Dietary Essential Fatty Acids in Early Postnatal Life: Long-Term Outcomes

Ricardo Uauy\textsuperscript{a,c}, Cecilia Rojas\textsuperscript{a}, Adolfo Llanos\textsuperscript{a,b}, and Patricia Mena\textsuperscript{a,b}

\textsuperscript{a}Institute of Nutrition and Food Technology, University of Chile and \textsuperscript{b}Hospital Dr. Sótero del Río, Santiago, Chile, and \textsuperscript{c}Nutrition and Public Health Interventions Research, Department of Epidemiology, London School of Hygiene and Tropical Medicine, London, UK

Introduction

The formation of long-chain (LC) polyunsaturated fatty acids (PUFAs) from the parent essential fatty acids (EFAs) in early life is limited, thus infants are dependent on the exogenous provision of LC-PUFAs from human milk or supplemented formula. LC-PUFAs are structural components of all tissues, they are indispensable for cell membrane synthesis and for the function of key organelles such as mitochondria, endoplasmic reticulum and synaptic vesicles; and also for membrane receptors and signal transduction systems. The brain, retina and other neural tissues are particularly rich in LC-PUFAs; if diet is deficient in LC-PUFAs during early life, neural structural development and function are affected. LC-PUFAs also serve as specific precursors for 20-carbon eicosanoid production (prostaglandins, prostacyclins, thromboxanes, and leukotrienes). Recently docosanoids derived from 22-carbon LC-PUFAs have been identified and their capacity to protect neural tissue from hypoxia-reperfusion injury characterized. Eicosanoids and docosanoids act as autocrine and paracrine mediators. They are powerful regulators of numerous cell and tissue functions (e.g. thrombocyte aggregation, inflammatory reactions and leukocyte functions, cytokine release and action, vasoconstriction and vasodilatation, blood pressure control, bronchial constriction, and uterine contraction).

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The evidence to date indicates that human infants who receive inadequate supply of LC-PUFAs have altered retinal rod function, delayed maturation of the visual cortex, and poorer auditory discrimination as compared to the infants fed human milk or LC-PUFA-supplemented formula. Some studies have also revealed altered mental development and cognitive function [1]. Over recent years, the role of LC-PUFAs in modulating signal transduction and regulating gene expression have been described, emphasizing the complexity of fatty acid (FA) effects on biological systems [2–6]. Dietary FAs, especially LC-PUFAs, have potentially significant effects in the modulation of developmental processes affecting short- and long-term health outcomes related to growth, body composition, mental development, immune and allergic responses, and the prevalence of nutrition-related chronic disease. Figure 1 illustrates the short- and long-term effects of nutrients, in this case LC-PUFAs, on health outcomes related to neurodevelopment, growth and body composition and nutrition related chronic diseases.

**Summary/Update on LC-PUFA Biochemistry and Metabolism**

*Nomenclature and Chemistry of LC-PUFAs*

FAs are classified by chain length as short- (<8 carbon), medium- (8–11 carbons), intermediate- (12–15 carbons) and long-chain (≥16 carbons). Based on their number of double bonds, they are classified as saturated (no double bonds), monounsaturated (1 double bond) or polyunsaturated (2 or more double bonds). The nomenclature indicates the total number of carbon atoms, the number of double bonds and the position of the terminal double bond. Thus stearic acid (18:0) is a saturated carbon chain with 18 carbons and no
double bonds, and oleic acid (18:1n-9) is a monounsaturated FA with 18 carbons and 1 double bond in the n-9 position. The position of the double bond is indicated by the carbon at which the double bond occurs; standardized nomenclature (International Union of Pure and Applied Chemistry) the numbering starts from the carboxyl terminus or delta carbon; traditional or common nomenclature starts from the methyl or n terminus (also called ω carbon). Most metabolic activity affecting FAs such as oxidation, desaturation and elongation affects the carboxyl end of the chain, thus changing the carbon position number relative to the Δ terminus. Conversely the n or ω terminus is rarely affected by metabolic activity and has the advantage of providing a stable base carbon position for numbering purpose. Thus, an ω-6 FA, also termed n-6 FA (such as LA, 18:2n-6), remains a member of the n-6 family independent of its metabolism. EFAs are those that cannot be made by humans and must be provided by the diet, they have double bonds in position n-3 or n-6. FAs that are chemically identical in terms of number of carbon atoms and unsaturation may present double bonds as cis or trans isomers; these have major differences in physical and biological characteristics. Animals and plants almost entirely use FAs with cis double bonds for metabolic and structural purposes. Cis isomers have both hydrogen atoms of the doubly bonded carbons in the same plane of symmetry, thus the molecule is bent and both acyl carbon chains may rotate using the double bond as an axis, allowing them to be less packed, be more flexible and fluid. Trans FAs have a straight carbon chain with a tertiary structure similar to saturated FAs. Recent work has demonstrated the importance of FA tertiary structure in defining lipid-protein interactions, for example FA transport proteins have selected affinity to specific FAs based on their special configuration, thus the FA structure confers functional properties to a given protein domain [7].

**LC-PUFA Metabolism and Requirements**

The need to include LA, the parent n-6 EFA in the early diet, has been recognized for over 50 years. Over the past decades the need to provide α-linolenic acid (α-LNA, 18:3n-3) as a source of the n-3 EFAs has been recognized. A need for LC-PUFAs (>18-carbon chain length) derived from EFAs has only recently been established, based on studies of preterm and term human infants. Animal tissues, especially the liver, can further elongate and desaturate the parent EFAs, generating a family of compounds for the respective families as shown in figure 2. The competitive desaturation of the n-3 and n-6 series by Δ6-desaturase is of major significance because this is the controlling step of the pathway leading to the formation of arachidonic acid (AA; 20:4 n-6) and docosahexaenoic acid (DHA; 22:6n-3) from linoleic acid (LA; 18:2n-6) and α-LNA; 18:3n- 3) respectively, further details can be found in recent reviews [8–11]. The n-6 PUFAs are abundant in commonly used vegetable oils (corn, sunflower, safflower), whereas n-3 PUFAs are
relatively low except in soy, canola and linseed oils. Presently, most infant formulas are designed to provide a similar FA composition to that found in mature human milk from omnivorous women. This precision is necessary because the FA composition of human milk will vary based on the maternal diet. The EFA content of human milk, especially the LC-PUFA content, will change according to the maternal diet. The evidence indicates that in early life 18 n-3 precursors are not sufficiently converted to DHA to allow biochemical and functional normalcy [12, 13]. Thus, not only LA and LNA but DHA and AA are now considered necessary nutrients for normal eye and brain development in the human.

The conversion of parent EFAs to LC-PUFAs is under active regulation; therefore, the effects of providing AA, eicosapentaenoic acid (EPA) or DHA are not replicated by providing the equivalent amount of LA or LNA. The uniqueness of the biological effects of feeding human milk on EFA metabolism is based on the direct supply of LC-PUFAs, bypassing the regulatory step of the \( \Delta^6 \)-desaturase. Excess dietary LA associated with some vegetable oils, particularly safflower, sunflower and corn oils, may decrease the formation of DHA from LNA because the enzyme, \( \Delta^6 \)-desaturase, is inhibited by excess substrate. In addition, AA formation is lower when excess LA is provided. The inhibitory effect of EPA on \( \Delta^5 \)-desaturase activity has been considered partly responsible for the lower AA observed when marine oil is consumed. Excess LA, as observed in infants receiving corn oil or safflower oil as the predominant FA supply, will inhibit the elongation and desaturation of the parent EFAs and thus lower the LC-PUFAs available for membrane synthesis. Human milk and LC-PUFAs from dietary sources provide preformed AA and substantial amounts of preformed n-3 LC-PUFAs such as DHA [8, 11]. Our recent stable isotope studies indicate that infants born with growth

![Fig. 2. Metabolism of n-9, n-6, and n-3 LC-PUFAs.](image)
retardation may in fact have impaired DHA formation relative to weight and gestational age-matched controls (unpublished work).

