Review

Nutritional role of polyunsaturated fatty acids

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In mammalian tissues there are four families of polyunsaturated fatty acids derived from the parent fatty acids: palmitoleic and oleic acids, which can be synthesized endogenously, and linoleic and linolenic acids, which must be obtained from the diet and are known as essential fatty acids. These four precursors are desaturated and chain elongated to form the long chain highly unsaturated fatty acids. The principal products of linoleic acid are arachidonic, with four double bonds (tetraene), and dihomogamma linolenic acids; those of linolenic acid are eicosapentaenoic and docosahexaenoic acids. These polyunsaturated acids derived from essential fatty acids when incorporated into membrane phospholipids can alter membrane fluidity, which determines the permeability of membranes and the behavior of membrane-bound enzymes and receptors. The dihomogammalinolenic, arachidonic, and eicosapentaenoic acids are also the precursors of eicosanoids, which influence many cellular processes. When the dietary amounts of linoleic and linolenic acids are inadequate, palmitoleate and oleate are desaturated and chain elongated to give rise to eicosatrienoic acids (triene). An elevated tissue triene/tetraene ratio is, therefore, used as a marker for essential fatty acid deficiency. The essential fatty acid deficiency symptoms include reduced growth rate, scaly dermatitis, impaired reproduction, and susceptibility to infection. The intake of 1 to 2% of the daily calories as linoleate and 0.2 to 0.5% as linolenate is widely acknowledged as the approximate amounts to meet the needs of essential fatty acids in humans

Keywords: essential fatty acids; functions; deficiency; requirements

Introduction

The first indication that fat may be essential for healthy growing animals was presented by Aron in 1918.[1] He proposed that butter, in addition to its contribution to calories, had a specific nutritive value possibly related to its content of certain lipid component(s). Evans and Burr[2] then showed that a deficiency of fat severely affected both growth and reproduction of experimental animals even though the then-known fat soluble vitamins A, D, and E were added in the diet. The active principle in fat was considered to be a new vitamin and was tentatively coined vitamin F. The nutritional importance of specific lipid molecule(s) in fat was first revealed through the pioneering work of Burr and Burr[3] in 1929. They fed weanling rats a fat-free diet and observed retarded growth, scaly skin, tail necrosis and even death. This disorder was reversed by feeding linoleic acid. A subsequent paper by the same authors[4] described impaired fertility, increased water consumption, and diminished urine production as further symptoms of deficiency in rats maintained on fat-free diet. They found that either linoleic acid or linolenic acid could provide the missing factor, and when either was included in the diet normal growth resumed. They coined the term "essential fatty acids" (EFA) for these components that were not synthesized in the animal organism and had to be supplemented in the diet for normal physiologic function.

In 1938 it was reported[5] that arachidonic acid was roughly about three times as effective as linoleic acid in relieving symptoms of deficiency and this fatty acid was added to the list of EFA. Linoleic acid was found to undergo transformation in the animal organism to give arachidonic acid[6] and was considered to be the principal unsaturated fatty acid required by animals. Burr's autobiographical account of the discovery of the EFA was published recently.[7]

Other investigators were able to produce EFA deficiency in a wide range of species from insects to birds and mammals feeding a diet lacking in these fatty acids, but it was difficult to produce deficiency symptoms in humans. The first experiment in a human was done by biochemist W.R. Brown, who himself went for 6 months on a diet extremely low in fat.[8] He was clinically well throughout the entire period, not having

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even a common cold. He did not develop scaly skin or other visible abnormalities seen in several species of animals maintained on EFA deficient diets and, therefore, the essentiality of these fatty acids, at least in adult humans, was in question. But any doubt that EFA were required in the human was dispelled when Hansen et al.[9] described severe skin symptoms in infants who were fed a milk-based formula diet in which EFA were virtually absent. Addition of EFA corrected the skin symptoms. This work proved conclusively that linoleic acid was required for proper growth and health of infants.[10] With the advent of parenteral nutrition based on a system of continuous infusion of fat-free solution containing glucose, EFA deficiency also occurred in human adults. This was characterized by a skin rash and changes in plasma levels of polyunsaturated fatty acids (PUFA). These changes were corrected by infusing an intravenous emulsion containing linoleate.[11,12]

Biosynthesis of polyunsaturated fatty acids

Mammalian tissues contain four families of PUFA: ω_9, ω_7, ω_6, and ω_3. The precursors of the first two are the monounsaturated fatty acids that can be synthesized endogenously from the saturated fatty acids. Monounsaturation in the Δ_9 position is the rule and the animal enzyme systems are incapable of inserting double bond between carbon atom 1 and 6 starting from the methyl (ω) carbon of the saturated fatty acid.[13] Thus there are only two saturated fatty acids available for desaturation: palmitic and stearic acids. An enzyme Δ_9 desaturase in the liver microsomes catalyzes the conversion of palmitate to palmitoleate ($C_{16:1},\omega_7$) and of stearate to oleate ($C_{18:1},\omega_9$). Monounsaturated fatty acids with double bonds occurring before the Δ_9 position are not synthesized to significant extent by animals because the required desaturase is absent and the trace amounts found in animal tissue lipids probably arise from the diet. The other two precursors are necessarily derived from dietary linoleic ($C_{18:2},\omega_6$) and linolenic ($C_{18:3},\omega_3$) acids. These four precursors are alternately desaturated (in which two hydrogen atoms are removed to create a new double bond) and elongated by the addition of two carbon atoms.[14] The desaturations are catalyzed by Δ_6, Δ_5, and probably Δ_4 desaturases[15] to form the principal PUFA found in animal tissues.

