1.1. Biochemistry of essential fatty acids

1.1.1. Introduction

Essential fatty acids (EFA) are important components of structural lipids and contribute to the regulation of membrane properties like fluidity, flexibility, permeability and modulation of membrane-bound proteins. Linoleic acid (LA, 18:2ω6) and α-linolenic acid (ALA, 18:3ω3) are the two parent EFA. The term 'essential' implies that they must be supplied in the diet because they are required by the human body and cannot be endogenously synthesised. The balance between ω3 and ω6FA in the diet is important because of their competitive nature and their different biological roles. Both parent EFA are metabolised to long chain polyunsaturated fatty acids (LCPUFA) of 20 and 22 carbon atoms. EFA and LCPUFA may together be referred to as polyunsaturated fatty acids (PUFA). Some LCPUFA, notably dihomo-γ-linolenic acid (20:3ω6), arachidonic acid (20:4ω6; AA), and eicosapentaenoic acid (20:5ω3; EPA) are precursors of a wide variety of short-lived regulatory molecules such as prostaglandins (PG), thromboxanes (TX) and leukotrienes (LT), together called eicosanoids. They are involved in inflammatory and anti-viral reactions, endothelial integrity and many more. LCPUFA, especially docosahexaenoic acid (22:6ω3; DHA), play important roles in the development of the central nervous system, including the retina [1-6]. Dietary (LC)PUFA and their derivatives gain increasing interest as modulators of gene expression by their capacity to act as ligands of peroxisome proliferator activated receptors (PPARs) and to suppress the expression of sterol regulatory element binding proteins (SREBPs). These are nuclear receptors that can be regarded as main switches in the co-ordinated expression or repression of a variety of (key) enzymes in FA synthesis and oxidation, lipogenesis, glucose utilisation and insulin sensitivity, thermoregulation, energy partitioning, reverse cholesterol transport, cholesterol synthesis, low-density-lipoprotein-receptor expression, growth and differentiation, and inflammatory responses [7-9].

1.1.2. Nomenclature

The systematic name for a fatty acid (FA) is derived from the name of its parent hydrocarbon by substitution of *oic* for the final *e*. For example, the C18 saturated FA is called octadecanoic acid. The common (trivial) name is stearic acid. Apart from these systematic and common names a shorthand notation can be used. The first number is the number of carbon atoms in the molecule. The second number, after the colon, is the number of double bonds. The last number indicates the number of methylene carbons from the methyl carbon (ω) end to the nearest double bond. Linoleic acid is designated 18:2ω6, which means 18 carbon atoms with two double bonds, the first one between carbon atoms 6 and 7 (Figure 1). The double bonds in almost all biologically occurring FA are in the *cis* configuration [4]. A list of common FA, including systematic and trivial names and shorthand notation is given in Table 1.

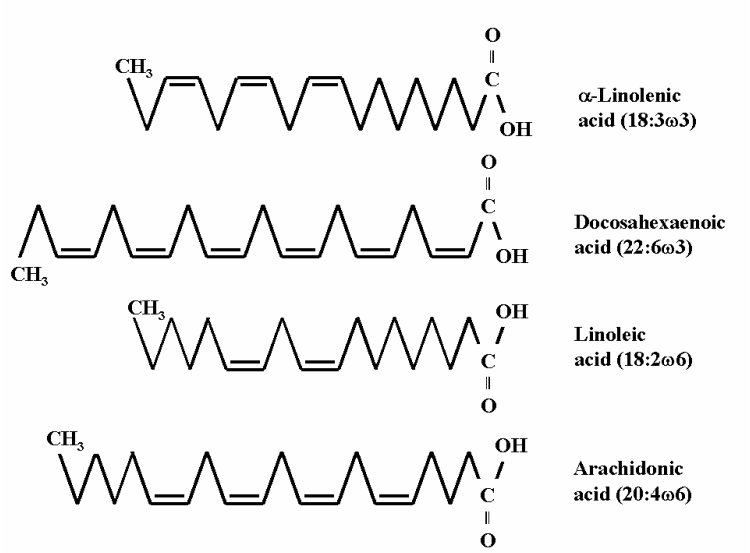

Figure 1. Structural formulas for α-linolenic acids (18:3ω3), docosahexaenoic acid (22:6ω3), linoleic acid (18:2ω6) and arachidonic acid (20:4ω6). The first number gives the number of carbon atoms, the second gives the number of double bonds. ω3 and ω6 indicate the posotion of the first double bond.