Up to a few years ago, the metabolism of LC-PUFAs beyond the 20-carbon step leading to the formation of DHA was considered to be an apparently simple reaction catalyzed by a Δ⁴-desaturase forming DHA from 22:5n-3. This enzyme proved elusive to purify using traditional biochemical methods and remained somewhat of an enigma in LC-PUFA metabolism. After conducting detailed analytical work using isotopic tracers and gas chromatography mass spectrometry, Sprecher et al. [14–16] found evidence that what appeared to be a Δ⁴-desaturase was in fact really a 3-step pathway as depicted in figure 2. The initial step is an elongation to a 24-carbon intermediate, which serves as substrate to a Δ⁶-desaturase; then, the chain is shortened to a 22-carbon six double bond FA through a peroxisomal β-oxidation. This partial β-oxidation is specific to the peroxisome and in fact is a 4-step biochemical reaction that includes an initial acyl-CoA oxidase, a two-step oxidation by the action of a peroxisomal D-bifunctional protein and finally is subjected to the action of a thiolase. Fibroblasts from Zellweger syndrome patients have now been demonstrated to be incapable of forming DHA from either labeled 18:3n-3 or the immediate precursor 22:5n-3. The specific genes responsible for the metabolic defect have been identified; they have altered acyl-CoA oxidase or D-bifunctional protein activity and are impaired (5–20% of normal values). The residual activity in fact suggests that very limited formation can occur outside the peroxisomes. In contrast DHA was formed in cells from patients with rhizomelic chondrodysplasia punctata, Refsum disease, X-ADL, and deficiency of mitochondrial medium chain and very long chain acyl-CoA dehydrogenase. These patients have altered mitochondrial FA oxidation but intact peroxisomal function [17]. These data are consistent with the retroconversion hypothesis proposed by Sprecher et al. [14–16] and demonstrate that peroxisomal enzymes acyl-CoA oxidase and D-bifunctional protein are essential for DHA synthesis. The Sprecher pathway has been verified for both DHA (22:6n-3) and docosapentaenoic acid (DPA; 22:5n-6) formation. The Δ⁶-desaturase in the Sprecher pathway is likely different from the enzyme responsible for the initial step of the parent EFA metabolism.

If n-3 FAs are absent or deficient in the diet, the elongation and desaturation of the n-6 compounds generate a significant elevation in DPA; if both EFAs are lacking, eicosatrienoic acid (ETA; 20:3n-9) accumulates (fig. 2). The ratio of n-6 DPA to DHA may be used as an index of isolated n-3 deficit, while if both n-6 and n-3 are lacking the ratio of ETA to AA+DHA is the best marker for combined EFA deficit [18].

**Dietary Supply of LC-PUFAs in Early Life**

The main source for the de novo synthesis of n-3 FAs in the marine environment are aquatic autotrophic bacteria, micro algae, protozoa, and small invertebrates which constitute the zooplankton and phytoplankton.
Fish, higher in the food chain, incorporate the n-3 PUFAs and further elongate them to form EPA and DHA. Thus fish will concentrate EPA and DHA as triglycerides, mainly in the adipose tissue and in the fat of muscle and visceral organs. The higher the fat content of fish, the higher its content of n-3 FAs [19, 20]. Another important source of LC-PUFAs is egg yolk phospholipids. The concentrations of PUFAs is different depending on the feed given to animals, the ample use of fish meal in chicken feed has increased egg yolk DHA [21]. LC-PUFA products for blending in infant foods can be successfully produced if chicken feed is carefully monitored and refined lipid extraction procedures are used. This is presently an important LC-PUFA source used in some infant formulas. Bacterial strains and micro-algae isolated from the intestinal content of some fish show a remarkably high content of EPA and DHA [22, 23]. Efforts to grow these microorganisms in natural or artificial seawater to obtain DHA for nutritional or pharmacological use have been successful. In addition, selected fungal strains produce concentrated AA, which is suitable for human consumption. The industrial production of AA and DHA from strains of single cell organisms has lead to an expanded use of this source. Purity and toxicological testing should be conducted on FA sources intended for use in commercial infant foods. Initial studies used a mixture of vegetable oils to supply LA and LNA and marine oil as a source of n-3 LC-PUFAs [24–26]. All recent studies have used nearly pure DHA from marine oil fractions or DHA and AA from single cell oils [27–32].

Limited research efforts have focussed on defining the implications of using different LC-PUFA sources in infant foods. Very recently, n-3 desaturase transgenic mice, obtained by inserting the n-3 desaturase (fat-1) gene from the invertebrate Caenorhabditis elegans, serve to illustrate the potential modification of n-3 content of animal tissues and milk, independent of the diet provided. This offers a novel alternative to change n-3 FA intake by genetically modifying the foods consumed without altering their selection [33].

Mechanisms for Potential Long-Term Effects of Dietary LC-PUFAs Consumed in Early Life

Changes in Lipid Membrane Properties

FA composition of structural membrane lipids can affect membrane function by modifying overall membrane fluidity, by affecting membrane thickness, by changing lipid phase properties, by specific changes in the membrane microenvironment, or by interaction of FAs with membrane proteins [34–41]. Most dietary n-3 FA-induced membrane changes are not reflected by an overall change in membrane fluidity but rather result in selective changes in membrane micro-domains affecting specific functions. The replacement of DHA by DPA usually results in the same overall lipid unsaturation level. Thus fluidity, on average, would remain unchanged.
Furthermore, the main changes in the physical state, induced by changes in the FA composition of lipid bilayers, occur after the first and second double bonds are introduced; namely when a saturated FA such as stearic acid (18:0) is replaced by oleic acid (18:1n-9) or by LA (18:2n-6) [42, 43].

Diet-induced changes in structural lipids affect the functional characteristics of excitable membranes in several animal species and in human neural cell lines [12, 35, 38, 39, 44, 45]. Electrocardiographic abnormalities, such as a notching in the QRS complex, indicating impaired electrical conduction, occur in LA and α-LNA deficiency before clinical signs appear [46]. Either LA or α-LNA corrected these abnormalities. More recently, it has been shown that dietary fat influences the susceptibility to cardiac arrhythmia, their incidence and severity [47]. Furthermore, studies with myocardial preparations have indicated that the vulnerability to catecholamine-induced arrhythmia is reduced in animals fed either n-6 or n-3 PUFA-enriched diets [33, 48]. Feeding fish oil from bluefin tuna rather than sunflower oil and saturated fat resulted in a marked reduction in induced arrhythmia in several animal species and in isolated papillary muscle [47]. Changes in cardiac electrophysiologic responses to β-mimetics and reduced excitability of cardiac myocytes and in the susceptibility to arrhythmia have also been noted [33, 49]. Myocytes form minimal amounts of cyclooxygenase products and no lipoxygenase products, thus the changes in excitability and conduction are probably related to structural lipid composition, and reflect changes in the function of ion channels [35]. n-3 FA supplementation ameliorates the liquefying effect of ethanol on neural membranes while LA and α-LNA deficiency enhanced a volatile anesthetic action in rats; LA supplementation specifically reverses this effect [50].

DHA supply modifies the phospholipid molecular species present in neural tissues, thus possibly affecting overall function [51]. Recently Litman and Mitchell [53] have reported that the type of LC-PUFAs present in membrane phospholipids has profound effects on G protein activation and on the development of rod outer-segment structure. The rhodopsin activation in response to light involves a transformation of the MI form to MII. This MI ↔ MII equilibrium constant is 6 times higher with di-DHA-acylated phosphatidyl choline (PC) than with di-myristic (saturated C14:0) PC. The di-DHA PC has an equilibrium constant that is almost identical to that of native rod disks. The effect is mostly explained by the increase in membrane free volume. The greater mobility of rhodopsin within the lipid microenvironment most likely explains the change in G protein activation and the corresponding enhanced signal transduction to photon stimuli [36]. Previous studies showed that both the decreased phospholipid acyl chain unsaturation and the increased cholesterol concentration reduce the formation of MII via a mechanism linked to the specific packing properties of polyunsaturated acyl chains and the effect of cholesterol on these packing properties [53, 54]. The sensitivity of the MII–Gt binding interaction to membrane composition demonstrates that
protein–protein interactions which occur on the hydrophilic surfaces of membrane proteins are affected by changes in membrane composition in the hydrophobic core of the membrane. This novel finding suggests that the protein–protein interactions, which occur on membrane surfaces in virtually all forms of signal transduction may be modulated by changes in the FA composition of the membrane. The results of these studies demonstrate that a membrane with a level of 22:6n-3 or 22:5n-3 phospholipid acyl chain equivalent to that found in a normal, healthy rod cell produces a response similar to that recorded in vivo, while a membrane in which the n-3 LC-PUFAs have been replaced by 22:5n-6 produces a much slower response. The dependence of phosphodiesterase (PDE) activity on the presence of DHA in the chain at the sn-2 position demonstrates that G protein-coupled signaling is exquisitely sensitive to phospholipid acyl chain unsaturation. The reduced activity of PDE when the phospholipid contained is 18:0 and 22:5n-6 compared with 18:0 and 22:6n-3 is a clear indication of the specific gain in function provided by DHA. This comparison is crucial to our understanding of the biochemical basis for the functional effects of dietary n-3 deficiency because deficit generally leads to the replacement of 22:6n-3 with 22:5n-6 [55]. Thus it is not the total number of double bonds but specifically the n-3 double bond that is crucial. The changes in MII formation and PDE activity in rod outer segments obtained from rats raised on an n-3-adequate or deficient diet are essentially identical to the results obtained with reconstituted vesicle systems and isolated rod outer segment disk membranes. These results provide an understanding at the molecular level of the changes in ERG associated with dietary n-3 FA deficiency in animals and in non-human primates. The delays in ERG response we have observed in human dietary n-3 deficiencies [24] are at least partially due to reduced MII–Gt coupling efficiency and slower rate of MII–Gt formation when 22:6n-3 phospholipid acyl chains are replaced by 22:5n-6. Assuming that the rates of Gt binding and Gt activation are closely related, a reduction in the rate of MII–Gt complex formation by 10% will delay the rod cell responses to light by 5%. In summary the effects of membrane composition on the rate and efficiency of receptor-G protein-coupling lateral diffusion would be sufficient to account for the delays in photoreceptor activity observed in dietary n-3 deficiency. Similar signaling motifs exist in various G protein-coupled sensory systems, thus the findings from rod photoreceptors should be applicable to other G protein-coupled sensory systems.