It is believed that the same enzymes catalyze the equivalent steps in ω_7, ω_9, ω_6, and ω_3 fatty acids pathways[16] and there is competition among substrates for the same enzyme system (*Figure 1*). The critical enzyme in these reactions is Δ_6 desaturase for which the greatest affinity appears to be conferred by the greatest number of double bonds in C_{18} substrate (provided the substrate concentrations are equal). Thus linolenic acid with three double bonds is desaturated at the highest rate followed by linoleic acid and oleic acid.[17] In the presence of either of the two dietary fatty acids little desaturation of oleate occurs. Linolenate effectively inhibits the desaturation of linoleate (at equal concentrations). In the absence of the members of the ω_6 families, however, oleate is desaturated and the members of the ω_9 family, particularly $C_{20:3},\omega_9$ or the "Mead acid"[18] appear in the tissues. This explains the well-documented finding that in EFA deficiency a trienoic acid (largely Mead acid) increases dramatically in the tissues and with feeding a diet containing EFA, it decreases.[19]

In the desaturation process additional double bonds are inserted between the pre-existing double bonds and the carboxyl group and the chain elongation always proceeds by the addition of two carbon units to the carboxyl terminus of the fatty acyl chain.[20] Therefore, the position of the double bond counting from the methyl (ω) end of the precursor fatty acid remains unaltered through all transformations. All transformation products of oleic acid possess the ω_9 configuration of oleate itself and are recognized as members of the ω_9 family. Nervonic acid ($C_{24:1},\omega_9$), a component of nerve tissue lipid that is derived by chain elongation of oleate and vaccenic acid ($C_{18:1},\omega_7$) that occurs in small amounts in animal lipids, is formed by chain elongation of pamitoleic acid.[21] Similarly linoleate and linolenate give rise respectively to ω_6 and ω_3 families of PUFA.[22] No interconversion between these families can occur.

In addition to the desaturases there is competition of the substrates for the chain elongation enzymes and for the acetyl transferases involved in the formation of phospholipids (which require PUFA). Lower members of a family may also be able to compete with some of its products for enzyme sites and limit the extension of its family. Long-chain highly unsaturated fatty acids can be shortened by two carbons, a phenomenon called retroconversion.[23] Because of the competition, retroconversion, etc., each family has characteristic end products that accumulate in tissue lipids while the intermediates are usually found in much smaller, often trace amounts. Thus for oleate, the major PUFA is $C_{20:3}\omega_9$, for palmitoleate $C_{20:3}$, ω_7, for linoleate arachidonic acid and some dihomogamma linolenic acid (DHGL), and for linolenate $C_{20:5}$ plus $C_{22:6}$, both ω_3. The carbon 22 hexaenoic acid is the most unsaturated fatty acid commonly found in the lipids of higher animals.

The activity of Δ_6 desaturase is the major controlling factor in the biosynthesis of PUFA and hence can affect the overall production of the principal PUFA. Available data from experimental animals suggest that the enzyme is sensitive to several factors.[13,24] Those which tend to increase the enzyme activity include high-protein diet, insulin, and EFA deficiency, while fasting, glucagon, glucocorticoids, and diabetes are known to decrease its activity.[25-27] Animal experiments suggest that Δ_6 desaturase activity decreases with age,[28] younger animals having higher activity perhaps because of the requirement of long-chain PUFA due to building of new tissues for growth.

It has been suggested that the enzyme Δ_6 desaturase is regulated by zinc. Some of the effects of zinc deficiency and essential fatty acid deficiency (particularly

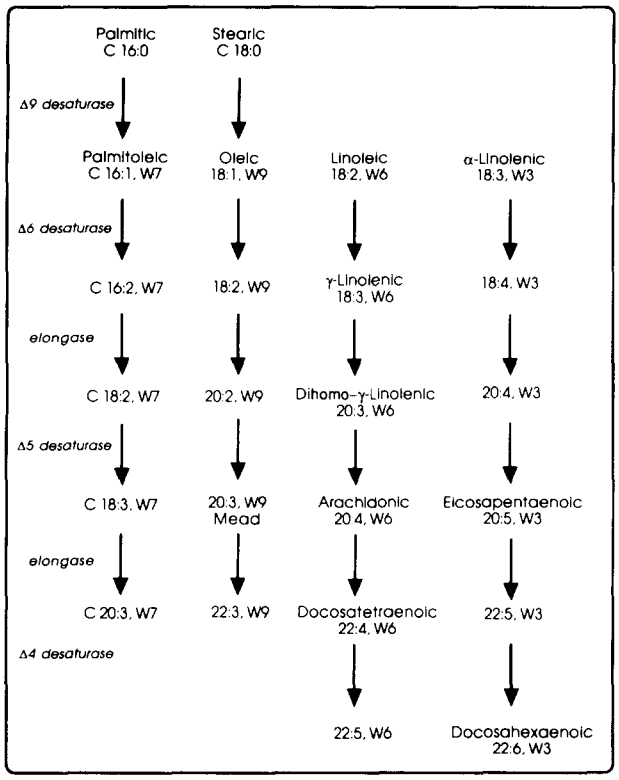

Figure 1 Biosynthesis of polyunsaturated fatty acids.

growth and dermal) are similar.[29] This is also supported by observations that most biologic effects of zinc deficiency are corrected by gamma-linolenic acid (which bypasses Δ_6 desaturase) but not by linoleic acid.[30] Reduction in the activity of this enzyme can limit the availability of DHGL and arachidonic acid required for normal physiologic functions. We acquire a good deal of arachidonic acid from meat, dairy products, etc., but little or no DHGL is found in our diet. There are also species differences in the rate of Δ_6 desaturase activity. Rat is perhaps the most efficient in the conversion of linoleic acid to DHGL. On the other hand, strict carnivores, such as the lion and the cat, lack Δ_6 desaturase.[31,32] These animals, therefore, require a source of preformed DHGL and perhaps arachidonic acid. In practice these species exhibit a specific requirement for PUFA of animal origin.

