pancreatic cancer cachexia. EPA appears to inhibit the up-regulation of the ATP-ubiquitin-dependent proteolytic pathway in skeletal muscle induced by PIF. The effect appears to be attributable to the inhibition of downstream signaling events. EPA also inhibits proteolysis, including growth factor production by the tumor, which may be evidence of a direct effect on tumor cell proliferation [141].

In another trial in pancreatic cancer, dietary supplementation with EPA combined with DHA, led to a significant median weight gain of 0.3 kg/month, accompanied by a temporary but significant reduction in acute-phase protein production and by stabilization of resting energy expenditure [142]. The acute-phase C-reactive protein has been shown, in particular, to be down-regulated by EPA in patients with pancreatic cancer cachexia, in a process involving the suppression of IL-6 production [143].

The profound cachexia associated with cancer also includes characteristic abnormalities in carbohydrate metabolism and marked peripheral insulin resistance. The abnormal metabolism of proteins, carbohydrates, and lipids in pancreatic cancer patients apparently arises from a complex interplay between cancer-derived factors and probably also involves inflammatory cytokines and circulating metabolic hormones.

The effect of PIF, produced by cachexia-inducing tumours on glucose utilization by different tissues and the effect of pretreatment with EPA, has been determined by Hussey [144]. Mice receiving PIF showed a profound depression of body weight (2.3 g) over a 24-hour period, and a marked hypoglycemia, which were completely abolished by pretreatment with a monoclonal antibody to PIF or by pretreatment with EPA for 3 days. These results suggest that in addition to a direct catabolic effect on skeletal muscle PIF has a profound effect on glucose utilization during cachexia.

Muscle protein degradation in cancer cachexia is also associated with a rise in PGE2 content. Experiments with the cachexia-inducing MAC16 tumor in mice, demonstrated that this increase is inhibited by EPA [145].

The Importance of PUFA in Brain Function

PUFA, especially AA and DHA are acylated into membrane phospholipids of vertebrates. PUFA account for 21%–36% of the fatty acids in the cell membrane, but the proportions of fatty acids with 20 or 22 carbon atoms varies considerably between tissues.

The nervous system is the organ with the second largest

concentration of lipids, only exceeded by adipose tissue. The adult brain contains approximately 50%–60% of its dry weight as lipid and approximately 35% of the lipids are PUFA [146], most of which are long-chain PUFA (EPA and DHA). Neuronal tissues, such as the brain, retina, and synaptic membranes are especially high in DHA [147]. In animal models, this relative distribution gives some indication of a possible important role in the membranes of these tissues: the high concentration of DHA in synaptic membranes correlates with development of the synapses [148]; chronic AA deficient rats had altered dopaminergic transmission in the frontal cortex [149]; n-3 PUFA in neuronal membranes affect the activity of ion pumps and channels [150]; the precise fatty acid composition of the membrane can affect the tertiary and quaternary structures of membrane-bound receptors such as cholinergic, adrenergic, dopaminergic and N-methyl-D-aspartate (NMDA) and associated neurotransmitter functioning [151–153]; deficiencies of EFA have been associated in animal models with disruption of neural integrity and function [150,154], visual and cognitive deficit [155].

PUFA for Bone Growth and Repair

Bone is a multifunctional organ that consists of a structural framework of mineralized matrix and contains heterogeneous populations of chondrocytes, osteoblasts, osteocytes, osteoclasts, endothelial cells, monocytes, macrophages, lymphocytes and hematopoietic cells. Bone growth is regulated by complex interactions between an individual's genetic potential, environmental influences and nutrition. Evidences suggests that the high intake of n-6 and inadequate amount of n-3 fatty acids in the diet contribute to the development of several pathologies, including those of the skeletal system (bone/joint diseases) [156].

Bone Modeling and Remodeling. The human skeleton is not static. Bone is a highly active metabolic tissue, continually changing throughout life. Bone remodeling is the process of bone growth associated with maintaining a fixed adult bone mass. In remodeling, only about 20 percent of the bone surface is active. Older bone tissue is destroyed (re-absorption) and replaced by new bone tissue (formation) in a cyclical process [156]. In the case of osteoporosis, the basic problem is that re-absorption gets ahead of formation, resulting in a net bone loss.

Bone produces various immune and blood cells, and is a "metabolic reservoir" for calcium, magnesium, and phosphorus. Bone metabolism is under the control of many hormones and growth factors, including activated vitamin D, estrogen, growth hormone, insulin, insulin-like growth factor, parathyroid hormone, and various eicosanoids, with PGE2 playing a major role [157]. At low levels, PGE2 apparently stimulates bone formation. The mechanism for this may be that PGE2 increases the production of insulin-like growth factor (IGF), a powerful "master" growth stimulator for bone, cartilage, and muscle, found in abnormally low levels in women with osteoporosis [158]. Surprisingly, high or excessive levels of PGE2 swamp this effect, and bone formation is reduced and re-absorption is increased [159]. Moreover, PGE2 has been shown to mediate, *in vitro*, the effects of 1,25-$(OH)_2$ Vitamin D3 [160], TNF-α [161], and growth factors [162], thus enhancing bone re-absorption. In bone modeling, this pattern leads to reduced skeletal growth. In bone remodeling, this pattern leads to osteoporosis. Growth opportunity lost in childhood can never be fully compensated for in adulthood and may put an individual at greater risk for osteoporosis later in life. Evidence exists that in osteomyelitis, lowering PGE2 levels can increase bone formation and reduce bone re-absorption rates [163]. Therefore, it is important to maintain low levels of PGE2 throughout one's lifetime.

Experimental Studies. PUFA supplementation may help optimize bone modeling and remodeling by moderately increasing production of series-1 and -3 prostaglandins at the expense of PGE2 [164].

In a study conducted at Purdue University, this nutritional approach was tested on bone modeling in growing rats [165]. For 42 days, groups of 15 rats were fed identical diets except that the n-6 to n-3 PUFA ratios differed. Safflower oil and fish oil were mixed to produce n-6 to n-3 ratios of 23.8, 9.8, 2.6, and 1.2. Rat liver and bone tissue samples showed both PGE2 levels and serum alkaline phosphatase (ALP) decreased as the proportion of n-6 to n-3 decreased. High levels of ALP indicate bone is being re-absorbed. Moreover, rats fed the 1.2 ratio diet had slightly higher rates of bone formation.

An increased production of bone PGE2 in tibia of chicks given a semipurified diet containing soybean oil, high in n-6 PUFA, was associated with a lower rate of bone formation compared with that of chicks fed a low dietary ratio of n-6/n-3 fatty acids [166]. Furthermore, dietary n-3 PUFA were reported to lower the concentration of AA in bone [165] and cartilage [167], and depress *ex vivo* PGE2 production in bone organ culture. One explanation for this phenomenon in bone is that dietary sources of PUFA that elevate AA cause an overproduction of PGE2 in bone that leads to a reduced bone formation rate.

Dietary lipids are known to affect the fatty acid composition of membrane phospholipids and influence cell function. Investigators have shown that rats fed with a lower dietary ratio of n-6/n-3 fatty acids had increased bone marrow cellularity [168] and bone strength [169]. Moreover, reports by Alam *et al*. [170] in rat alveolar bone and Xu *et al*. [167] in chicken cartilage corroborate the findings that dietary PUFA alter the fatty acid composition and PGE2 production in these tissues.

Sakaguchi *et al*. [171] were the first to report on the interaction of estrogen deficiency, EPA, and bone activity in rats. Ovariectomy and low calcium diet caused a decrease in bone weight and bone strength. EPA prevented the loss of bone weight and bone strength induced by ovariectomy in the low

calcium diet group, but it failed to show an increase in bone weight and strength in the normal calcium group.