Gene Expression

Over the last decade the role of LC-PUFAs in regulating gene expression has been extensively studied given the potential of dietary FA to affect several developmental and metabolic processes with relevant short- and long-term health outcomes [56–59]. Understanding how FAs regulate transcription factors that play a major role in carbohydrate and lipid metabolism will
provide an opportunity to develop nutritional interventions and therapeutic strategies with potential effects on several chronic diseases such as coronary artery disease and atherosclerosis, obesity and type-2 diabetes, cancer, major depressive disorders and schizophrenia. The mechanism for the regulation of gene expression by FA involves members of the superfamily of nuclear receptors that function as transcription factors. Several types of transcription factors account for the transcriptional effects of FA, namely, the peroxisome proliferator-activated receptor (PPAR), the liver nuclear receptor (LXR), hepatic nuclear factor-4α (HNF-4α), sterol regulatory element-binding protein (SREBP) and NFκB. An indirect effect of FA in gene expression can also be obtained by their action on enzyme-mediated pathways such as cyclooxygenase, lipoxygenase and protein kinase C pathways that involve changes in membrane lipid composition affecting G-protein receptor or kinase-linked receptor.

The FA effects on gene expression are cell-specific and influenced by FA structure and metabolism. FAs act as ligands and promote dimerization of nuclear receptors, specifying homodimer or heterodimer formation. In addition, binding of specific FAs may also specify the interaction of the homodimer or heterodimer with co-activator or co-repressor proteins that determine the activity of the protein complex, as illustrated in figure 3. Activated nuclear receptor dimers modulate the transcription of specific genes through binding to cis-regulatory elements that are present in target genes.
The PPAR family of nuclear receptors has received considerable attention due to its major role in the regulation of lipid and glucose metabolism and adipocyte differentiation. A model depicting the regulation of PPAR activation is presented in figure 4. PPARs form heterodimers with RXR and binds to typical cis elements named PPAR response elements (PPRE). Transcription-activation assays demonstrate that PPAR transcription factor activity is regulated by polyunsaturated FAs or by their metabolites, apparently by direct binding of the ligand to a hydrophobic pocket in the receptor. However, it is not possible to rule out an indirect modulation of PPAR activity by a FA-dependent phosphorylation of the protein. As mentioned above, the type of ligand determines the interaction of PPAR with co-activators or co-repressors (fig. 3). The evidence is that the interaction of PPARα with the co-activator is facilitated by FAs, whereas the acyl-CoA derivative stimulates its association to the co-repressor [60]. Conversely, the transcriptional activity of the transcription factor HNF-4α is activated by acyl-CoA thioester derivatives of the LC-PUFA and not the FA itself. Agonistic ligands for HNF-4α include saturated acyl-CoAs with 14–16 carbon chain length; whereas antagonistic ligands include n-3 and n-6 polyunsaturated fatty acyl-CoAs [61].

Fig. 4. Regulation of gene expression by PPARγ. Ligand binding to PPARγ promotes its dimerization with RXR bound to its own ligand, 9-cis retinoic acid. The interaction of the PPAR-RXR heterodimer with a co-activator protein promotes binding to a specific cis element known as the PPAR response element (PPRE) that is present in PPAR-responsive genes. This mechanism allows PPARγ to regulate the transcription of genes that participate in the metabolism of adipocytes (glucose uptake, lipid trafficking, accumulation of triglycerides) and in the differentiation of adipose cells.
Three PPAR isoforms, \( \alpha, \beta \) or \( \delta \) and \( \gamma \) encoded by individual genes, are known to date. These isoforms display distinct tissue distribution and biological function. PPAR\( \alpha \) is expressed mainly in liver, kidney, skeletal and cardiac muscle, where it regulates the catabolism of FAs. The PPAR\( \alpha \) null mice display defective expression of genes encoding several mitochondrial FA oxidation enzymes as well as lower expression of inducible mitochondrial and peroxisomal FA \( \beta \)-oxidation enzymes compared with wild-type mice, thus establishing a role for the receptor in FA homeostasis [62]. PUFA activation of PPAR\( \alpha \) results in a reduction of hepatic lipogenesis by decreasing the content of enzymes involved in lipid synthesis (FA synthetase, acetyl-CoA carboxylase, stearoyl-CoA carboxylase, malic enzyme). This effect is explained by diminished gene transcription; that is, the level of the mRNAs for these enzymes is decreased [63]. PPAR\( \gamma \) is expressed at high levels in white adipose tissue where it controls lipogenesis and glucose homeostasis in adipocytes, and also regulates their cellular differentiation (fig. 4). PPAR\( \gamma \) is also expressed in monocytes and macrophages. The activation of PPAR\( \gamma \) by FAs and/or their derived compounds induces the expression of gene products related to lipogenesis (adipocyte FA-binding protein, PEPCK, acyl-CoA synthetase and, lipoprotein lipase) and to adipocyte terminal differentiation [64–66].

There are two PPAR\( \gamma \) isoforms that derive from the same gene by alternative promoter usage and splicing. Specific mutations for PPAR\( \gamma2 \) associated with enhanced adipocyte differentiation but with a marginal effect on insulin sensitivity have recently been identified in humans [67]. PPAR\( \beta \) is ubiquitous, and its biological function is beginning to be unraveled. Recent data suggest that it plays a role in regulating skeletal muscle lipid metabolism and insulin sensitivity [68] and also in VLDL signaling in macrophages [69].

Growing data show that LC-PUFAs affect the expression of genes that regulate cell differentiation and growth; therefore, early diet may influence structural development of organs, as well as founding of neurologic and sensory functions. The evidence that supports the activation of the transcription factor retinoid X receptor (RXR) by DHA in brain tissue offers a possible mechanism to explain the effect of DHA in neural functioning [3]. Specifically, a possible role of DHA on retinal neuronal differentiation has been proposed [70]. Our experiments with human fetal retina explants show that supplementation of culture medium with DHA modifies the expression of genes related to neurogenesis and neuronal function [71]. Transcripts for ion channels involved in retinal synaptogenesis [72, 73], such as the N-methyl-D-aspartate (NMDA)- and \( \gamma \)-amino butyric acid (GABA)-activated \( \text{Ca}^{2+} \) channels, are highly expressed in retinal explants treated with DHA. Calcium influx through these channels increases the intracellular \( \text{Ca}^{2+} \) concentration in specific cells [74], which appears to trigger different cellular responses that contribute to the establishment of neuronal connections. The evidence indicates that the neurotransmitter GABA is present at early
stages of neurogenesis, even before synapses are formed. During human embryogenesis, GABA synthesis apparently uses putrescine instead of glutamate as a substrate [75]. Accordingly, the expression of the gene for ornithine decarboxylase (the enzyme that catalyzes putrescine biosynthesis at early stages of development) is also increased by DHA in human fetal explants. These experiments with human fetal retinal explants also demonstrate changes in the expression of genes involved in apoptosis. This process takes place during the embryonic development of the vertebrate retina, particularly at stages when synaptic connections are established. Exposure to DHA caused an increased expression of some genes that repress apoptosis; but also reduce the expression of genes that encode for pro-apoptotic factors, presumably due to the simultaneous degeneration and differentiation processes undergoing the morphologic and functional remodeling of the retina during development. Taken together, these results support the idea that the effects of DHA on gene expression contribute to the development and maturation of the human retina.