Functions

Fatty acids in general are utilized in the body as the principal sources of energy and hence a proportion of EFA (linoleic and linolenic) also contributes to provide energy. The PUFA are oxidized more rapidly than saturated or monounsaturated fatty acids.[33] After ingestion, linoleic and linolenic acids have been shown to be distributed between adipose triglycerides, other tissue stores, and tissue structural lipids. By contrast the long-chain PUFA derived from EFA, eg, DHGL, arachidonic acid, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids are spared from oxidation.[34] These acids, if present in the diet as preformed, are incorporated into structural lipids about 20 times more efficiently than they are incorporated after synthesis from dietary linoleic and linolenic acids.[35,36] The PUFA are incorporated into phospholipids on the glycerol moiety almost exclusively in the 2-acyl position. Arachidonic acid is by far the most important long-chain PUFA in tissue lipids. DHGL is present in concentrations much lower than arachidonic acid.

Recently it has been reported[37] that normal young cartilages of several species studied (young chicken, fetal calf, newborn pig, rabbit, and human) have unusually high levels of Mead acid and low levels of PUFA of ω_6 family, a characteristic of EFA deficiency. But the levels of ω_6 fatty acids in their blood and other tissues are normal. Mead acid is found to be particularly abundant in phosphatidyl ethanolamine, phosphatidyl inositol, and free fatty acid fractions from the young cartilage. ω_9 fatty acid probably plays some functional role such as inhibition of response known to be involved in cartilage destruction. The high levels of Mead acid are found to progressively decrease with increasing age and are accompanied by a steady increase in ω_6 fatty acids. This trend is particularly pronounced in oesteoarthritic cartilage.[38]

The biologic functions of EFA include stimulation of growth, maintenance of skin and hair growth, regulation of cholesterol metabolism, lipotropic activity, maintenance of reproductive performance, and other physiologic and pharmacologic effects. On a molecular level these fatty acids help maintain membrane integrity and optimum level of unsaturation in tissue lipid and are components of specific lipids.[39,40]

Membrane integrity

The first function to be widely attributed to EFA was as an essential component of the phospholipids that serve as structural units of biomembranes. PUFA are the major components of structural lipids of membranes of cells, mitochondria, and nuclei and they play a major and vital role in the properties of most membranes. The physical properties (such as the fluidity) of phospholipids are in large part determined by the chain length and the degree of unsaturation of their component fatty acids. The physical properties, in turn, affect the phospholipid's ability to perform structural function such as the maintenance of normal activities of membrane bound enzymes such as adenyl cyclase,[41] 5-nucleotidase, and Na^+/K^+ ATPase.[42] The dietary fatty acid may modify the insertion, aggregation, and diffusional movements of membrane components, the activity and affinity of receptors, membrane permeability,[43] and transport properties.[44] Several cellular functions such as secretion, chemotaxis, signal transmission, and susceptibility to microorganism invasion depend on membrane fluidity.[45,46]

The regulatory function of PUFA is also suggested in part by the heterogeneity and selectivity in their tissue membrane distribution. The fatty acid profiles of complex lipids vary with the type of lipid, position within a phospholipid, body organ region, and cell

type.[42] The highly unsaturated fatty acids are particularly concentrated when there is a requirement for rapid movement at a cellular level such as may be required in transport mechanism in the brain, its synaptic junction, and the retina where only the long-chain derivatives of EFA are found.[47,48]

Skin

The most abundant PUFA in human skin are linoleic acid and arachidonic acid.[49,50] There is substantial evidence that at least one essential function of linoleic acid is in the skin to maintain the integrity of epidermal water barrier.[51] The physical structure of the epidermal water barrier was ascribed to sheets of stacked lipid bilayers that fill the intercellular spaces of the uppermost layer of the epidermis.[52] These lipid bilayers contain large amounts of sphingolipids of which the linoleate-rich species have been characterized as acylceramide, acylglucosyl ceramide, and a unique acyl acid. For linoleate of the sphingolipid to attain barrier function it must first be metabolized by lipoxygenase type reaction.[53] In EFA deficiency the linoleic acid in ceramides is replaced by oleic acid and results in severe water loss from the skin.[54]

Immunity and infection

Increased susceptibility to infection is a well-documented consequence of EFA deficiency in animals[55] and infection is a common clinical problem for patients undergoing fat-free hyperalimentation.[56] Certain PUFA are known to be effective in killing those viruses that have a lipid component in the envelope.[57,58] But the mechanism by which EFA deficiency causes infection is not clear.

Mice on a diet deficient in EFA showed significant reduction in immune responses.[59] EFA deficiency also diminished responses to T cell-dependent and T cell-independent antigens in mice.[60] Full restoration of these responses occurred upon switching to a control diet. However, others[61-63] have reported that mice fed an EFA deficiency diet showed potentiation of cell-mediated immunity indicated by accelerated rejection of skin allografts. Several investigators found that the effect of PUFA depended on the concentration, ie, low concentration of PUFA were stimulatory and high concentration inhibitory.