Claassen *et al.* [172] studied the effects of feeding different ratios of DGLA and EPA on bone status and parameters of bone collagen breakdown by assessing free urinary pyridinium in growing rats, aged 5–12 weeks. Pyridinium excretion was significantly lower in all the groups receiving the diets containing DGLA and EPA. No abnormal bone growth stimulation or restriction was seen in any of the supplemented groups. After six weeks of supplementation the 3:1 and 1:1 (DGLA:EPA) diet groups showed significantly higher levels of bone calcium than controls, and bone calcium was significantly higher in the 3:1 diet group than in the 1:1 diet group.

Claassen *et al.* [172] further explored the effect of DGLA: EPA on calcium absorption in the same groups of rats. Calcium absorption (calcium intake minus fecal excretion) after the six-week supplementation period was significantly higher in the 3:1 and 1:3 supplemented groups as compared to the control group. This study shows that essential fatty acid supplementation may have a role in reducing the age-related decline in calcium absorption.

Schlemmer *et al.* [173] used ovariectomized female rats to study the relationship between EFAs, bone turnover, and bone calcium. The rats were supplemented from age 12–18 weeks with a semi-synthetic diet containing different ratios of DGLA: EPA+DHA (9:1, 3:1, 1:3, 1:9) added to the diet. All diets increased bone calcium content and reduced urinary deoxypyridinoline and hydroxyproline excretion.

Clinical Studies. In a single-blind, randomized study, Kruger *et al.* [174] tested the interactions between calcium and DGLA + EPA in osteoporotic or osteopenic women. All of the women were living in the same institution for the elderly and fed the same low-calcium, non-vitamin D enriched foods, and had similar amounts of sunlight. Subjects were randomly assigned to DGLA + EPA or coconut oil (placebo group); in addition, all received 600 mg/day of calcium. Markers of bone formation/degradation and bone mineral density (BMD) were measured at baseline, 6, 12 and 18 months. At 18 months, osteocalcin and deoxypyridinoline levels fell significantly in both groups, indicating a decrease in bone turnover, whereas bone specific ALP rose indicating beneficial effects of calcium given to all the patients. Lumbar and femoral BMD, in contrast, showed different results in the two groups. Over the first 18 months, lumbar spine density remained the same in the treatment group, but decreased 3.2% in the placebo group. Femoral bone density increased 1.3% in the treatment group, but decreased 2.1% in the placebo group. During the second period of 18 months with all patients now on active treatment, lumbar spine density increased 3.1% in patients who remained on active treatment, and 2.3% in patients who switched from placebo to active treatment; femoral BMD in the latter group showed an increase of 4.7%.

Another human study has specifically examined the effects of PUFA supplementation on osteoporosis [175]. Forty elderly women with age-related osteoporosis were divided into four groups. They received one of four treatments daily for 16 weeks: 4 g evening primrose oil; 4 g fish oil; 4 g of a fish and evening primrose oil mixture; or 4 g olive oil placebo. The women took no other medications, supplements, or special foods. In this study fish oil increased serum calcium, osteocalcin and collagen, and decreased ALP. Evening primrose oil alone had no significant effects, but the positive results from the fish oil group were also seen in the fish oil plus evening primrose oil group. According to the research team, evening primrose oil may have potentiated the effects of fish oil.

Mood and Bone. Clinical depression in both women and men has been correlated with reduced bone density. In a National Institutes of Health study, 24 women with a history of major depression were compared to 24 controls. Subjects were matched for age, race, body-mass index, and menopausal status. Upon testing, various bone sites showed densities 6.5 to 13.6 percent lower in the depressed women [176]. Clinical depression is known to be associated with strongly reduced levels of n-3 PUFA, and clinically depressed people have been found to respond to fish oil supplementation. Deficiencies of n-3 PUFA may be a common link between depression and reduced bone density, both prevalent in older people [177].

PUFA AS A PHYSIOLOGICALLY-IMPORTANT NUTRIENT DURING PREGNANCY AND FETAL DEVELOPMENT

PUFA are vitally important structural elements of cell membranes and, therefore, essential for the formation of new tissues, as occurs during pregnancy and fetal development. The central nervous system is particularly rich in AA and DHA and the cerebral accretion of these fatty acids may have been decisive in the evolution of *Homo sapiens* [178].

The brain has its growth spurt in the third trimester of pregnancy and during early childhood. Therefore, an appropriate pre- and post-natal supply of PUFA is thought essential for normal fetal and neonatal growth [179], neurologic development and function, activity of retinal photoreceptors [180], and learning and behavior [181].

Maternal concentrations of PUFA, especially DHA, are associated with sleep and wake states of newborns. Sleep and wake rhythm provides a tool for assessing the functional integrity of the central nervous system. It has been demonstrated that higher maternal plasma DHA during pregnancy is associated with more mature neonatal sleep-state patterning [182].

In addition, intrauterine nutrition may influence the adult risk for chronic diseases [183], suggesting that early nutrition has an imprinting effect on later life. This further emphasizes the importance of an adequate supply of essential PUFA during pregnancy, lactation, and infancy.

Several studies indicate that it may be necessary to increase

the dietary PUFA intakes of pregnant women to prevent a decrease in their essential PUFA concentrations during pregnancy and to optimize the fetal PUFA status, particularly in preterm infants because they have a significantly lower PUFA status than do term neonates [184].

In addition, the PUFA status of pre-term infants drops considerably during the first postnatal weeks, even when infants are fed breast milk [185,186]; however, the EFA status increases considerably during the same gestational period *in utero* [187]. Consequently, during the growth spurt of the brain, the availability of PUFA is much lower in infants born preterm than in intrauterine fetuses of comparable gestational age.

It has been also showed in pre-term infants that AA and DHA concentrations at birth are positively related to PUFA concentrations at the expected date of delivery [188]. Therefore, a high intrauterine PUFA status would particularly benefit a fetus born pre-term because it would result in a higher postnatal PUFA status.

As DHA and AA have been shown to be associated with increased prenatal and neonatal growth, several observations indicate that enhancement of the fetal PUFA status may promote fetal and early neonatal development, thereby improving the "starting condition" and general prognosis of infants born pre-term [184,189,190].

Moreover, the type of fat received in early life (both *in utero* and in early life) determined the level of lipoprotein lipase activity and gene expression which are maintained into later life. It has been demonstrated that fiber type-specific decrease of skeletal muscle LPL activity caused by aging [191] is reversed in animals fed with PUFA. Adult rats fed with a diet high in n-3 PUFA had higher levels of lipoprotein lipase activity and gene expression, and lower plasma triglyceride concentrations following a test meal challenge, than young animals [192]. The DHA content of maternal plasma phospholipids is significantly lower in multiparous than in primiparous women [193]. In this study it appeared that infants born to multiparous women had significantly less DHA in their umbilical tissue phospholipids than did infants born to primiparous women. Whether this lower DHA content has functional consequences for these infants is not known; however, prenatal and early postnatal DHA status is thought to have important consequences on the growth and function of the central nervous system and, consequently, on neurologic and cognitive development. Therefore, incomplete replenishment of maternal DHA stores after delivery may, at least in part, explain the observation that first-born children generally do better than their younger siblings on several developmental, behavioral, and intelligence tests [194–197].