Potential Effects Mediated by Eicosanoid and Docosanoid Production

The effect of LC-PUFAs in the early diet can modulate eicosanoid (derived from 20-carbon FAs, AA and EPA) and possibly docosanoid (derived from 22-carbon FAs, DHA) production affecting multiple physiologic functions that may explain both acute and long-term health effects.

Depending on nature of the dietary supply, membrane phospholipases liberate AA and/or EPA from phospholipids. Thus, LC-PUFAs through the action of cyclooxygenase or lipoxygenase form eicosanoid products, prostaglandins, prostacyclins, thromboxanes and leukotrienes, that play key roles in modulating inflammation, cytokine release, immune response, platelet aggregation, vascular reactivity, thrombosis, and allergic phenomenon. The balance between AA (n-6) and EPA (n-3) in biological membranes is regulated based on dietary supply and tissue-specific factors. The n-6/n-3 ratio in phospholipids modulates the balance between prostanoids of the 2 and 3 series derived from AA and EPA, respectively. Series-3 prostanoids are weak agonists or in some cases antagonize the activity of series-2 prostanoids.

Eicosanoids of the 2 series promote inflammation, platelet aggregation and activate the immune response, on the contrary series-3 prostanoids tend to ameliorate these effects [76, 77]. Figure 5a summarizes the role of n-6/n-3 balance in regulating eicosanoid effects of potential interest. This balance affects health outcomes modifying the severity, progression and recovery from diseases that are mediated by prostanoids resulting from the ratio of n-6:n-3 FAs released from membranes by the activation of specific phospholipases.
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Fig. 5. **a** Role of n-6/n-3 LC-PUFAs in regulating eicosanoids effects. **b** The effect of DHA in regulating the expression of genes for the synthesis of eicosanoids in human fetal retina explants is consistent with an anti-inflammatory role. Exposure of retina explants to DHA resulted in increased expression of the genes for lipocortin (inhibitor of AA release) and phospholipid hydroperoxide glutathione peroxidase (a negative regulator of lipoxygenase activity). In addition, the expression of the gene for leukotriene A4 hydrolase (the enzyme that catalyses leukotriene B4 synthesis) is reduced. Numbers indicate the fold change in the corresponding mRNA in retina explants exposed to DHA as compared to those treated with oleic acid (OA).
Potential Long-Term Effects of LC-PUFA Supply

Growth and Body Composition

The classic LA deficit is accompanied by growth failure. In fact, recent studies suggest that if the LA:LNA ratio is very low, LA may be insufficient to support normal growth [78]. Observational studies from malnourished populations are not conclusive of EFA deficiency as evidenced by plasma and RBC FA composition. Studies in Sudan compared EFA blood levels in normal children under 4 years of age to those suffering from marasmic protein energy malnutrition (PEM) or Kwashiorkor. The n-6 EFAs, including LA and AA, were significantly lower in plasma phospholipids and cholesterol esters relative to controls, there was a corresponding increase in the nonessential FAs such as oleic acid [18:1n-9] in the malnourished. No differences were found for the n-3 series EFAs. The different diets used during the recovery of PEM may influence the findings from different studies exploring the effect of PEM on EFA status [79]. Studies from rural China, where soy oil is consumed and diets are low in total protein and energy, human milk has a low DHA content (<0.2%) with an AA to DHA ratio of 2.4:3.1 revealing a relationship between growth and EFA content of human milk. At 3 months of age weight gain was significantly related to the AA content of human milk (r = 0.46) while linear growth was related to DHA content (r = 0.80). Studies in fully breast-fed infants from industrialized countries where human milk has an AA to DHA ratio of 1.6 indicate a direct relationship between AA+DHA in human milk and head circumference growth, suggesting a possible effect of brain growth [80].

Experimental studies with variable amounts of parent EFA added to the formula, LA:LNA ranges from 17:1 to 5:1, have served to demonstrate effects on growth. Jensen et al. [78] reported decreased growth rates with high LNA formulas, while Markrides et al. [30] using similar formulas with LA:LNA 10:1 to 5:1 did not show adverse effects on growth. Formulas supplemented with DHA and GLA have not demonstrated adverse effects on growth [81].

Several studies providing balanced DHA+AA LC-PUFA supplements to preterm or term infants have failed to demonstrate effects on weight, linear or head growth [26, 52, 82–96]. The initial studies by Carlson et al. [25] in 1993 demonstrated a direct relationship between AA levels and growth in terms of weight and length. The high EPA in the fish oil supplement has been proposed as a potential cause of the growth effects, since EPA can displace AA from membranes and AA is necessary for growth. In a second study in preterm infants with BPD Carlson et al. [97] demonstrated that low EPA LC-PUFA supplementation could also interfere with growth at specific ages. Additional studies have failed to demonstrate adverse effects on growth [85, 96]. This issue has recently been addressed by conducting a meta-analysis of all available studies in both term and preterm infants. Randomized trials involving 1,680 term infants and 1,647 preterm infants met criteria for
inclusion in the meta-analysis. Term infants allocated to any type of LC-PUFA supplementation were not statistically different at 4 or 12 months of age. A subgroup analysis of infants allocated to an n-3 LC-PUFA alone group (no AA) also showed no effect of supplementation on any growth parameter at either 4 or 12 months of age. Preterm trials provided raw data for 1,624 preterm infants, the growth of preterm infants was explored through the generation of growth curves of infants in control, n-3 LC-PUFA+AA treatment and n-3 LC-PUFA alone treatment. No difference in the pattern of growth for weight, length or head circumference was noted. A multiple regression analysis to assess the determinants of growth in these infants at 40, 57 and 92 weeks post-menstrual age (PMA) found a significant effect of size at birth, gender and the actual age of assessment. The overall influence of LC-PUFA supplementation accounted for less than 3% of the variance in growth.

**Allergic and Inflammatory Responses**

Asthma is considered a good example of allergic disease. The main features of obstructive airway disease are related to alterations in the airway and air trapping in the lung. Airway obstruction due to bronchoconstriction and increased mucous production leads to air trapping and loss of gas exchange. Virtually all these features correspond to known actions of AA metabolites, prostanoids and leukotrienes C4, D4, E4. Moreover, leukotrienes have been postulated to amplify oxygen radical-mediated lung injury by inducing chemotactic mediators, which attract polymorphonuclear cells and increase vascular permeability. These findings indicate an important role for inflammatory mediators in the pathophysiology of this disease. The specific FA composition of the diet can modulate cytokine production while preserving cell-mediated immunity [98]. Similarly a study in preterm infants fed formula supplemented with LC-PUFAs (AA+DHA) demonstrated in a post hoc analysis a lower incidence of bronchopulmonary dysplasia [97]. The control group (n = 45) had a 40% prevalence while the supplemented group (n = 49) had a 24% prevalence (p < 0.1). The small number of infants studied may limit the conclusions of this study [97]. The use of medium-chain triglycerides and a more balanced n-6/n-3 ratio in the PUFA supply could be justified on the basis of existing knowledge. The advent of a parenteral fat emulsion containing EPA and DHA to provide n-3 LC-PUFAs in the management of critically ill pulmonary patients is presently undergoing clinical evaluation [99, 100]. How can we explain the potential benefits of LC-PUFAs in airway obstructive disease? Cell membrane phospholipase A2 (PLA2) releases AA from glycerophospholipids, which are converted into different eicosanoids (prostaglandins, thromboxane and lipoxins) involved in inflammatory signaling as shown in figure 5a). Inhibition of PLA2 can be expected to block the synthesis of all eicosanoids and several features of the inflammatory response. Our experiments with retinal explants exposed to
DHA show a 4-fold increase in lipocortin mRNA (fig. 5b), a protein that belongs to the group of antiflammins, that comprises the annexin and lipocortin families [71]. These endogenous proteins are among the most potent anti-inflammatory drugs known to date. Indeed, anti-inflammatory corticosteroids induce the expression of several of these proteins. Therefore, according to our results, DHA is likely to contribute to a decrease in eicosanoid biosynthesis by increasing the expression antiflammins (lipocortin). Moreover, a 5-fold increase in the mRNA for phospholipid hydroperoxide glutathione peroxidase (a negative regulator of lipoxygenases) and the reduced expression of the leukotriene A4 hydrolase mRNA in the explants treated with DHA, would also contribute with an anti-inflammatory effect.