There are two possible ways by which EFA may affect immune function: 1) via membrane structural changes, and/or 2) via chemical mediators such as eicosanoids. PUFA may cause change in the membrane fatty acid composition of lymphoid cells. This may cause a change in membrane fluidity leading to alteration in the activity of enzymes, receptor expression, and intercellular signaling, which in turn can influence lymphocyte responsiveness.[64] Evidence is accumulating that eicosanoids, especially prostaglandins E type, are operating at several levels of immune response as intercellular mediators[65,66] inhibiting the action of suppressor T cells and leading to increased antibody production, at least in vitro. However, the effect of PUFA on the immune response is controversial and the mechanism through which PUFA may influence immune system is still not clearly understood.

Dietary arachidonic acid is preferentially incorporated into the phospholipids of activated lymphocytes[67] and arachidonic acid constitutes over 20% of total fatty acid content in phospholipids of macrophages.[61,68] Immune competent cells from animals fed EFA-deficient diet or a diet with increased ω_3 fatty acids relative to arachidonic acid synthesize fewer prostaglandins.[69,70] In EFA deficiency there is a lack of eicosanoid precursors. Feeding high ω_3 fatty acids results in substitution of arachidonic acid by EPA. The PGE_3 formed from EPA has less of an inflammatory effect than PGE_2 derived from arachidonic acid. Likewise the leukotriene derived from EPA (eg, B_5) is approximately 30 times less potent than that formed from arachidonic acid (eg, B_4) in aggregation of neutrophils and release of lysosomal enzymes.[71] Diet rich in ω_3 fatty acids may produce change in the production of eicosanoids in a way more favorable with regard to tendency toward severity of inflammatory reactions.[72,73]

Eicosanoids

DHGL, arachidonic acid, and EPA are the precursors of eicosanoids[74] that are formed by the action of membrane bound cyclooxygenase or specific lipoxygenase enzyme systems.[48,75] The eicosanoid family includes prostaglandins, thromboxanes, prostacyclins leukotrienes, lipoxins, and other hydroxy fatty acids.[76-79] These compounds participate in many physiologic and pathologic processes and are potent regulators of cell function. They act locally in the tissues in which they are formed and are rapidly converted to their inactive forms.[80]

Specific role for ω_3 fatty acids

Of the two parent PUFA, linoleic and linolenic acids, linoleic acid has long been known to be essential for animals and humans. It is generally considered that linoleic acid satisfies most of the EFA requirements of mammals and any EFA activity expressed by linolenic acid is also expressed by linoleic acid, which is more potent. While both linoleic and linolenic acids support growth, development, and reproduction, dermal integrity requires only linoleic acid. A specific requirement for ω_3 fatty acids has only been shown in fish.[81] Because of the inability of linolenic acid to normalize all physiologic functions during EFA deficiency, several investigators have designated ω_3 fatty acid as nonessential or partially essential. However, during the last few years studies have been published to suggest that ω_3 fatty acids may be essential in addition to the requirement for ω_6 fatty acids for which they can partially substitute.[82]

ω_3 fatty acids are important components of structural lipids in many tissues, notably the brain and the retina[83] and the levels in these tissues are depleted with extreme difficulty. Biologic structures involved in

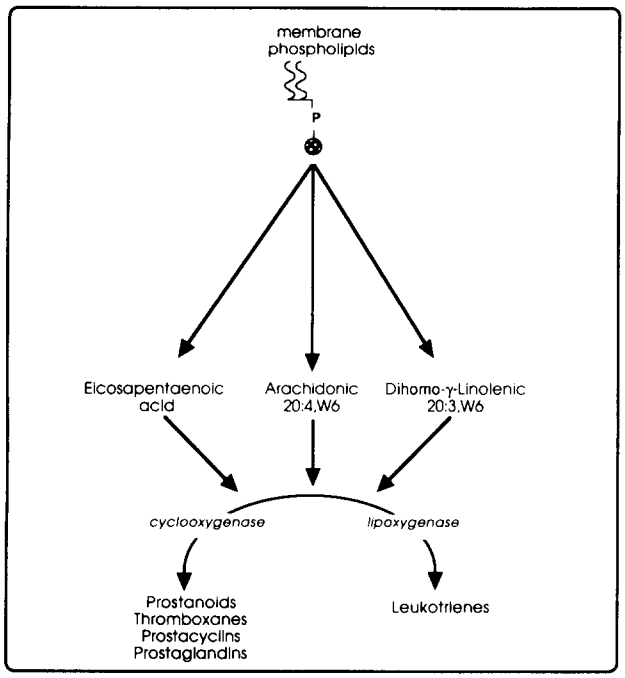

Figure 2 Formation of eicosanoids.

fast movement or signal transmission appear to have a requirement for highly unsaturated fatty acids. For example, the synaptic junctions have more unsaturated DHA. In several species studied, including humans, retinal rod outer segment disk membrane in which rhodopsin rests, the major phopholipid contains 40%–60% of the total fatty acid as DHA.[84,85] In the cerebral cortex of humans, monkeys, and rats DHA accounts for approximately one-third of the fatty acid content of ethanolamine and serine phosphoglycerides.[86] Changes in physiologic functions have been observed in monkeys selectively deprived of ω_3 fatty acids for prolonged periods and the altered changes have been corrected by the administration of linolenic acid.[87] Rats exposed to ω_3 fatty acid-deficient diet exhibited depressed learning ability[88] and abnormalities in the electroretinogram,[89] which measures the retinal-evoked response to flashes of light.