DRUGS MODULATED BY PUFA

Prostanoids produced via the action of COX-2 appear central to many inflammatory conditions. In lipopolysaccharide (LPS)-treated rats however, COX-2 induction alone does not greatly increase prostanoid production *in vivo*. For this, a second AA liberating stimulus is also required. Thus, only after intravenous injection of bradykinin or exogenous AA was a marked increase in prostanoid formation seen. There is, therefore, synergy between proinflammatory mediators; both induction of COX-2 protein and increase in the supply of AA are required to greatly enhance prostanoid production. Second, the supply of AA to increase prostanoid production reduces the effectiveness of both currently used nonsteroidal antiinflammatory drugs (NSAIDs) (diclofenac) and novel COX-2-selective inhibitors (celecoxib) as inhibitors of COX-2 activity. This clearly indicates that: 1) increased prostanoid production in inflammation is a two-component response: increased COX-2 expression and increased AA supply; 2) the supply of AA to COX-2 determines the effectiveness of NSAIDs. NSAIDs and selective COX-2 inhibitors, therefore, will generally be less effective at more inflamed sites, providing a rationale for the very high doses of NSAIDs required in human conditions such as rheumatoid arthritis [198].

GUIDELINES FOR THE ASSESSMENT OF PUFA STATUS

For the assessment of the essential PUFA status of an individual, the total amount of the various EFA and PUFA in plasma or erythrocyte phospholipids is a useful indicator [199]. It should be realized that the plasma content of essential PUFA does not necessarily guarantee the proper use of these fatty acids by cells and tissues. Therefore, additional status markers are required to reliably assess the functional PUFA status of a given individual.

In general, if insufficient essential PUFA are available to meet PUFA requirements, the body starts to synthesize certain fatty acids that are hardly present if the EFA and PUFA status is adequate. Therefore, these fatty acids can be essential PUFA status markers.

The best known marker is Mead acid (C20:3n-9). The synthesis of this fatty acid is promoted if there are insufficient concentrations of LA and LNA to meet the need for the synthesis of long-chain PUFA. EPA and DHA inhibit Mead acid synthesis; the presence of Mead acid indicates a general shortage of all essential PUFA.

Another suitable indicator of essential PUFA status is the essential PUFA status index, which is the ratio between all essential PUFA (the sum of all n-3 and n-6 fatty acids) and all nonessential unsaturated fatty acids (the sum of all n-7 and n-9 fatty acids). The higher the essential PUFA status indexes the better the essential PUFA status.

Finally, if there is a functional shortage of DHA, the body starts to synthesize the most comparable long-chain PUFA of the n-6 family, osbond acid (22:5n-6). Therefore, under steady

state conditions, the ratio between DHA and osbond acid is a reliable indicator of the functional DHA status [200].

CONCLUSIONS

Dietary fatty acids are of significant importance for several of the most common diseases in modern societies. To obtain more specific knowledge about health consequences of dietary PUFA we depend on better understanding of the mechanisms of action of these fatty acids in the body.

One of the most interesting aspects of PUFA biology is related to their interactions with nuclear receptor proteins. These mechanisms of action, behind the well known interactions on eicosanoids metabolism may open a new dietary approach to diseases.

Supported by clinical evidence, many organizations and governments now recognize and provide research to find in PUFA a new therapeutic approach to a wide spectrum of modern illness.

REFERENCES

1. Simopoulos AP: Executive Summary. In Galli C, Simopoulos AP (eds): "Dietary Omega-3 and Omega-6 Fatty Acids: Biological effects and Nutritional Essentiality. Series A: Life Sciences." New York: Plenum-Press, Vol 171, pp 391–402, 1989.
2. Simopoulos AP, Kifer RR, Martin RE (eds): "Health Effects of Polyunsaturated Fatty Acids in Seafoods." Orlando, FL: Academic Press, 1986.
3. Adam O: Linoleic and linolenic acids intake. In Galli C, Simopoulos AP (eds): "Dietary Omega-3 and Omega-6 Fatty Acids: Biological effects and Nutritional Essentiality. Series A: Life Sciences," vol 171. New York: Plenum-Press, pp 391–402, 1989.
4. Phinney SD, Odin RS, Johnson SB, Holman RT: Reduced arachidonate in serum phospholipids and cholesteryl esters associated with vegetarian diet in humans. Am J Clin Nutr 51:385–392, 1990.
5. Raper NR, Cronin FJ, Exler J: Omega-3 fatty acid content of the US food supply. J Am Coll Nutr 11:304–308, 1992.
6. FAO/WHO: Fats and oils in human nutrition report of a joint expert consultation. Food and Agriculture Organization of the United Nations and the World Health Organization. FAO. Food Nutr Pap 57:1–147, 1994.
7. British Nutrition Foundation. Task Force on Unsaturated Fatty Acids. London: Chapman and Hall, 1992.
8. Kris-Etherton P, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD: Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 71:179–188, 2000.
9. Sugano AM, Hirata F: Polyunsaturated fatty acids in food chain in Japan. Am J Clin Nutr 71(Suppl):S189–S196, 2000.
10. Hughes CL, Dhiman TR: Dietary compounds in relation to dietary diversity and human health. J Med Food 5:51–68, 2002.
11. Holman RT: The slow discovery of the importance of omega 3 essential fatty acids in human health. J Nutr 128:427S–433S, 1998.
12. Sprecher H, Luthria DL, Mohammed BS, Baykousheva SP: Re-evaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J Lipid Res 36:2471–2477, 1995.
13. Sprecher H, Chen Q, Yin FQ: Regulation of the biosynthesis of 22:5n-6 and 22:6n-3: a complex intracellular process. Lipids 34:S153–S156, 1999.
14. Infante JP, Huszagh VA: On the molecular etiology of decreased arachidonic (20:4n-6), docosapentaenoic (22:5n-6) and docosahexaenoic (22:6n-3) acids in Zellweger syndrome and other peroxisomal disorders. Mol Cell Biochem 168:101–115, 1997.
15. Infante JP, Huszagh VA: Analysis of the putative role of 24-carbon polyunsaturated fatty acids in the biosynthesis of docosapentaenoic (22:5n6) and docosahexaenoic (22:6n-3) acids. FEBS Lett 431:1–6, 1998.
16. Mohrhauer H, Christiansen K, Gan MV, Deubig M, Holman RT: Chain elongation of linoleic acid and its inhibition by other fatty acids *in vitro.* J Biol Chem 242:4507–4517, 1967.
17. Jump DB, Clarke SD: Regulation of gene expression by dietary fat. Annu Rev Nutr 19:63–90, 1999.
18. Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF: The essentiality of long-chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 40:1–94, 2001.
19. James MJ, Gibson RA and Cleland LG: Dietary polyunsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr 71:343S–348S, 2000.
20. Petersen C, Baumann M, Petersen S: New targets for the modulation of radiation response—selective inhibition of the enzyme cyclooxygenase 2. Curr Med Chem Anti-Canc Agents 3:354–359, 2003.
21. Corey EJ, Shih C, Cashman JR: Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis. Proc Natl Acad Sci USA 80:3581–3584, 1983.
22. Smith WL, Garaviti M, and DeWitt DL: Prostaglandin Endoperoxide H Synthase (Cyclooxygenase)-1 and -2. J Biol Chem 271: 33157–33160, 1996.
23. Porter NA, Caldwell SE, Mills KA: Mechanisms of free radical oxidation of unsaturated lipids. Lipids 30:277–290, 1995.
24. Capdevila JH, Falck JR, Harris RC: Cytochrome P450 and arachidonic acid bioactivation. Molecular and functional properties of the arachidonate monooxygenase. J Lipid Res 41:163–181, 2000.
25. Serhan CN and Oliw E: Unorthodox routes to prostanoid formation: new twists in cyclooxygenase-initiated pathways. J Clin Invest 107:1481–1489, 2001.
26. Waber PC, Fischer S, von Schacky C: Dietary omega-3 polyunsaturated fatty acids and eicosanoid formation in man. In Simopoulos AP, Kifer RR, Martin RE (eds): "Health Effects of Polyunsaturated Fatty Acids in Seafoods." Orlando, FL: Academic Press, pp 49–60, 1986.
27. Lewis RA, Lee TH, Austen KF: Effects of omega-3 fatty acids on the generation of products of the 5-lipooxygenase pathway. In Simopoulos AP, Kifer RR, Martin RE (eds): "Health Effects of Polyunsaturated Fatty Acids in Seafoods." Orlando, FL: Academic Press, pp 227–238, 1986.
28. Jump DB, Clarke SD, Thelen A, Liimatta M, Ren B, Badin M:

Dietary polyunsaturated fatty acid regulation of gene transcription. Prog Lipid Res 35:227–241, 1996.
29. Takada R, Saitoh M, Mori T: Dietary gamma-linolenic acid enriched oil reduces body fat content and induces liver enzyme activities relating to fatty acid beta-oxidation in rats. J Nutr 124:469–474, 1994.
30. Power GW, Newsholme EA: Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. J Nutr 127: 2142–2150, 1997.
31. Miller CC, Ziboh VA: Induction of epidermal hyperproliferation by topical n-3 polyunsaturated fatty acids on guinea pig skin linked to decreased levels of 13-hydroxyoctadecadienoic acid (13-HODE). J Invest Dermatol 94:353–358, 1990.
32. Kidweel WR: Fatty acid growth requirements of normal and neoplastic mammary epithelium. Prog Clin Biol Res 222:699–707, 1986.
33. Lambe KG, Tugwood JD: A human peroxisome-proliferator-activated receptor-gamma is activated by inducers of adipogenesis, including thiazolidinedione drugs. Eur J Biochem 239:83–98, 1996.
34. Jump DB, Clarke SD, MacDougald OA, Thelen A: Polyunsaturated fatty acids inhibit S14 gene transcription in rat liver and cultured hepatocytes. Proc Natl Acad Sci USA 90:8454–8458, 1993.
35. Jump DB, Clarke SD, Thelen AT, Liimatta M: Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. J Lipid Res 35:1076–1084, 1994.
36. Clarke SD, Jump DB: Fatty acid regulation of gene expression; a unique role for polyunsaturated fats. In Berdanier CD, Hargrove JL (eds): "Nutrition and Gene Expression." Boca Raton, FL: CRC Reviews, pp 227–245, 1992.
37. Desvergne B, Wahli, W: Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 20, 649–688, 1999.
38. Mascaro C, Acosta E, Ortiz JA, Marrero PF, Hegardt FG, Haro D: Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. J Biol Chem 273:8560–8563, 1998.
39. Rodriguez JC, Gil-Gomez B, Hegradt FG, Haro D: Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. J Biol Chem 269:18767–18772, 1994.
40. Varanasi U, Chu R, Huang Q, Castellon R, Yeldandi AV, Reddy JK: Identification of a peroxisome proliferator-responsive element upstream of the human peroxisomal fatty acyl CoA oxidase gene. J Biol Chem 271:2147–2155, 1996.
41. Aubert J, Champigny O, Saint-Marc P, Negrel R, Collins S, Ricquier D, Ailhaud G: Upregulation of UCP-2 gene expression by PPAR agonists in preadipose and adipose cells. Biochem Biophys Res Commun 238:606–611, 1997.
42. Ye JM, Doyle PJ, Iglesias MA, Watson DG, Cooney GJ, Kraegen EW: W. Peroxisome proliferator-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats: comparison with PPAR-gamma activation. Diabetes 50:411–417, 2001.
43. Guerre-Millo, M, Gervois P, Raspe E, Madsen E, Poulain P, Derudas B, Gerbert JM, Winegar DA, Willson TM, Fruchart JC, Berge RK, Staels B: Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. J Biol Chem 275:16638–16642, 2000.
44. Storlien L, Hulbert AJ, and Else PL: Polyunsaturated fatty acids, membrane function and metabolic diseases such as diabetes and obesity. Curr. Opin. Clin. Nutr. Metab. Care 1:559–563, 1998.
45. Duplus E, Glorian M, and Forest C: Fatty acid regulation of gene transcription. J Biol Chem 275:30749–30752, 2000.
46. de Urquiza AM, Liu S, Sjoberg M, Zetterstrom RH, Griffiths W, Sjovall J, Perlmann T: Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. Science 290:2140–2144, 2000.
47. Hertz R, Magenheim J, Berman I, Bar-Tana J: Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4alpha. Nature 392:512–516, 1997.
48. Ou J, Tu H, Shan B, Luk A, DeBose-Boyd RA, Bashmakov Y, Goldstein JL, Brown MS: Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. Proc Natl Acad Sci USA 98:6027–6032, 2000.
49. Hannah VC, Ou J, Luong A, Goldstein JL, Brown MS: Unsaturated Fatty Acids Down-regulate SREBP Isoforms 1a and 1c by Two Mechanisms in HEK-293 Cells. J Biol Chem 276:4365–4372, 2001.
50. DeBose-Boyd RA, Ou J, Goldstein JL, Brown MS: Expression of sterol regulatory element-binding protein 1c (SREBP-1c) mRNA in rat hepatoma cells requires endogenous LXR ligands. Pro Natl. Acad Sci USA 98:1477–1482, 2001.
51. Osborne TF: Sterol Regulatory Element-binding Proteins (SREBPs): Key Regulators of Nutritional Homeostasis and Insulin Action. J Biol Chem 275:32379–32382, 2000.
52. Ren B, Thelen AP, Peters JM, Gonzalez FJ, Jump DB: Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor alfa. J Biol Chem 272:26827–26832, 1997.
53. Xu J, Nakamura MT, Cho HP, Clarke S: Sterol Regulatory Element Binding Protein-1 Expression Is Suppressed by Dietary Polyunsaturated Fatty Acids. J Biol Chem 274:23577–23583, 1999.
54. Kim HJ, Takahashi M, Ezaki O: Fish Oil Feeding Decreases Mature Sterol Regulatory Element-binding Protein 1 (SREBP-1) by Down-regulation of SREBP-1c mRNA in Mouse Liver. J Biol Chem 274:25892–25898, 1999.
55. Mater MK, Thelen AP, Pan DA, Jump DB: Sterol Response Element-binding Protein 1c (SREBP1c) Is Involved in the Polyunsaturated Fatty Acid Suppression of Hepatic S14 Gene Transcription. J Biol Chem 274:32725–32732, 1999.
56. Hertz R, Magenheim J, Berman I, Bar-Tana J: Fatty acid-CoA esters are ligands of hepatic nuclear factor-4. Nature 392:512–516, 1998.
57. Liimatta M, Towle HC, Clarke SD, Jump DB: Dietary polyunsaturated fatty acids interfere with the insulin/glucose activation of L-type pyruvate kinase gene transcription. Mol Endocrinology 8:1147–1153, 1999.
58. Van der Klis FR, Schmidt ED, van Beeren HC, Wiersinga WM: Competitive inhibition of T3 binding to alpha 1 and beta 1 thyroid hormone receptors by fatty acids. Biochem Biophys Res Commun. 179:1011–1016, 1991.