Classic studies in rodents have demonstrated a tumor necrosis factor (TNF)-induced gut injury that is nearly identical to necrotizing enterocolitis (NEC). These effects can be modulated by dietary n-6/n-3. More recently interleukin-6 has been demonstrated to play a key role in the activation sequence leading to tissue injury [101]. Elevated interleukin-6 in amniotic fluid and in umbilical cord blood has been associated with NEC and other neonatal morbidity including sepsis and intraventricular hemorrhage. Whether diet modulation of cytokine release can prevent NEC deserves further study. Accumulation of AA and increased prostanoid production have been demonstrated during reperfusion of ischemic myocardium and ischemic gut in adults. This has been shown in newborn pigs with gut ischemia induced by occluding the superior mesenteric artery [102]. Efflux of 6-keto-PGF1α, an AA prostanoid derivative, represents a component of the response to mesenteric ischemia. In this study oxygen free radical scavengers did not alter the prostanoid increase after ischemia. The mechanisms by which dietary LC-PUFA modulate cytokine production have not been fully elucidated. Changes in the production of the eicosanoids PGE2 and leukotriene B4 and a reduction in the intracellular signal transduction pathways involved in the synthesis of cytokines have been suggested as an explanation for the protective effects of n-3 PUFAs [76, 103]. Others propose that n-3 FAs modulate protein kinase C activity, which may participate in transcriptional control of TNF gene expression via the activation of transcription factors NF-κB [98]. A recent study by Caplan et al. [104] using a rat model, found that PUFA reduced the incidence of death, had lower endotoxin levels and decreased intestinal phospholipase A2-II and platelet-activating factor (PAF) receptor expression and other markers of intestinal inflammation.

Present views on the pathogenesis of NEC include a final common pathway of mucosal injury linked to feeding, bacterial proliferation, hypoxia and or ischemia. Mucosal injury is thought to be mediated by cytokine release activated by any of these factors. Recent controlled clinical observations using a randomized and masked design in premature infants suggest that a formula containing egg phospholipids as a source of LC-PUFAs (DHA and
AA) may reduce the incidence of NEC [105]. The control formula infants (n = 85) had a 17.6% prevalence of proven NEC while the egg phospholipid (n = 34) formula-fed infants had only 2.9%. They speculate that one or more components present in the egg phospholipids enhanced gut maturation. LC-PUFAs, phospholipids or choline could potentially mediate this protective response. A larger scale clinical trial is presently in progress in an attempt to validate this initial observation. The balance between AA and EPA could play a role in defining prostanoid synthesis and thus preventing intestinal mucosal injury. In a previous study, the same investigator reported a nonsignificant association between LC-PUFA supplementation and an increase NEC incidence [97].

The tissue response to allergy and inflammation involves multiple cellular and molecular interactions that are tightly regulated [1]. In early steps, circulating leukocytes must sense appropriate signals and migrate from the blood stream through blood vessel walls to reach the site of tissue damage. The contact between monocytes and stimulated endothelial cells is a critical step for the inflammation response to proceed. Endothelial cells produce and present on their surface the PAF that binds the PAF receptor (a G protein-coupled receptor) on the surface of leukocytes, monocytes and platelets. Binding of PAF to its receptor triggers a program of cell adhesion (monocyte-endothelial cell, neutrophil-endothelial cell, monocyte- activated platelet), gene expression and cytokine/chemokine secretion [106] (fig. 6). The intracellular response to PAF includes translocation of the transcription factor NF-κB to the nucleus and consequently, the expression of early immediate genes such as monocyte chemotactic protein-1, cell adhesion molecules (ICAM-2), cytokines (GM-CSF and TNF-α). It is apparent that PAF exerts the control of the duration and magnitude of the inflammatory response. In fetal retina explant experiments, exposure to DHA produced an increase in the mRNA for PAF acetylhydrolase, the enzyme that degrades PAF (fig. 6; table 1). This effect would presumably increase PAF degradation [107] in the vasculature, and therefore reduce PAF-mediated effects. Table 1 summarizes changes in the expression of genes involved in inflammation. The mRNA for transcription factor NF-κB, an important mediator of PAF effects, increased in retinal tissue exposed to DHA; however, the significance of this result is not clear, given that translocation of the protein into the nucleus is the critical step to regulate its activity as a transcription factor.

The control of cell–cell interactions related to the inflammatory response could also be mediated by PAF-independent pathways. It has been reported that oxidized lipids are able to differentially regulate endothelial cell binding to monocytes and to neutrophils, and to play a role in chronic inflammation. An early event following peroxidation is the activation of phospholipase A2, which leads to the formation of several pro-inflammatory compounds, as discussed before. Systemic infections due to bacterial, fungal or viral agents are common in the small premature infants. Experimental studies suggest
that n-3 LC-PUFAs may blunt the response to endotoxin and modulate undesirable sequelae secondary to sepsis by decreasing the production of inflammatory cytokines [103]. IL-1 and TNF produced from stimulated mononuclear cells have a potent inflammatory and catabolic effect. The feeding of n-3 LC-PUFA supplements to young piglets given endotoxin reduces the lactic acidosis, maintains or improves tissue perfusion of the intestine, heart, and lung. Studies in critical adult patients, using an enteral nutrition product that contains n-3 LC-PUFAs, arginine and nucleic acids, demonstrate a beneficial effect on clinical outcome possibly mediated by a modulation of the inflammatory and immune response. However, these data cannot be ascribed to isolated LC-PUFA supplementation [108, 109].

Neurologic and Sensory Development

The effect of LC-PUFAs on brain development was the topic of a recent meeting published as a supplement to the Journal of Pediatrics (October 2003), thus we will only discuss selected aspects. Preterm infants are considered particularly vulnerable to EFA deficiency given the virtual absence...
of adipose tissue at birth, the possible immaturity of the FA elongation/desaturation pathways and the inadequate α-LNA and DHA intake provided by formula. Over the past decades, several studies have examined effect of LC-PUFAs on plasma and tissue lipid composition, retinal electrophysiologic function, on the maturation of the visual cortex as measured by pattern

Table 1. Expression analysis of genes related to inflammation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genebank Acc. No.</th>
<th>DHA/BSA</th>
<th>OA/BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAF family member-associated</td>
<td>U63830</td>
<td>0.3</td>
<td>1.3</td>
</tr>
<tr>
<td>NF-κB activator TANK</td>
<td>AB000509</td>
<td>3.9</td>
<td>0.8</td>
</tr>
<tr>
<td>TRAF5</td>
<td>L19067</td>
<td>3.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Monocyte-derived neutrophil chemotactic factor (MDNCF)</td>
<td>Y00787</td>
<td>6.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Macrophage colony-stimulating factor (M-CSF1)</td>
<td>M27087</td>
<td>2.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Monocyte to macrophage differentiation</td>
<td>X85750</td>
<td>3.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Endothelial cell protein C/AC receptor (EPCR)</td>
<td>L35545</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Endothelial-monocyte activating polypeptide II</td>
<td>U10117</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Endothelial differentiation protein (edg-1) gene</td>
<td>M31210</td>
<td>1.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Platelet activating factor acetylhydrolase</td>
<td>D63391</td>
<td>5.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Leukocyte platelet-activating factor receptor</td>
<td>M76676</td>
<td>2.1</td>
<td>0.5</td>
</tr>
<tr>
<td>GM-CSF receptor</td>
<td>M64445</td>
<td>5.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Transforming growth factor-b type III receptor</td>
<td>L07994</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Transforming growth factor-β (TGF-β)</td>
<td>M60315</td>
<td>4.1</td>
<td>0.6</td>
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<tr>
<td>TGF-β receptor interacting protein 1</td>
<td>U36764</td>
<td>2.3</td>
<td>0.5</td>
</tr>
<tr>
<td>TGF-βIR α</td>
<td>D50683</td>
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<td>0.3</td>
</tr>
<tr>
<td>TGF-β inducible early protein (TIEG)</td>
<td>U21847</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Tumor necrosis factor receptor</td>
<td>M33294</td>
<td>1.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Heparin-binding EGF-like growth factor</td>
<td>M60278</td>
<td>3.3</td>
<td>0.7</td>
</tr>
<tr>
<td>MAPKAP kinase (3pK)</td>
<td>U09578</td>
<td>5.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Transcription factor IL-4 Stat</td>
<td>U16031</td>
<td>3.4</td>
<td>0.6</td>
</tr>
<tr>
<td>ICAM-2 cell adhesion ligand for LFA-1</td>
<td>X15606</td>
<td>5.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Tissue inhibitor of metalloproteinases-3</td>
<td>U14394</td>
<td>3.1</td>
<td>1.1</td>
</tr>
<tr>
<td>MEK5</td>
<td>U25265</td>
<td>3.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Cyclin-dependent protein kinase</td>
<td>U79269</td>
<td>4.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Cdk-inhibitor p57KIP2 (KIP2)</td>
<td>U22398</td>
<td>0.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

The ratio DHA/BSA or OA/BSA refers to transcript abundance in explants exposed to DHA or oleic acid (OA) complexed to BSA, compared to the level in their corresponding controls exposed only to BSA.
reversal visual evoked potentials (VEPs) and behaviorally by the forced-choice preferential looking (FPL) visual acuity response [24, 25, 110–112]. The largest collaborative multicenter study of a large group of preterm infants included 450 preterm infants fed LC-PUFA formula supplemented with different AA and DHA sources: fish oil and egg phospholipids or fungal oil. The level of DHA was 0.25% of total fat in preterm formulas and 0.15% in follow-up formula, both formulas contained 0.4% AA. Significant differences were found in sweep VEP at 6 months favoring the LC-PUFA-supplemented formula group as compared to the control formula group. No differences in behavioral test of visual acuity (Teller cards), or in Fagan habituation or Macarthur vocabulary tests were found. For infants below 1,250 g at birth an advantage in the LC-PUFA-supplemented group in the Bayley score was observed at 12 months. Long-term follow-up of these infants or from other studies has not been reported [28].