Epidemiologic studies have provided data associating seafood consumption with reduced risk of coronary heart disease and inflammatory disease.[90-92] Seafood is rich in two ω_3 fatty acids, EPA and DHA, and low in arachidonic acid. Clinical studies have shown that the exchange of marine fish oil for vegetable oil in an otherwise typical Western diet leads to a more favorable pattern of serum lipids. It causes a reduction in very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in both normal and hyperlipidemic patients.[93] Fish-oil consumption also reduces platelet aggregability and prolongs bleeding time.[94,95] Experimental and epidemiologic studies also suggest a negative relationship between certain types of cancer and dietary ω_3 fatty acids. A high intake of these fatty acids in the diet is associated with a decrease in the incidence, growth, and spread of a variety of experimental tumors.[96] Recently is has been reported[97] that the formation of interleukin-1 and tumor necrosis factor (TNF), the principal polypeptide mediators of inflammation, can be suppressed by dietary supplementation with long-chain ω_3 fatty acids.

The essentiality of linolenic acid in humans is controversial but because of the high concentration of DHA derived from it in the brain and retina it appears that ω_3 fatty acids may be required in the diet. However, more studies are needed in clarifying the role of linolenic acid in human nutrition.

Deficiency

The detailed studies on the symptoms of EFA deficiency have been done in young rats.[98] They include reduced growth rate, scaly dermatitis, impaired reproduction, kidney abnormalities, abnormal swelling and function of mitochondria, decreased capillary resistance and increased fragility of erythrocytes, increased water consumption, and increase in triene/tetraene ratio above 0.4. Rats deficient in EFA have lower brain arachidonic acid and DHA and increase in Mead acid and they are more sensitive to all volatile anesthetics.[99] Most normal diets contain enough EFA or their metabolic products to meet daily requirements and the deficiency is rare in humans. But when it exists some of the symptoms characteristic in animals, such as abnormal skin condition, increased susceptibility to infection, and an increase in triene:tetraene ratio have also been observed in EFA-deficient humans.

The first study of the EFA deficiency in human adults maintained on a diet extremely low in fat for 6 months did not produce dramatic symptoms.[8] It was suggested that because adults contained little more than two pounds of linoleic acid in body stores, more than 6 months were necessary for the depletion of EFA stores and to produce deficiency symptoms. The first definitive evidence for the dietary requirement of EFA in humans was provided by the study of Hansen et al.[100] They fed 428 healthy infants one of the five proprietary milk mixtures adequate in protein, minerals, and vitamins but varying in linoleic acid content from less than 0.1% to 7.3% of total calories. A high proportion of babies who were fed milk mixtures low in linoleic acid (< 1% of total calories) for 3 months developed dry, thick, desquamated skin and retarded growth. The clinical manifestations disappeared after the administration of diets that provided 1% or more of the calories as linoleic acid. On this basis it was concluded that the minimum requirement of EFA in humans was 1% of total calories or more to cure symptoms.

Parenteral nutrition

The most common cause of deficiency in all age groups is the long-term intake of fat-free parenteral nutrition (PN). The PN was technologically developed

in 1968.[101] It has helped many patients (who were unable to tolerate oral food for extended periods and, therefore, were difficult to treat) to survive. PN solutions were fat free until 1979 because of the toxic reactions to the then available corn oil and castor oil emulsions and the patients receiving these PN solutions became EFA deficient.

PN is commonly administered as a continuous infusion of glucose-containing solution that results in a constant elevation of serum insulin. This depresses the release of fats including EFA from adipose fat stores.[102,103] Normally adipose tissue fat contains approximately 10% EFA.[104] A 70 Kg man has about 12 Kg adipose tissue of which a little more than 1000 g is EFA. Assuming the adult daily requirement for EFA as 7.5 g, his body stores of EFA can last more than 6 months and he is not likely to show deficiency symptoms even if he remains on fat-free diet. However, if he is on PN without fat, the continuous glucose infusion is expected to cause inhibition of the release of EFA from adipose stores. Thus continuous fat-free PN seems to provide optimal conditions for development of EFA deficiency. It supplies no fat and inhibits the mobilization of body's fat stores. Plasma free fatty acids originate from endogenously synthesized lipids derived from glucose and these do not include EFA. On the other hand, PN containing only amino acids and completely free of glucose do not produce biochemical evidence of EFA deficiency.[104]

Studies in infants who were maintained on long-term fat-free PN demonstrated the development of clinical signs together with biochemical evidence of EFA deficiency.[105-107] The administration of diets containing linoleic acid reversed both clinical and biochemical abnormalities. Some premature infants developed very rapid biochemical changes in the plasma as early as second and third days of life.[107,108] Borderline stores of EFA characteristic of the premature and the high caloric expenditure might have been responsible for the early onsets of EFA deficiency.

In the neonate maintained on fat-free "glucose-containing" PN, biochemical and clinical signs of EFA may become apparent in 5–10 days after the start of PN. In adults receiving similar PN the biochemical evidence of deficiency is generally seen 2 weeks[11] after the initiation of PN and by the end of 7 weeks all patients exhibit clinical signs of deficiency. Manifestations of EFA deficiency in these patients include alopecia, brittle nails, desquamating dermatitis, increased capillary fragility, indolent wound healing, increased platelet aggregation due to reduced prostaglandin synthesis, increased susceptibility to infection, fatty liver infiltration, and growth retardation in infants and children. Linoleic acid is the primary and perhaps the only essential fatty acid, at least for human adults.[109] To correct or prevent the deficiency when oral intake is denied, linoleic acid must be provided intravenously. The minimum linoleate dose to prevent deficiency state is about 5% of the total calories for adults and 2% for pediatric patients. Deficiency is accentuated by the increased metabolic demands associated with growth and the hypermetabolism following injury, sepsis, or stress. These patients should receive 500 mL of 10% lipid emulsion[110] 2–3 times per week.