59. Juge-Aubry CE, Gorla-Bajszczak A, Pernin A, Lemberger T, Wahli W, Burger AG, Meier CA: Peroxisome proliferator-activated receptor mediates cross-talk with thyroid hormone receptor by competition for retinoid X receptor. Possible role of a leucine zipper-like heptad repeat. J Biol Chem. 270:18117–18122, 1995.
60. Brenner RR: Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog Lipid Res 20:41–47, 1981.
61. Helge JW, Wu BJ, Willer M: Training affects muscle phospholipid fatty acid composition in humans. J Appl Physiol 90:670–677, 2001.
62. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV: The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med 328:238–244, 1993.
63. Storlien LH, Baur LA, Kriketos Ad, Pan DA, Cooney GJ, Jenkins AB, Calvert GD, Campbell LV: Dietary fats and insulin action. Diabetologia 39:621–631, 1996.
64. Vessby B, Tengblad S, and Lithell H: Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. Diabetologia 37:1044–1050, 1994.
65. Eck MG, Wynn JO, Carter WJ, Faas FH: Fatty acid desaturation in experimental diabetes. Diabetes 28:479–485, 1979.
66. Mimouni V, Poisson JP: Altered desaturase activities and fatty acid composition in liver microsomes of spontaneously diabetic Wistar BB rat. Biochim Biophys Acta 1123:296–302, 1992.
67. Bassi A, Avogaro A, Crepaldi C, Zambon S, Marin R, Macdonald I, Manzato E: Short-term diabetic ketosis alters n-6 polyunsaturated fatty acid content in plasma phospholipids. J Clin Endocrinol Metab 81:1650–1653, 1992.
68. Ginsberg BH, Jabour J, and Spector AA: Effects of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. Biochim Biophys Acta 690:157–164, 1982.
69. Grunfeld C, Baird KL, and Kahn CR: Maintenance of 3T3-L1 cells in culture media containing saturated fatty acids decreases insulin binding and insulin action. Biochem Biophys Res Commun 103:219–226, 1981.
70. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW: Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. Diabetes 40:280–289, 1991.
71. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV: The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med 328:238–244, 1993.
72. Clore JN, Li J, Gill R, Gupta S, Spencer R, Azzam A, Zuelzer W, Rizzo WB, Blackard WG: Skeletal muscle phosphatidylcholine fatty acids and insulin sensitivity in normal humans. Am J Physiol Endocrinol Metab 275:E665–E670, 1998.
73. Pan DA, Lillioja S, Milner MR, Kriketos AD, Baur LA, Bogardus C, Storlien LH: Skeletal muscle membrane lipid composition is related to adiposity and insulin action. J Clin Invest 96:2802–2808, 1995.
74. Ayre KJ, Hulbert AJ: Dietary fatty acid profile influences the composition of skeletal muscle phospholipids in rats. J Nutr 126:653–662, 1996.
75. Baur LA, O'Connor J, Pan DA, Kriketos AD, Storlien LH: The fatty acid composition of skeletal muscle membrane phospholipid: its relationship with the type of feeding and plasma glucose levels in young children. Metabolism 47:106–112, 1998.
76. Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A: Desaturation and elongation of fatty acids and insulin action. Annals of the New York Academy of Sciences 967:183–195, 2002.
77. Lillioja S, Young AA, Culter CL, Ivy JL, Abbott WG, Zawadzki JK, Yki-Jarvinen H, Christin L, Secomb TW, Bogardus C: Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. J Clin Invest 80:415–424, 1987.
78. Saltin B, Henriksson J, Nygaard E, Andersen P, Jansson E: Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners. Ann NY Acad Sci 301:3–29, 1977.
79. Kriketos AD, Pan DA, Sutton JR, Hoh JF, Baur LA, Cooney GJ, Jenkins AB, Storlien LH: Relationships between muscle membrane lipids, fiber type, and enzyme activities in sedentary and exercised rats. Am J Physiol Regulatory Integrative Comp Physiol 269:R1154–R1162, 1995.
80. Bordoni A, Hrelia S, Lorenzini A, Bergami R, Cabrini L, Biagi PL, Tolomelli B: Dual influence of aging and vitamin B6 deficiency on delta-6-desaturation of essential fatty acids in rat liver microsomes. Prostaglandins Leukot Essent Fatty Acids 58:417–420, 1998.
81. Despret S, Dinh L, Clement M, Bourre JM: Alteration of delta-6 desaturase by vitamin E in rat brain and liver. Neurosci Lett 145:19–22, 1992.
82. Narce M, Poisson JP, Bellenger J, Bellenger S: Effect of ethanol on polyunsaturated fatty acid biosynthesis in hepatocytes from spontaneously hypertensive rats. Alcohol Clin Exp Res 25:1231–1237, 2001.
83. Maxwell WJ, Keating JJ, Hogan FP, Kennedy NP, Keeling PW: Prostaglandin E2 and leukotriene B4 synthesis by peripheral leucocytes in alcoholics. Gut 30:1270–1274, 1989.
84. Gudbjarnason S: Dynamics of n-3 and n-6 fatty acids in phospholipids of heart muscle. J Int Med 225 (Suppl 1):117–128, 1989.
85. Wahl HG, Kausch C, Machicao F, Rett K, Stumvoll M, Haring HU: Troglitazone Downregulates delta-6 Desaturase Gene Expression in Human Skeletal Muscle Cell Cultures. Diabetes 51: 1060–1065, 2002.
86. Hope A, Fitzpatrick-Wong S: Dietary long-chain polyunsaturated fatty acids minimize dexamethasone-induced reductions in arachidonic acid status but not bone mineral content in piglets. Pediatric Research 51:282–289, 2002.
87. Kris-Etherton P, Daniels SR, Eckel RH, Engler M, Howard BV, Krauss RM, Lichtenstein AH, Sacks F, St Jeor S, Stampfer M, Eckel RH, Grundy SM, Appel LJ, Byers T, Campos H, Cooney G, Denke MA, Howard BV, Kennedy E, Krauss RM, Kris-Etherton P, Lichtenstein AH, Marckmann P, Pearson TA, Riccardi G, Rudel LL, Rudrum M, Sacks F, Stein DT, Tracy RP, Ursin V, Vogel RA, Zock PL, Bazzarre TL, Clark J: Summary of the scientific conference on dietary fatty acids and cardiovascular health. Circulation 103:1034–1039, 2001.