In term infants the question of whether healthy full-term infants need LC-PUFAs in their formula has received great attention over the past decade. The finding of lower plasma DHA concentrations in infants fed formula compared to that of breast-fed infants suggests that formulas provide insufficient LNA or that chain elongation-desaturation enzymes are not sufficiently active during early life to support optimal tissue accretion of DHA. Full-term infants also appear to be dependent on dietary DHA for optimal functional maturation of the retina and visual cortex [29, 30, 32, 88, 93, 113–126]. In an attempt to control for the confounding effect of artificial feeding Gibson et al. supplemented mothers to produce breast milk with a DHA concentration ranging from 0.1 to 1.7% of total FA. Infants’ plasma and erythrocytes phospholipid DHA content was related to breast milk DHA in a saturable manner, no significant increase was noted in blood DHA content with a breast milk DHA of >0.6% of total FA. Infant VEP acuity had no relationship with DHA groups and the developmental quotient at 12 months was significantly but weakly correlated to breast milk DHA [121]. At 24 months this was no longer evident. A behavioral study on 44 term infants fed a combined DHA and AA-supplemented formula or a control formula during the first 4 months demonstrated that habituation tests at 4 months are better in the LC-PUFA-supplemented formula [122]. Infant cognitive behavior was assessed at 10 months of age by a means-end problem-solving test [29]. The LC-PUFA-supplemented group had significantly more intentional solutions and scored higher than infants fed a non-LC-PUFA-containing formula. Higher problem-solving scores in infancy are related to higher childhood IQ scores [123]. Both these studies are limited in their extrinsic validity because of small sample size and rather homogenous populations. Birch et al. have shown a persistent benefit on visual acuity development for the first year of life in DHA-supplemented formula-fed infants compared with infants fed formula with ample LNA but devoid of LC-PUFA. In the supplemented groups the formula given for the first 17 weeks of life contained 0.35% DHA with or without 0.72%
AA derived from single cell oil sources. The dietary effects on visual acuity development were evident using sweep VEP acuity but not evident if the behavioral measure of acuity, the FPL method, was used. Supplemented groups receiving DHA or DHA+AA and the breast milk group had better acuity. The differences were significant during the periods of rapid changes in development of acuity, the first 20 weeks and after 35 weeks of age [27]. The developmental outcome of formula-fed infants was also reported [32]. The Bayley mental developmental index (MDI) at 18 months was also significantly better in both groups with DHA. A 7-point normalized MDI score difference between formula with or without LC-PUFAs was noted despite the relatively small sample size ($n = 20$ per group). The small variability in developmental score obtained was likely due to the highly homogenous population studied and the fact that one observer evaluated behavior in all subjects. This is the first randomized controlled study that reports a LC-PUFA effect on mental development at 18 months of age. Moreover, positive significant correlations between blood DHA levels with measures of visual acuity during the first year of life and mental development at 18 months were noted [32]. The existence of a relationship between biochemical and functional data suggests that both phenomena be causally related. Lucas et al. did not find a beneficial effect of LC-PUFAs supplementation in a large group of infants ($n = 309$) randomized to controlled formula diets with and without LC-PUFAs, or breast fed ($n = 138$). Detailed follow-up study of term infants through 18 months of life revealed no benefit or adverse effect of LC-PUFA supplementation on cognitive, motor development, infection, atopy or formula tolerance. However, the interpretation of these data is limited by the fact that formulas differed in several FAs and not only by the presence or absence of LC-PUFAs. In addition, the expected higher IQ of breast-fed infants was not apparent in this study and no biochemical evaluation of how dietary LC-PUFAs affected infant EFA status was included [91].

No study on the long-term follow-up of term infant has been reported, but hopefully results will be forthcoming. Unfortunately, as described in our recent publication, the experimental designs, the level and mix of EFA and LC-PUFAs tested differed greatly among studies. Some term studies provide 0.35% DHA while others have provided as low as 0.1%. These values are in the very low to mid range of the mean DHA content derived from combined data on human milk composition of omnivorous women determined around the world.

Several studies have demonstrated significant effects of the dietary LC-PUFAs on visual maturation in the first 4 months of life, but in most cases the delayed response normalizes at 6 months or at most by 1 year of age. These phenomena should not be dismissed as transitory and of limited significance; we should assume that we failed to detect a significant change at a year because our tools were not sensitive enough, or that in fact other related functions are indeed affected. For example, in our studies we failed to
detect differences in visual acuity at 6 months but space perception assessed by stereoacuity responses was different at 3 years of age [93]. From this, we can conclude that unless sensitive outcome measures are used and a sufficient follow-up interval is provided, it is impossible to fully discard the possible long-term consequences of early developmental effects. The duration and reversibility of diet-induced effects are important considerations in evaluating diet-induced changes in developmental outcomes. There may be transient effects that reflect the acceleration or the slowing of a maturational process with a fully normal final outcome. This is of special relevance during the first few months of life when visual maturation is progressing rapidly.

Several potential mechanisms by which early dietary EFA supply may affect visual and brain maturation and long-term function can be outlined based on the available experimental data. The potential role of DHA as a modulator of membrane properties can be supported by the in vitro studies of membrane fluidity and transport in neural cells modified in their membrane FA. The role of DHA in amplifying the photo transduction cascade is supported by the electrophysiological findings in animals and humans. Decreased retinal rod cell threshold means that less light is required to trigger a response, higher maximum amplitude means that more signal is being transmitted to the visual pathway. Moreover the discovery of biochemical differences in phosphorylated microtubular associated proteins in neurons from the visual cortex of light-deprived kittens during early development provide a mechanism for the classical observations by Hubel and Wiesel [127]. Microtubular proteins play a key role in the dendritic arborization and interconnections in the cortex; darkness inhibited the expression of this gene product [128, 129]. Gene expression is modulated by both sensory stimuli as well as by specific nutrients. The latter effect is shown by our experiments with human fetal retinal explants treated with DHA or oleic acid. The expression of 14% of all retinal genes studied were overexpressed when retinal explants were provided DHA at physiologic concentrations, while less than 1% was overexpressed with oleic acid exposure. Transcripts displaying changes in abundance encode proteins involved in a variety of biological functions; however, housekeeping genes were minimally affected.

Rotstein et al. have reported that in rats deficient in n-3 FAs, both rod outer segment growth and the amount of rhodopsin are effected by DHA in n-3 FAs [70]. The results suggest that DHA action during retinal development could in part be explained by direct or indirect modification of gene expression. Thus the interactive role of LC-PUFAs and light-mediated stimuli offers a plausible explanation for the phenomenon of a critical period for ocular dominance that has a biochemical basis as well as structural and functional correlates [130–132]. We speculate that, by affecting light transduction early on in life, DHA may have long-lasting effects on the organization and function of the visual cortex (fig. 7). The fact that human milk-fed infants exhibit more
mature stereo-acuity at 3 years relative to formula-fed infants shows that this phenomenon may be indicative of long-term effects relevant to the human [93].