Other conditions

Because of the very sensitive procedure for the measurement of triene:tetraene ratio it has been possible to demonstrate EFA deficiency in elderly patients with peripheral vascular disease,[111] due to malabosorption after major intestinal resection,[112,113] and in patients with serious accidents and burns.[114] In all these conditions oral or intravenous feeding of linoleic acid has been found to correct the biochemical and skin abnormalities. Patients with acquired immune deficiency syndrome (AIDS) have been found to have low total plasma PUFA[115] and the 20- and 22-carbon EFA of the ω_3 series are selectively and significantly reduced. The reduction in ω_3 fatty acids may be responsible for the elevated circulating level of TNF in these patients[116] and may have relevance to the pathogenesis of cachexia.[117] Normalization of the levels of these fatty acids in AIDS patients may be a worthwhile therapeutic aim.

Linolenic acid deficiency

In 1982 the first case involving specific deficiency symptoms attributed to linolenic acid deficiency was described by Holman et al.[118] A 6-year-old girl who sustained an accidental gunshot wound to the abdomen, underwent repeated resections of the small intestine and recovered sufficiently. She was maintainted on home PN that included safflower oil emulsion rich in linoleic acid. After 5 months, she experienced episodes of numbness, paresthesia, weakness, inability to walk, leg pain, and blurred vision. Analysis of serum revealed very low levels of linolenic acid and other ω_3 acids derived from it. PN was then changed to include soybean oil emulsion, which contains linolenic acid. All the symptoms of deficiency disappeared over the next 3 months and plasma level of ω_3 fatty acids returned to normal levels. This neuropathy was correlated with linolenic acid deficiency and not with linoleic acid. In 1984 Neuringer et al.[119] described linolenic acid deficiency in Rhesus monkeys. They found decreased amounts of linolenic acid and PUFA derived from it in plasma phospholipids of the offsprings who also showed a loss in visual activity. Bjerve et al.[120-122] reported linolenic acid deficiency in nine patients who were fed by gastric tube for 2.5–12 years and had received 0.02%–0.09% of calories as ω_3 acid. Total ω_3 fatty acids in their plasma and erythrocytes were decreased. They had slight but definite scaly dermatitis that disappeared with supplementation of linolenic acid.

Requirements

The exact requirement of EFA in humans is not clearly defined. Arachidonic acid with four double bonds (tetraene) is the major metabolite of linoleic acid and eico-

satrienoic acid (triene) is the major product derived from non-essential fatty acids. As stated earlier, dietary intake of adequate amounts of EFA decreases the formation of triene as a consequence of competitive inhibition among families of PUFA for desaturases and possibly acyl transferases. If EFA are not available, the biosynthesis of PUFA with three double bonds derived from oleic and palmitoleic acids continue. It has been shown in all species tested including humans that triene:tetraene ratio in plasma is below 0.4 when dietary EFA are adequate and is increased above 0.4 in relation to the degree of deficiency.[19] The optimum dietary linoleate required to give a ratio of less than 0.4 and to prevent symptoms of EFA deficiency is 1%–2% of total calories. It has been suggested that those functions dependent on eicosanoid formation may show an optimum response with dietary linoleate at higher levels perhaps as high as 6%–10% of total calories. An absolute amount required is not yet known, but because no ill effects have been reported up to this level (6%–10%) the tendency is to consider it as optimal. The Food and Nutrition Board of the National Academy of Sciences recommends that at least 3% of daily calories be provided as linoleate but it is recognized that larger amounts may be desirable to control blood lipids in certain individuals. The average daily intake of linoleic acid by adults in most industrialized western countries is about 10 grams.

Pregnancy

There are no studies reported on the EFA requirements during pregnancy and lactation. The approximate accumulation of EFA during pregnancy is estimated to be about 620 g, which includes the demand for uterine, placental, mammary gland, and fetal growth and the increased maternal blood volume.[123] Most of the fat in fetal organs such as liver and brain is structural and contains a high proportion of phospholipids requiring long-chain PUFA derived from EFA. To meet these needs, 4.5% of the expected caloric intake in the form of EFA is recommended during pregnancy.[124-126]

Lactation

Approximately 4%–5% of total energy in human milk is present as linoleic and linolenic acids and 1% as long-chain PUFA derived from these acids, amounting to about 6% of total energy as EFA and its metabolites.[127,128] The fat stored during normal pregnancy is utilized during lactation at the rate of about 300 calories per day. Between 3–5 g of EFA are secreted in milk per day. The efficiency of conversion of dietary EFA into milk fatty acid is not known, but an additional 1%–2% of energy in the form of EFA is recommended during the first 3 months of lactation and an additional 2%–4% of the energy above the basic requirements is recommended thereafter.[123]