88. Demaison L, Moreau D: Dietary n-3 polyunsaturated fatty acids and coronary heart disease-related mortality: a possible mechanism of action. Cell Mol Life Sci 5:463–477, 2002.
89. Charnock JS: Antiarrhythmic effects of fish oils. In: Simopoulos AP, Kifer RR, Martin RE, Barlow SM (eds): "Health Effects of Omega-3 Polyunsaturated Fatty Acids in Seafoods," vol. 66. Basel: Karger, pp 278–291, 1991.
90. Reibel DK, Holahan MA, Hock CE: Effects of dietary fish oil on cardiac responsiveness to adrenoceptor stimulation. Am J Physiol 254:H494–H499, 1988.
91. Christensen JH, Skou HA, Fog L, Hansen V, Vesterlund T, Dyerberg J, Toft E, Schmidt EB: Marine n-3 fatty acids, wine intake, and heart rate variability in patients referred to coronary angiography. Circulation 103:651–657, 2001.
92. Bigger JT, El-Sherif T: Polyunsaturated fatty acids and cardiovascular events. A fish tale. Circulation 103:623–625, 2001.
93. Kang JX, Leaf A: Prevention of fatal cardiac arrhythmias by polyunsaturated fatty acids. Am J Clin Nutr 71:202–207, 2000.
94. Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett WC, Ma J: Blood levels of long-chain n-3 fatty acids and the risk of sudden death. N Engl J Med 346:1113–1118, 2002.
95. Siscovick DS, Raghunathan TE, King I, Weinmann S, Wicklund KG, Albright J, Bovbjerg V, Arbogast P, Smith H, Kushi LH: Dietary intake and cell membrane levels of long-chain omega-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. JAMA 274:1363–1367, 1995.
96. Albert CM, Hennekens CH, O'Donnell CJ, Ajani UA, Carey VJ, Willett WC, Ruskin JN, Manson JE: Fish consumption and risk of sudden cardiac death. JAMA 279:23–28, 1998.
97. De Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N: Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Heart Study. Circulation 99:779–785, 1999.
98. Singh RB, Rastoqi SS, Verma R, Laxmi B, Singh R, Ghosh S, Niaz MA: Randomised controlled trial of cardioprotective diet in patients with recent acute myocardial infarction: results of one year follow up. Br Med J 304:1015–1019, 1992.
99. Burr ML, Fehily AM, Gilbert JF: Effects of changes in fat, fish and fibre intakes on death and myocardial reinfarction. Diet And Reinfarction Trial (DART). Lancet 2:757–761, 1989.
100. GISSI Investigators: Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione TRIAL: Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 354: 447–455, 1999.
101. Phillipson BE, Rothrock DW, Connor WE, Harris WS, Illingworth DR: Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N Engl J Med 312:1210–1216, 1985.
102. Nestel PJ: Fish oil attenuates the cholesterol-induced rise in lipoprotein cholesterol. Am J Clin Nutr 43:752–757, 1986.
103. Nestel PJ, Connor WE, Reardon MR, Connor S, Wong S, Boston R: Suppression by diets rich in fish oil of very low density lipoprotein production in man. J Clin Invest 74:72–89, 1984.
104. Simopoulos AP: Fatty acids. In Goldberg (ed): "Functional foods". New York, Chapman & Hall, pp 355–391, 1994.
105. Thies F, Garry JM, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC, Grimble RF: Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomized controlled trial. Lancet 361:477–485, 2003.
106. Eritsland J, Arnesen H, Gronseth K, Fjeld NB, Abdelnoor M: Effect of dietary supplementation with n-3 fatty acids on coronary artery bypass graft patency. Am J Cardiol 77:31–36, 1996.
107. Bairati I, Roy L, Meyer F: Double-blind, randomized, controlled trial of fish oil supplements in prevention of recurrence of stenosis after coronary angioplasty. Circulation 85:950–956, 1992.
108. Appel LJ, Miller ER, III, Seidler AJ, Whelton PK: Does supplementation of diet with "fish oil" reduce blood pressure? A meta-analysis of controlled clinical trials. Arch Intern Med 153:1429–1438, 1993.
109. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV: The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med 328:238–244, 1993.
110. Simopoulos AP: Is insulin resistance influenced by dietary linoleic acid and trans fatty acids? Free Radic Biol Med 17:367–372, 1994.
111. Yam D, Eliraz A, Berry EM: Diet and disease: the Israeli paradox: possible dangers of a high omega-6 polyunsaturated fatty acid diet. Isr J Med Sci 32:1134–1143, 1996.
112. Simopoulos AP: Fatty acid composition of skeletal muscle membrane phospholipids, insulin resistance and obesity. Nutr Today 2:12–16, 1994.
113. Baur LA, O'Connor J, Pan DA, Storlien LH: Relationships between maternal risk of insulin resistance and the child's muscle membrane fatty acid composition. Diabetes 48:112–116, 1999.
114. Harris WS: Do omega-3 fatty acids worsen glycemic control in NIDDM? ISSFAL Newsletter 3:6–9, 1996.
115. Connor WE, Prince MJ, Ullmann D, Riddle M, Hatcher L, Smith FE, Wilson D: The hypotriglyceridemic effect of fish oil in adult-onset diabetes without adverse glucose control. Ann N Y Acad Sci 683:337–340, 1993.
116. Rojo-Martinez G, Soriguer FJ, Gonzalez-Romero S, Tinahones F, Moreno F, de Adana SR, Garriga MJ, Esteva I, Garcia-Arnes J, Gomez-Zumaquero JM, Garcia-Almeida JM: Serum leptin and habitual fatty acid dietary intake in patients with type 1 diabetes mellitus. Eur J Endocrinol 142:263–268, 2000.
117. Reseland JE, Haugen F, Hollung K, Solvoll K, Halvorsen B, Brude IR, Nenseter MS, Christiansen EN, Drevon CA: Reduction of leptin gene expression by dietary polyunsaturated fatty acids. J Lipid Res 42:743–750, 2001.
118. Shapiro AC, Wu D, Meydani SN: Eicosanoids derived from arachidonic and eicosapentaenoic acids inhibit T cell proliferative response. Prostaglandins 45:229–240, 1993.
119. Meydani SN, Dinarello CA: Influence of dietary fatty acids on cytokine production and its clinical implications. Nutr Clin Pract 8:65–72, 1993.
120. Wander RC, Hall JA, Gradin JL, Du SH, Jewell DE: The ratio of (n-6) to (n-39 fatty acids influences immune system function, eicosanoid metabolism, lipid peroxidation and vitamin E status in aged dogs. J Nutr 127:1198–1205, 1997.
121. Curtis CL, Hughes CE, Flannery CR, Little CB, Harwood JL, Caterson B: n-3 fatty acids specifically modulate catabolic factors involved in articular cartilage degradation. J Biol Chem 275:721–724, 2000.