**Hypoxia Reperfusion Injury**

Brain injury (ischemia and hemorrhage) due to hypoxic and hemorrhagic insults to the neonatal brain is not infrequent, especially in preterm infants. Most ischemic injury occurs prior to or at birth; intraventricular hemorrhage is detected mostly in the first hours of life. Thus, it is difficult to propose a nutritional prevention for these conditions, unless the intervention is given to the mother. Whether maternal dietary LC-PUFA supply could play a role in defining the occurrence and severity of brain hemorrhagic injury is not known. Based on limited data from animal observations, Crawford et al. [133] have speculated that poor maternal dietary LC-PUFA supply could be responsible for the high prevalence of hemorrhagic injury observed in small preterm neonates. In addition the possibility of dietary modulation of cytokine release should be considered, since cytokines mediate much of the vascular and tissue damage observed during and after reperfusion. Evidence of a specific role of inflammation and cytokine release in periventricular leukomalacia has been proposed [134, 135]. A pharmacological modulation of AA metabolism is presently used in the form of indomethacin, a potent inhibitor of cyclooxygenase. The effect of early lipid supply on brain injury deserves further research.
At a pathological level, there are experimental data suggesting a protective role of DHA in brain ischemic damage. Okada et al. [136] have demonstrated that chronic administration of a DHA-rich diet reduces the brain ischemic damage observed through a reduced spatial cognitive deficit and decreases in the amount of damaged neurons in the hippocampus. In a different model, Glozman et al. [137] evaluated the generation of thiobarbituric acid (TBARS) as an indicator of malondialdehyde generated by lipoperoxidation which plays a key role in the pathogenesis of the ischemia. The brain of newborn rats provided DHA by injection into the amniotic sac generates less TBARS after an ischemic episode. This reduction in TBARS generation is paralleled by a significant increase in esterified DHA in brain phospholipids. The results indicate that EFA components of brain membrane phospholipids serve as targets for reactive oxygen species, which participate in the onset of neurodegenerative disease. The question is whether appropriate amounts of EFAs would prevent or retard the onset of these conditions. The results have shown that the consumption of EFAs does not increase the damage induced by reactive oxygen species. In contrast, Calviello et al. [138] reported that low doses of DHA and EPA substantially modify membrane composition without increasing susceptibility to oxidative stress.

An increase in brain DHA content decreases lipid peroxidation and protects from ischemia [137, 139]. Furthermore, Hossain et al. [140] have shown that the intake of DHA increases brain glutathione peroxidase and catalase enzyme activity by 25%, and also increases reduced glutathione in aged hypercholesterolemic rats. This is correlated with an increase in the ratio DHA/AA in brain phospholipids [140]. The antioxidant effect of DHA has also been reported in liver. Venkatraman et al. [141] showed that, following consumption of a 10% fish oil diet, there were significant increases in rat liver antioxidant defenses as compared with those receiving a corn oil diet. In addition AA has been demonstrated to be involved in memory and learning functions, particularly in the long-term potentiation (LTP) paradigm where AA is thought to have a function as a retrograde messenger. The finding that the AA concentration is decreased in the hippocampus of aged rats in which LTP is compromised is consistent with a role for AA in memory. This is supported by the observation that dietary supplementation of aged rats with AA and its precursor GLA restored the AA concentration to that observed in the hippocampus of young rats and reversed the loss of LTP [142, 143].

As discussed above, DHA is a substrate for lipid peroxidation. Given its high content in the retina and brain, DHA has been considered the major source for lipid peroxides that would participate in neural injury and inflammation. However, experiments based on different neuronal cell models suggest a protective role for DHA, which is also consistent with data derived from studies in vivo. A likely explanation for the discrepancy has been provided by a recent report showing that selected docosanoids derived from DHA, such as 10,17S-docosatriene (DCT), play a potent anti-inflammatory
role. These DHA derivatives inhibit leukocyte infiltration, inflammatory gene expression and cytokine production in hypoxia-reperfusion injury, decreasing by half the damage induced by arterial occlusion if DCT is infused during the recovery from hypoxia [144].

**Nutrition-Related Chronic Diseases: Obesity, Diabetes, Hypertension, Dyslipidemias (Metabolic Syndrome)**

As discussed above (p 111), LC-PUFAs affect the expression of genes subject to transcriptional activation by PPARs, and thus may contribute to the regulation of fuel oxidation, lipid and glucose metabolism, fuel partitioning, adipocyte growth and maturation. The long-term effects of LC-PUFA supply on nutrition-related chronic diseases (NRCDs) in addition to their effect on gene expression with direct bearing on glucose and lipid metabolism, and on adipose tissue growth and maturation are depicted in figure 8. The increased risk of NRCDs in infants born with intrauterine growth restriction (IUGR) is presently being recognized. The hormonal metabolic adaptation to IUGR during fetal life is associated with an increased risk of the metabolic syndrome (insulin resistance, hypertension, visceral obesity and cardiovascular disease) in later life. Thus present research efforts to prevent NRCDs in IUGR include the promotion of optimal lean body mass growth and the supply of n-3 LC-PUFAs. IUGR results from the failure of the placenta to provide the necessary nutrients required by the fetus to maintain adequate growth.

We have recently completed studies of LC-PUFA metabolism using stable isotopes that suggest a defect in DHA biosynthesis in IUGR infants (unpublished work). Thus, altered LC-PUFA metabolism should be considered as a potential mechanism to explain the observed increased prevalence

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**Fig. 8.** Effect of PPAR transcriptional control of genes related to risk factors for chronic disease.
of the metabolic syndrome in adults born as IUGR infants. During the last trimester of gestation there is a significant accumulation of LC-PUFAs in the fetus and an increase in the relative content of both n-6 and n-3 LC-PUFAs in the human brain and retina. The intrauterine accretion and the supply of LC-PUFAs in early life are critical in determining the concentration in plasma and tissue pools. Fetal tissue content of LC-PUFAs is dependent upon maternal intake and on an adequate placental transfer. Thus, a greater risk of insufficient LC-PUFA supply occurs in pregnancies complicated with abnormal placental function affecting nutrient transfer. Results obtained from IUGR animal models indicate that placental insufficiency is associated with abnormalities in fetal lipid metabolism of skeletal muscle and liver. A possible mechanism for the observed changes is the abnormal expression of PPAR proteins [145, 146]. Cetin et al. reported that IUGR fetuses have a lower proportion of long-chain n-6 and n-3 FAs (AA and DHA) relative to the precursors (LA and LNA) in fetal blood in comparison to maternal blood than is found in appropriate for gestational age control infants [147]. Studies evaluating EFA status in IUGR infants, most of them premature, compared to controls using either umbilical cord blood, the umbilical artery vein wall or the placenta reported lower EFA status in IUGR infants [148–150]. These studies suggest an impaired LC-PUFA formation or increased catabolism in fetuses affected by IUGR. Our studies in human neonates using stable isotope (2H or 13C)-labeled LA and LNA to assess in vivo metabolic formation of AA and DHA, respectively, provide clear evidence that term and preterm infant are able to synthesize AA and DHA from the parent EFAs, albeit in small amounts [151]. Our results indicate that after receiving labeled LA and LNA, IUGR infants have a significantly lower conversion of precursors to LC-PUFAs relative to the control groups matched by body weight and by gestational age. There is a significantly decreased formation of DHA from LNA to DHA for the body weight-matched control group (p < 0.01) but not for the gestational age-matched control group (p = 0.07). The ratio of DHA to LNA is 2-fold greater in the gestational age controls and 3-fold higher in the body weight controls relative to the IUGR infants. The elongation step forming DPA from EPA appears to be relatively insensitive to body weight or gestational age, while the marked differences in the DHA/DPA ratio suggests that the peroxisomal partial β-oxidation step is affected in IUGR infants. Consistent with this, the biosynthesis of AA from the precursors did not differ significantly in the IUGR and the two comparison groups. The formation of AA does not require peroxisomal β-oxidation as presented above (p 104). The finding of abnormal DHA biosynthesis could explain why IUGR infants are more vulnerable to impaired glucose and lipid metabolism in later life. In fact, recent observations on infants who received LC-PUFAs early on in life revealed a lower systolic blood pressure as compared to the randomized control formula-fed, and no difference with the human milk-fed babies [152]. These results suggest that IUGR infants could benefit from nutritional
intervention in order to improve their LC-PUFA supply in utero as well as in early postnatal life to secure optimal growth, glucose and lipid metabolism, and improved developmental outcomes.

**Conclusions**

The data from animal studies and the preliminary data from human studies presented in this review suggest that the supply of essential lipids in early life may condition not only short-term effects related to growth, neurosensory maturation and mental development but could also contribute in determining the susceptibility to allergic disease and immune responses, condition the severity of inflammatory responses, and possibly modify the risk of diet-related chronic disease linked to the metabolic syndrome (hypertension, insulin resistance, obesity, and cardiovascular disease). The long-term clinical significance of the experimental findings discussed is hard to determine from the existing data, additional information from long-term follow-up of controlled feeding studies is needed before this issue can be resolved.