Infancy

Normal growth of infants depends on an adequate supply of EFA.[100] Human gray matter and retinal membranes contain significant amounts of long-chain PUFA, especially DHA. Rapid accretion of these fatty acids occurs in the central nervous system during the last trimester of gestation and first months of life.[129,130] Therefore preterm infants, due to their low fat stores, may be susceptible to EFA deficiency. Lower accumulation of DHA in neural and retinal membranes during development is associated with behavioral abnormalities[131] and impaired visual development.[119] Long-chain PUFA may not be synthesized from parent EFA at optimal rates during the first few weeks after birth and especially in low birth-weight infants. At present there is insufficient information to determine whether term or preterm infants have sufficient enzymatic activity to synthesize their own long-chain PUFA from EFA to meet their requirement for brain growth and development. Because of the importance of long-chain PUFA for development, it is essential to provide brain cells with adequate dietary intake of EFA and their long-chain derivatives. Human milk provides both EFA and their long-chain derivatives. The ideal recommendation for milk substitutes would be to match the EFA of human milk from well-nourished mothers with respect to linoleic and linolenic acid and the long-chain PUFA derived from them.[132] Formulas containing fat of vegetable origin with high linoleic acid content provide adequate EFA of the ω_6 family. Human milk also contains linolenic acid and DHA that are often absent from infant formulas. A diet deficient in linolenic acid but adequate in linoleic acid results in reduced amounts of DHA and increased amounts of PUFA derived from linoleic acid in the brain cells of rats.[133] The long-chain derivatives of EFA are highly susceptible to oxidative damage and incorporating them into synthetic formulas is a matter of serious concern. It is not known precisely what are the optimum requirements for EFA of ω_6 and ω_3 families for infants. However, using the fatty acid composition of red blood cell phospholipids as an index of cerebral membrane composition Carlson et al.[134] found that infants fed human milk have significantly better DHA status than formula-fed infants. Recent work of Innis et al[135] suggests that infants fed formula containing at least 2 % of the total fatty acid as linolenic acid (0.95% of calories) and a ratio of linoleic:linolenic acid similar to that of human milk may permit incorporation of ω_3 fatty acids in low birth-weight infants equivalent to those fed mother's milk.

Aging

Most individuals have enzymes required to derive long-chain PUFA from linoleic and linolenic acids. Some authors have suggested that the activity of Δ_6 desaturase may decrease with aging,[28,136] which may contribute to the deficiency of DHGL and arachidonic acid. However, the data of Siguel and Schaefer[137] suggest that this may not be true for most elderly people,

because the levels of plasma PUFA derived from EFA of elderly are similar to those of young individuals. In some people the formation of PUFA may not meet body needs (perhaps due to the metabolic block) and such individuals may have increased requirements for EFA derivatives in the diet. The usual diets contain adequate amounts of arachidonic acid, therefore the main problem in those with decreased desaturase activity is likely to be the deficiency of DHGL, the precursor of prostaglandins of the "1" series.[138] Evening primrose oil contains gamma linolenic acid that bypasses the step requiring Δ_6 desaturase.[139] The amount of DHA, a product derived from linolenic acid, is found to decrease with aging in the retina of rats[140] and appears to be the result of decreased Δ_4 desaturase activity with aging. Therefore some have suggested that there may be increased requirements of EFA and some long-chain PUFA derived from EFA in the elderly; but at present there is not sufficient clinical evidence to warrant this conclusion.

ω_3 fatty acids

There is still controversy regarding the requirement of linolenic acid. A recent case of peripheral neuropathy and blurred vision in a child receiving parenteral nutrition has been attributed to ω_3 fatty acid deficiency[118] and the deficiency has also been described in adults.[120,121] There is now experimental evidence for a dietary requirement of ω_3 fatty acids in primates and the visual defect associated with ω_3 fatty acid depletion supports its essentiality in humans. A role of these fatty acids in the development of neural tissue and visual function is evident.[141] In healthy humans plasma levels of ω_6 fatty acids are about 10 times greater than ω_3 fatty acids.[142] Based on these data and some observations in elderly patients it has been suggested that linolenic acid requirements should be 0.2%–0.3% of total energy for adults[120] and 0.56% for children.[118]

Factors affecting requirement

Several substances are known to affect the requirements due to their interaction with EFA, which can affect their utilization or metabolism.

Saturated fatty acids have been found to increase requirement of EFA as measured by growth, dermal symptoms of deficiency, and triene:tetraene ratio in plasma.[143] This action may be related to the effect of saturated fatty acids in raising plasma cholesterol that forms esters with PUFA and deplete the EFA pool available for phospholipids. Dietary EFA are effective in reducing plasma cholesterol levels.[144,145]

Monounsaturated fatty acids can replace EFA in the lipids of deficient animals and humans. As stated earlier, they also suppress the desaturation of EFA if present at high dietary levels. Most of the unsaturated fatty acids occuring naturally and present in our diets possess double bonds in the cis configuration. Partial hydrogenation of vegetable oils is done for production of margarines and shortenings. This process forms saturated fatty acids and a variety of trans and positional isomers of unsaturated fatty acids in varying amounts. Stick margarine contains 25%–35%, tub margarine 15%–25% and shortening 20%–30% of trans isomers. It has been estimated that the average daily trans fatty acid intake is 8–10 g or 6%–8% of the total dietary fatty acids. The trans monounsaturated acids have been found to increase the EFA requirement in animals when included at moderate amounts in the diet.[146] The position and geometry of unsaturation influence the inhibition of desaturase reactions critical to the metabolism of PUFA. The trans fatty acids also raise plasma levels of LDL and total cholesterol and HDL cholesterol.[147,148]