122. Mayser P, Grimm H, Grimminger F: n-3 fatty acids in psoriasis. Br J Nutr 87(Suppl 1):S77–S82, 2002.
123. Stenson WF, Cort D, Rodgers, Burakoff R, DeSchryver-Kecskemeti K, Gramlich TL, Beeken W: Dietary supplementation with fish oil in ulcerative colitis. Ann Interr Med 116:609–614, 1992.
124. Rose DP: Effects of dietary fatty acids on breast and prostate cancer: evidence from in vitro experiments and animal studies. Am J Clin Nutr 66:1513S–1522S, 1997.
125. Hilakivi-Clarke L, Stoica A, Raygada M, Martin MB: Consumption of a high-fat diet alters estrogen receptor content, protein kinase C activity, and mammary gland morphology in virgin and pregnant mice and female offspring. Cancer Res 58:654–660, 1998.
126. Fay MP, Freedman LS, Clifford CK, Midthune DN: Effect of different types and amounts of fat on the development of mammary tumors in rodents: a review. Cancer Res 57:3979–3988, 1997.
127. Hilakivi-Clarke L, Onojafe I, Raygada M, Cho E, Clarke R, Lippman ME: Breast cancer risk in rats fed a diet high in n-6 polyunsaturated fatty acids during pregnancy. J Natl Cancer Inst 88:1821–1827, 1996.
128. Hilakivi-Clarke L, Clarke R, Onojafe I, Raygada M, Cho E, Lippman M: A maternal diet high in n-6 polyunsaturated fats alters mammary gland development, puberty onset, and breast cancer risk among female rat offspring. Proc Natl Acad Sci USA 94:9372–9377, 1997.
129. Caygill CPJ, Hill MJ: Fish, n-3 fatty acids and human colorectal and breast cancer. Eur J Cancer Prev 4:329–332, 1995.
130. Caygill CPJ, Charlett A, Hill MJ: Fat, fish, fish oil and cancer. Br J Cancer 74:159–164, 1997.
131. Schloss I, Kidd MSG, Tichelaar HY, Young GO, O'Keefe SJ: Dietary factors associated with a low risk of colon cancer in coloured West Coast fishermen. S Afr Med J 87:152–158, 1997.
132. Gaard M, Tretli S, Loken EB: Dietary factors and risk of colon cancer: a prospective study of 50,535 young Norwegian men and women. Eur J Cancer Prev 5:445–454, 1996.
133. Jiang WG, Bryce RP, Horrobin DF: Essential fatty acids: molecular and cellular basis of their anti-cancer action and clinical implications. Crit Rev Oncol Hematol 27:179–209, 1998.
134. Lecker SH, Solomon V, Mitch WE, Goldberg AL: Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. J Nutr 129(1S Suppl):227S–237S, 1999.
135. Guttridge DC, Mayo MW, Madrid LV, Wang CY, Baldwin AS Jr: NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. Science 289(5488):2363–2366, 2000.
136. Tisdale MJ: Biomedicine. Protein loss in cancer cachexia. Science 289(5488):2293–2294, 2000.
137. Smith HJ, Lorite MJ, Tisdale MJ: Effect of a cancer cachectic factor on protein synthesis/degradation in murine C2C12 myoblasts: modulation by eicosapentaenoic acid. Cancer Res 59: 5507–5513, 1999.
138. Kawamura I, Morishita R, Tomita N, Lacey E, Aketa M, Tsujimoto S, Manda T, Tomoi M, Kida I, Higaki J, Kaneda Y, Shimomura K, Ogihara T: Intratumoral injection of oligonucleotides to the NF kappa B binding site inhibits cachexia in a mouse tumor model. Gene Ther 6:91–97, 1999.
139. Whitehouse AS, Smith HJ, Drake JL, Tisdale MJ: Mechanism of attenuation of skeletal muscle protein catabolism in cancer cachexia by eicosapentaenoic acid. Cancer Res 61:3604–3609, 2001.
140. Barber MD, Ross JA, Voss AC, Tisdale MJ, Fearon KC: The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. Br J Cancer 81: 80–86, 1999.
141. Kern S, Hruban R, Hollingsworth MA, Brand R, Adrian TE, Jaffee E, Tempero MA: The product of a pancreas cancer think tank. Cancer Research 61:4923–4932, 2001.
142. Wigmore SJ, Ross J, Falconer JS, Plester CE, Tisdale MJ, Carter DC, Fearon KC: The effect of polyunsaturated fatty acids on the progress of cachexia in patients with pancreatic cancer. Nutrition 12:S27–S30, 1996.
143. Wigmore SJ, Fearon KC, Maingay JP, Ross JA: Down-regulation of the acute-phase response in patients with pancreatic cancer cachexia receiving oral eicosapentaenoic acid is mediated via suppression of interleukin-6. Clin Sci (Lond) 92:215–221, 1997.
144. Hussey HJ, Tisdale MJ: Effect of a cachectic factor on carbohydrate metabolism and attenuation by eicosapentaenoic acid. Br J Cancer 80:1231–1235, 1999.
145. Tisdale MJ: Inhibition of lipolysis and muscle protein degradation by EPA in cancer cachexia. Nutrition 12:S31–S33, 1996.
146. Yehuda S, Rabinovitz S, Mostofsky DI: Essential fatty acids are mediators of brain biochemistry and cognitive functions. J Neurosci Res 56:565–570, 1999.
147. Sun GY, Sun AY: Synaptosomal plasma membranes: acyl group composition of phosphoglycerides and (Na+ plus K+)-ATPase activity during fatty acid deficiency. J Neurochem 22:15–18, 1974.
148. Martin RE, Bazan NG: Changing fatty acid content of growth cone lipids prior to synaptogenesis. J Neurochem 59:318–325, 1992.
149. Delion S, Chalon S, Herault J: Chronic dietary gamma-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. J Nutr 124:2466–2476, 1994.
150. Neuringer A, Anderson GJ, Connor WE: The essentiality of n-3 fatty acid for the development and function of the retina and brain. Ann Rev Nutr 8:517–541, 1988.
151. L'Hirondel M, Cheramy A, Godeheu G, Glowinski J: Effects of arachidonic acid (AA) on dopamine synthesis, spontaneous release, and uptake in striatal synaptosomes from the rat. J Neurochem 64:1406–1409, 1995.
152. Matsuo T, Sumida H, Suzuki M: Beef tallow diet decreases norepinephrine turnover rates in rat hypotalamus and cerebral cortex. Metabolism 44:1377–1379, 1995.
153. Methot N, Demers CN, Baezinger JE: Structure of both the ligand-and-lipid-dependent channel-inactive states of the nicotinic acetylcholine receptor probed by FTIR spectroscopy and hydrogen exchange. Biochemistry 34:15142–15149, 1995.
154. Yamamoto N, Saitoh M, Moriuchi A, Nomura M, Okuyama H: Effect of dietary alpha linolenate/linoleate balance on brain lipid composition and learning ability in rats. J Lipid Res 28:144–151, 1987.
155. Neuringer M, Reisbeck S, Janowsky J: The role of n-3 fatty acids in visual and cognitive development: Current evidence and methods of assessment. J Pediatr 125:S39–S47, 1994.