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Dietary Essential Fatty Acids in Early Postnatal Life

Dietary Essential Fatty Acids in Early Postnatal Life

Dietary Essential Fatty Acids in Early Postnatal Life


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Dietary Essential Fatty Acids in Early Postnatal Life


Dietary Essential Fatty Acids in Early Postnatal Life

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Discussion

Dr. Steenhout: Thank you for your extensive talk. If I summarize, I am convinced as you are about the benefit of docosahexaenoic acid (DHA), but I am a little bit concerned about the role of arachidonic acid. There are recent publications on the role of long-chain polyunsaturated fatty acids (LC-PUFAs) in the development of obesity, for example the article published by Hsu and Ding [1] in the *British Journal of Nutrition* showing how important DHA is in the transformation of pre-adipocyte in adipocyte, or the recent publication by the Massiera et al. [2] in France showing that arachidonic acid and prostacyclin signaling promote adipose tissue development and raising the question whether it is a human health concern. So what are we doing when we start supplementing infant formula with both n-6 and n-3 for all the infants? To come back to a question raised earlier by Dr. Di Renzo about what should be the optimal ratio between n-3 and n-6. In the US, based on breast milk data, it is a common consensus to go for supplementation with a ratio of 2 for arachidonic acid to 1 for DHA. What could be the influence of such a high ratio on the different metabolic pathways? You mentioned data on the evolution of breast milk. Considering the dogma of breast milk as a reference, are we not just transposing some data on the actual evolution of the mother’s diet and should we not reverse this evolution by trying to have a diet with less inflammatory lipids, more DHA, and perhaps some additional γ-linoleic acid as mentioned by Dr. Hornstra? What are your comments?

Dr. Uauy: The point I think we need to have very clear is that the outcomes can no longer be weight. We need outcomes that relate to body composition early on, we need long-term outcomes. In the absence of control studies to provide for that we have to rely on ecological data. If we look at the Orient there is less obesity; although now with the changes in diet there is more obesity. For example human milk, DHA in the Orient is around 0.6%, if not higher. Arachidonic acid is about 0.6% or 0.5%, the ratio is 1 to 1. There has been an uncontrolled experiment changing the Western diet in terms of fat for the last 200 years. Who knows what the consequences are. If you look at the ratio in Japan, their daily diet has a ratio of n-6 to n-3 of 4 to 1. A typical Western diet has 20 to 1, corn oil has 50 to 1, sunflower has 150 to 1. These changes may be good to lower cholesterol but in fact they may not be good for the rest of the effects derived from n-6 fatty acid intake. By the way, the effect of DHA and n-3 supplementation is in fact less adiposity in all of the animal data we have. Whether we reproduce that at the levels provided in human milk, who knows. We know that there are metabolic effects. Bauer [3] has confirmed that the membrane composition of breast-fed babies is different, and insulin sensitivity has been related to the fatty acid composition of the muscle and adipose tissue of human babies. We will need better studies before we have the right ratio. If I were you, I would be looking at what is maternal milk, human milk, in areas that have low morbidity and mortality and healthy life years ahead. I would be looking at China and Japan more than Texas or Central USA or other populations that are not the paradigm of health.

Dr. Hornstra: Perhaps to follow up this particular discussion, in our studies in which we try to relate fatty acid exposure during gestation with obesity 7 years later, there was no relation with arachidonic acid during gestation. There was no relationship, at least not a significant one, with DHA either. The only fatty acid that was related to body fatness, obesity, insulin sensitivity, etc., was γ-linolenic acid. We investigated other fatty acids as well, but found no relationship [4].

Dr. Steenhout: In your studies on pregnancy, you are not speaking about supplementation?

Dr. Hornstra: I am speaking about my observational studies in which there is a wide difference in exposure. As I demonstrated in one of my slides, the difference in
exposure for arachidonic acid was 2-fold and the difference in exposure for DHA was 3-fold. For γ-linolenic acid, the concentration range was between 0 and 0.1%.

*Dr. Bleker:* If I remember well at the start of your impressive talk you showed immense differences in DHA metabolism between growth-retarded and preterm infants with the same gestational age or even same birth weight and longer gestational age. Have you ever studied the therapeutic or interventional consequences of that?

*Dr. Uauy:* I think these are very early data that were presented at the SRP last year and have now been accepted for publication. They actually correspond with data on blood levels, but of course this is much stronger evidence that there may be an impaired peroxisomal function in intrauterine growth-retarded (IUGR) infants, which means that perhaps, rather than keeping measuring and weighing IUGR infants and trying to optimize catch-up growth, we probably should do something more and potentially start to look at how we can optimize lean body mass growth and do more than just look at weight gain. Possibly, changing the quality of the fat supply might do something to prevent the metabolic syndrome. I think for now it is a hypothesis worth testing because we know there are animal data. Perhaps it is good that we do control studies or we look at IUGR babies who have predominantly been fed human milk versus cow’s milk formula. Even most developing countries are just using routine cow’s milk formula.

*Dr. Kramer:* Just an additional point about breast feeding versus breast milk. All of the discussions focus on differences in n-3 LC-PUFA content between various formulas or between breast milk and formula. I just want to add that breast feeding is different from formula feeding, not just in the composition of the milk but also in the physical act of breast feeding. I am sure you are familiar with some of the studies by Meaney [5] on the effects of maternal grooming. There are epigenetic mechanisms that affect gene expression, which may have as much or more to do with metabolic programming and cognitive and brain development than the LC-PUFA contents.

*Dr. Uauy:* I fully agree with that. The whole hormonal milieu that is generated for mother and infant is not reproducible. This is not about the comparing formula to human milk, but in fact is about what to do with formula. Should we model formula based on the properties of human milk. For example in terms of anti-inflammatory properties, breast milk is loaded with acetylhydrolase, there are also growth factors that may be contributing to the maturation of the gut. We know that several brush border enzymes are affected by human milk in unique ways. So this is not to do with saying that we can do as good as breast milk, but I think we should be inspired by breast milk for the non-breast-fed infants, which should be the exception.

*Dr. Kramer:* What I am saying is that it would be a good idea in some of these experiments to have an additional group that gets the same breast milk but in a bottle or a nipple versus being breast-fed, just to see what the difference is in terms of gene expression. It might be interesting.

*Dr. Hornstra:* To follow up on that – a study has been done by Lucas et al. [6] investigating the relationship between the type of feeding and infant intelligence. They added a group that received breast milk by bottle.

*Dr. Uauy:* That is an everyday group in the newborn nursery because babies cannot be put to the breast before 34 weeks.

*Dr. Hornstra:* That is right, and it was very clear that also in these human milk, bottle-fed babies there was a cognitive advantage, but whether that has to do with the fatty acids only or with different substances in the milk is not know for sure.

*Dr. Butte:* Is there any evidence of a detrimental effect of DHA supplemented during organogenesis? Of course I am concerned that DHA is very popular, it is sold over the counter as a brain food. Is there any possible abuse during this critical period?

*Dr. Uauy:* The obvious issue, and when you look at the eicosanoid cascade it is bleeding because of the effects on platelets and in fact if you give over 3 g to an adult
you have a dose response of increased bleeding time that is dependent on the n-3 fatty acid load given. Now what the optimal bleeding time is, is also arguable. The normal bleeding time that we have is modeled also by the type of fat we consume. At the level we are talking about, we measured bleeding time in the tiny premature being studied when we were using fish oil as a source on long chain n-3 fatty acids and were giving 0.4 DHA, which meant 0.6 eicosapentaenoic acid (EPA), so 1% of the formula was long-chain n-3 PUFAs. We observed a very transient effect in the premature, so in the first 6 weeks of life, slightly increased well within the norm, the change was about 10% but because we had a very precise system to measure the bleeding time this was significant. In fact in hypercholesterolemic and hypertriglyceridemic people are actually given 3, 4, 5, 12 g per day in the case of children some started to develop epistaxis (nasal bleeding). I think that the key issue here is that I would advise people to stay within the normal variations of diet, so if you go to fish-eating populations people would be consuming a couple of grams at most of EPA + DHA. (This sentence is not needed, is an adult study and has not been published). For hypertriglyceridemia you have to use between 3 and 12 g/day, so there is an evidence base to look at the safety side. I don’t think we have a dose response yet for a lot of these effects, but obviously that is something that we have to explore.

Dr. Butte: But what about an animal model? Is there any evidence during organogenesis? I am thinking of the dramatic effect on gene expression and the such.

Dr. Uauy: The data from Japan and most of the animal studies are replacing all saturated fat by n-3 or n-6 PUFAs. So it is probably not translatable to a human setting. Under those conditions, they have found a decrease in lipogenesis with fish oil at high intakes. Mainly decreasing visceral fat, this may be advantageous, and in fact that is why I think doing things within the ranges of common human dietary exposures, where you have epidemiologic data on relative health or relative morbidity of the population is the best way to advance research in this area. But I am sure that if you look in detail there will be some effects.

References