Effects of high doses of essential fatty acids

Studies on EFA have been mainly concerned with the symptoms of deficiency and the minimal requirements to prevent or treat the deficiency state. Little is known about the adverse effects of high doses of these nutrients in the diet. Excess linoleic and linolenic acids and other fatty acids compete for the site on Δ_6 desaturase and will have an effect on the formation of long-chain PUFA derived from them. Dietary EFA can act as depressors of some enzymes such as fatty acid synthetase and glucose-6-phosphate dehydrogenase[149] but we do not know whether this action is beneficial or harmful. It has been suggested that a high intake of PUFA may be an environmental factor in some types of cancer. Animal studies have shown that when fed with a carcinogen, PUFA are more co-carcinogenic than saturated fatty acids.[150] Some studies have been reported that show a proportionate increase in cancer rate as the amount of ingested PUFA increase.[151] It has been established that as the intake of PUFA increases the antioxidant capacity of the body is challenged. The oxidation of excess PUFA results in the formation of peroxidized free radicals that can damage and destroy cells, cellular components, and other body proteins. The end result is the formation of lipofuscin pigment granules that are polymers of peroxidized PUFA. Lipofuscin seems to be associated with the aging process because the amount of this pigment increases with age.[152] Vitamin E works as effective antioxidant and inhibits oxidation of PUFA. Vegetable oils generally are rich in this vitamin but PUFA in the body have a longer half-life than vitamin E and, therefore, the requirement of the vitamin is increased with excess intake of PUFA.[153]

EFA are the precursors of eicosanoids and each of the three 20-carbon fatty acids DHGL, arachidonic acid, and EPA give rise to different series of eicosanoids, some with opposite effects. Thromboxane A_2 derived from arachidonic acid causes aggregation of platelets, while prostacyclin I_2 derived from the same precursor has a diametrically opposite effect. The aggregation of platelets seems to be beneficial at peripheral wounds but may be harmful within the coronary or cerebral vessels. It is not known what effect the excess PUFA of the ω_6 family will have on the formation of different eicosanoids. Excess ω_3 fatty acids

may have adverse effects from the suppression of thrombogenic activity.

Several hazardous effects have been reported in newborn infants: a reduced clearance rate in small-for-date infants as well as premature infants born before 32 weeks of gestation[154] and displacement of bilirubin from albumin binding sites and increased risk of kernictirus in jaundiced newborns[155] are observed. The deposition of lipid material in macrophages that may alter immunity,[156,157] immunosuppressive effect,[158] and altered pulmonary gas exchange[159] have also been described.

Conclusions

Four families of PUFA occur in mammalian tissues. The precursors of the two are palmitoleic (ω_7) and oleic (ω_9) acids that can be synthesized endogenously from palmitic and stearic acids, respectively. The precursors of the other two are linoleic (ω_6) and linolenic (ω_3) acids (from plant sources) and are considered dietarily essential for both humans and animals. The long-chain highly unsaturated PUFA are formed from these precursors by a process involving desaturation and chain elongation. The principal products of the ω_6 family are arachidonic acid and DHGL and those of the ω_3 family are EPA and DHA. The major products derived from non-essential fatty acids (when the dietary intake of linoleate and linolenate are inadequate) are eicosatrienoic acids (ω_7 and ω_9).

The long-chain PUFA derived from EFA are primarily esterified in the carbon-2 position of bilayer phospholipids of mammalian plasma, nuclear, and mitochondrial membranes. These PUFA, when incorporated into membrane phospholipids, can alter the physicochemical characteristics (microviscosity and fluidity) of the membrane lipid matrix which, in turn, can influence the conformation, mobility, and function of a variety of membrane-bound proteins. Linoleic acid is specifically required in the skin to maintain the integrity of epidermal water barrier and the 20-carbon fatty acids (DHGL, arachidonic acid, and EPA) when released from their phospholipids can also be transformed into eicosanoids that influence many cell processes and organ functions. Other than these roles the specific functions of individual PUFA are not clearly understood.

Mammals have an absolute requirement for the ω_6 family of fatty acids. If deprived of these nutrients a state of deficiency is induced. Features include dermatitis with increased transepidermal water loss, reproductive inefficiency, susceptibility to infection, and depressed inflammatory response. Biochemically, EFA deficiency is characterized by a decrease in tissue ω_6 fatty acids including arachidonic acid and the accumulation of ω_9 fatty acids, specifically eicosatrienoic acid (Mead acid), leading to the increase in the plasma triene:tetraene ratio above 0.4.

The role of the ω_3 family of fatty acids is less clear. Amounts of ω_3 fatty acids in mammalian tissues are generally much lower than ω_6 fatty acids and it has been difficult to demonstrate its essentiality in animal studies. It is generally considered that linoleic acid satisfies most of the EFA requirements and any EFA activity attributed to linolenic acid is also expressed by linoleic acid that is equal or more active. But the consistent presence of large amounts of ω_3 fatty acids, especially DHA, in such tissues as retina and brain, and the extreme stability of the composition of PUFA in the brain, even in the face of wide variation in the diet, suggest a specific role for ω_3 in nervous system and retinal function. The recent reports on the neurologic symptoms in an infant associated with parentally fed linolenic acid-poor formula and the deficiency symptoms in adults that were corrected by linolenic acid supports the essentiality of this fatty acid in the diet. The requirement may be more critical during developmental stage because of the presence of DHA in the brain. More studies are required to understand the biochemical mechanisms underlying the essentiality of individual long-chain EFA–derived PUFA and the amounts needed to meet the minimal requirements in humans.

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