156. Watkins BA: Regulatory effects of polyunsaturates on bone modeling and cartilage function. World Rev Nutr Dietetics 83:38–51, 1998.
157. Watkins BA, Seifert MF, Allen KG: Importance of dietary fat in modulating PGE2 responses and influence of vitamin E on bone morphometry. World Rev Nutr Dietetics 82:250–259, 1997.
158. Watkins BA, Seifert MF: Conjugated linoleic acid and bone biology. J Am Coll Nutr 19:478S–486S, 2000.
159. Rawlinson SC, Mohan S, Baylink DJ, Lanyon LE: Exogenous prostacyclin, but not prostaglandin E2, produces similar responses in both G6PD activity and RNA production as mechanical loading, and increases IGF-II release, in adult cancellous bone in culture. Calcif Tissue Int 53:324–329, 1993.
160. Collins DA, Chambers TJ: Prostaglandin E2 promotes osteoclast formation in murine hematopoietic cultures through an action on hematopoietic cells. J Bone Miner Res 7:555–561, 1992.
161. Tashjian AH, Voelkel EF, Lazzaro M, Goad D, Bosma T, Levine L: Tumor necrosis factor-alpha (cachectin) stimulates bone resorption in mouse calvaria via a prostaglandin-mediated mechanism. Endocrinology 120:2029–2036, 1987.
162. Hurley MM, Lee SK, Raisz LG, Bernecker P, Lorenzo J: Basic fibroblast growth factor induces osteoclast formation in murine bone marrow cultures. Bone 22:309–316, 1998.
163. Plotquin D, Dekel S, Katz S, Danon A: Prostaglandin release by normal and osteomyelitic human bones. Prostaglandins Leukot Essent Fatty Acids 43:13–15, 1991.
164. Broadhurst CL, Winther M: Evening primrose oil: pharmacological and clinical applications. In Mazza JG, Ooma BD (eds): "Functional Foods: Herbs, Botanicals and Teas." Lancaster, PA: Technomic Publishing, pp 213–264, 2000.
165. Watkins B, Li Y, Allen KG, Hoffmann WE, Seifert MF: Dietary ratio of (n-6)/(n-3) polyunsaturated fatty acids alters the fatty acid composition of bone compartments and biomarkers of bone formation in rats. J Nutr 130:2274–2284, 2000.
166. Watkins BA, Shen CL, Allen K, Seifert MF: Dietary (n-3) and (n-6) polyunsaturates and acetylsalicylic acid alter *ex vivo* PGE2 biosynthesis, tissue IGF-I levels, and bone morphometry in chicks. J Bone Miner Res 11:1321–1332, 1996.
167. Xu H, Watkins BA, Adkisson HD: Dietary lipids modify the fatty acid composition of cartilage, isolated chondrocytes and matrix vesicles. Lipids 29:619–625, 1994.
168. Atkinson TG, Barker HJ, Meckling-Gill KA: Incorporation of long-chain n-3 fatty acids in tissues and enhanced bone marrow cellularity with docosahexaenoic acid feeding in post-weanling Fischer 344 rats. Lipids 32:293–302, 1997.
169. Kokkinos PP, Shaye R, Alam BS, Alam SQ: Dietary lipids, prostaglandin E2 levels, and tooth movement in alveolar bone of rats. Calcif Tissue Int 53:333–337, 1993.
170. Alam SQ, Kokkinos PP, Alam BS: Fatty acid composition and arachidonic acid concentrations in alveolar bone of rats fed diets with different lipids. Calcif Tissue Int 53:330–332, 1993.
171. Sakaguchi K, Morita I, Murota S: Eicosapentanoic acid inhibits bone loss due to ovariectomy in rats. Prostaglandins Leukot Essent Fatty Acids 50:81–84, 1994.
172. Claassen N, Coetzer H, Steinmann CM, Kruger MC: The effect of different n-6/n-3 essential fatty acid ratios on calcium balance and bone in rats. Prostaglandins Leukot Essent Fatty Acids 53:13–19, 1995.
173. Schlemmer CK, Coetzer H, Claassen N, Kruger MC: Oestrogen and essential fatty acid supplementation corrects bone loss due to ovariectomy in the female Sprague Dawley rat. Prostaglandins Leukot Essent Fatty Acids 61:381–390, 1999.
174. Kruger MC, Coetzer H, de Winter R, Gericke G, van Papendorp DH: Calcium, gamma-linolenic acid and eicosapentaenoic acid supplementation in senile osteoporosis. Aging (Milano) 10:385–394, 1998.
175. Terano T: Effect of omega 3 polyunsaturated fatty acid ingestion on bone metabolism and osteoporosis. World Rev Nutr Diet 88:141–147, 2001.
176. Michelson D, Stratakis C, Hill L, Reynolds J, Galliven E, Chrousos G, Gold P: Bone mineral density in women with depression. New Engl J Med 335:1176–1181, 1996.
177. Horrobin DF, Bennet CN: Depression and bipolar disorder: relationships to impaired fatty acid and phospholipid metabolism and to diabetes, cardiovascular disease, immunological abnormalities, cancer, ageing and osteoporosis. Prostaglandins Leukot Essent Fatty Acids 60:217–234, 1999.
178. Broadhurst CL, Cunnane SC, Crawford MA: Rift Valley lake fish and shellfish provided brain-specific nutrition for early Homo. Br J Nutr 79:3–21, 1998.
179. Innis SM: Essential fatty acids in growth and development. Prog Lipid Res 30:39–103, 1991.
180. Uauy R, Peirano P, Hoffman D, Mena P, Birch D, Birch E: Role of essential fatty acids in the function of the developing nervous system. Lipids 31:S167–S176, 1996.
181. Stevens LJ, Zentall SS, Deck JL, Abate ML, Watkins BA, Lipp SR, Burgess JR: Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. Am J Clin Nutr 62:761–768, 1995.
182. Cheruku SR, Montgomery-Downs HE, Farkas SL, Thoman EB, Lammi-Keefe CJ: Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning. Am J Clin Nutr 76:608–613, 2002.
183. Barker DJP: Mothers, babies and disease in later life. London: BMJ Books, 1994.
184. Foreman-van Drongelen MM, Al MD, van Houwelingen AC, Blanco CE, Hornstra G: Comparison between the essential fatty acid status of preterm and full-term infants, measured in umbilical vessels. Early Hum Dev 42:241–251, 1995.
185. Foreman-van Drongelen MMHP, van Houwelingen AC, Kester AD, de Jong AE, Blanco CE, Hasasrt TH, Hornstra G: Long-chain polyene status of preterm infants with regard to the fatty acid composition of their diet: comparison between absolute and relative fatty acid levels in plasma and erythrocyte phospholipids. Br J Nutr 73:405–422, 1995.
186. Foreman-van Drongelen MM, Houwelingen AC, Kester AD, de Jong AE, Blanco CE, Hasaart TH, Hornstra G: Influence of feeding artificial-formula milks containing docosahexaenoic and arachidonic acids on the postnatal long-chain polyunsaturated fatty acid status of healthy preterm infants. Br J Nutr 76:649–667, 1996.
187. van Houwelingen AC, Foreman-van Drongelen MMHP, Nicolini U, Nicolaides KH, Al MD, Kester AD, Hornstra G: Essential fatty acid status of fetal phospholipids: similar to postnatal values obtained at comparable gestational ages. Early Hum Dev 46:141–152, 1996.

188. Foreman-van Drongelen MMHP, van Houwelingen AC, Kester ADM, Hasaart TH, Blanco CE, Hornstra G: Long-chain polyunsaturated fatty acids in preterm infants: status at birth and its influence on postnatal life. J Pediatr 126:611–618, 1995.
189. Koletzko B, Braun M: Arachidonic acid and early human growth: is there a relation? Ann Nutr Metab 35:128–131, 1991.
190. Carlson SE, Werkman SH, Peeples JM, Cooke RJ, Tolley EA: Arachidonic acid status correlates with first year growth in preterm infants. Proc Natl Acad Sci USA 90:1073–1077, 1993.
191. Hamilton MT, Areiqat E, Hamilton DG, Bey L: Plasma triglyceride metabolism in humans and rats during aging and physical inactivity. Int J Sport Nutr Exerc Metab S97–S104, 2001.
192. Chapman C, Morgan LM, Murphy MC: Maternal and early dietary fatty acid intake: changes in lipid metabolism and liver enzymes in adult rats. J Nutr 130:146–151, 2000.
193. Al MDM, van Houwelingen AC, Hornstra G: Relation between birth order and the maternal and neonatal docosahexaenoic acid status. Eur J Clin Nutr 51:548–553, 1997.
194. Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C: Breast milk and subsequent intelligence quotient in children born preterm. Lancet 339:261–264, 1992.
195. Rodgers B: Feeding in infancy and later ability and attainment: a longitudinal study. Dev Med Child Neurol 20:421–442, 1978.
196. Gale CR, Martyn CN: Breast feeding, dummy use and intelligence. Lancet 347:1072–1075, 1996.
197. Emken EA, Adlof RO, Gulley RM: Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochim Biophys Acta 1213:277–288, 1994.
198. Hamilton LC, Mitchell JA, Tomlinson AM, Warner TD: Synergy between cyclo-oxygenase-2 induction and arachidonic acid supply *in vivo*: consequences for nonsteroidal antiinflammatory drug efficacy. FASEB 13:245–251, 1999.
199. Hornstra G: Essential fatty acids, pregnancy, and pregnancy complications: a roundtable discussion. In Sinclair A, Gibson R (eds): "Essential Fatty Acids and Eicosanoids." Champaign: American Oil Chemists' Society, pp 177–182, 1992.
200. Neuringer M, Connor WE, Lin DS, Barstad L, Luck S: Biochemical and functional effects of prenatal and postnatal omega-6 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci USA 83:4021–4025, 1986.

Received August 6, 2003; revision accepted March 1, 2004.