1.1.3. Digestion, absorption and transport

Triglycerides (TG) constitute the majority of lipids in the diet. They must be broken down into glycerides and FA, before they can be absorbed in the duodenum. Hydrolysis by gastric and pancreatic lipase produces free FA (FFA), monoglycerides (MG) and diglycerides (DG). Most of these are incorporated into bile micelles, which are tiny particles, composed of bile salts, phospholipids (PL), MG and FFA. Micelles are water-soluble and carry the FFA and MG to the jejunal brush border for uptake. Within the mucosal cell the FFA and MG are re-esterified to TG. The latter are incorporated into chylomicrons and secreted into the lymph to be transported to the subclavian vein. Via the bloodstream the lipoproteins transport the lipids through the body to tissues where they are needed as energy source, membrane components, precursors of biological active metabolites or storage [4].

1.1.4. Metabolism

1.1.4.1 *Endogenous synthesis*

When the fat content of the diet is low, rates of FA synthesis in the liver increases. Endogenous synthesis yields mainly palmitic and stearic acid (16:0 and 18:0, respectively), which can subsequently be desaturated by Δ9-desaturase to the monounsaturated FA (MUFA) palmitoleic and oleic acids (16:1ω7 and 18:1ω9, respectively). LA limits 18:1ω9 synthesis by inhibiting desaturation of 18:0 [4].

Table 1. Systematic and common names of selected fatty acids and their shorthand notation.

Systematic name	Common name	Shorthand notation
Butanoic	butyric	4:0
Hexanoic	caproic	6:0
Octanoic	caprylic	8:0
Decanoic	capric	10:0
dodecanoic	lauric	12:0
tetradecanoic	myristic	14:0
hexadecanoic	palmitic	16:0
heptadecanoic	margaric	17:0
octadecanoic	stearic	18:0
eicosanoic	arachidic	20:0
docosanoic	behenic	22:0
tetracosanoic	lignoceric	24:0
hexacosanoic	cerotic	26:0
9-hexadecenoic	palmitoleic	16:1ω7
11-octadecenoic	*cis*-vaccenic	18:1ω7
9-octadecenoic	oleic	18:1ω9
11-eicosenoic	eicosenoic	20:1ω9
5,8,11-eicosatrienoic	Mead's	20:3ω9
15-tetracosenoic	nervonic	24:1ω9
9,12,15-octadecatrienoic	α-linolenic, ALA	18:3ω3
6,9,12,15-octadecatetraenoic	stearidonic	18:4ω3
5,8,11,14,17-eicosapentaenoic	timnodonic, EPA	20:5ω3
7,10,13,16,19-docosapentaenoic	clupanodonic, DPA	22:5ω3
4,7,10,13,16,19-docosahexaenoic	cervonic, DHA	22:6ω3
9,12-octadecadienoic	linoleic, LA	18:2ω6
6,9,12-octadecatrienoic	γ-linolenic, GLA	18:3ω6
11,14-eicosadienoic		20:2ω6
8,11,14-eicosatrienoic	dihomo-γ-linolenic	20:3ω6
5,8,11,14-eicosatetraenoic	arachidonic, AA	20:4ω6
7,10,13,16-docosatetraenoic	adrenic	22:4ω6
4,7,10,13,16-docosapentaenoic	DPA	22:5ω6

1.1.4.2 *Desaturation and elongation*

Oleic acid, LA and ALA are metabolised by a series of alternating steps of desaturation (removal of two hydrogen atoms and thereby insertion of an extra double bond) and elongation (addition of two carbon atoms), which take place in the endoplasmatic reticulum (Figure 2). The desaturase enzymes show preference for FA from the various series in the order ω3>ω6>ω9. The Δ6- and Δ5-desaturation steps are generally considered to be rate limiting in LCPUFA biosynthesis [1,3,4]. Delta-6-desaturase activity is inhibited by high levels of both its products and precursors and influenced by dietary factors and a number of hormones [10,11]. High intake of carbohydrates decreases Δ6-desaturation activity,

whereas proteins are activators [11-13]. Deficiency of the minerals iron, zinc, selenium and magnesium all seem to reduce Δ6- and/or Δ5-desaturase activity [3,14]. The hormones glucagon, epinephrine and thyroxine are depressors of Δ6-desaturase activity, while insulin can be regarded as an activator [11]. It should however be kept in mind that almost all of these observations are based on animal studies and that they cannot be readily extrapolated to humans [15].

diet		**diet**		**biosynthesis or diet**
				18:0
			Δ9	↓
18:3ω3		18:2ω6		18:1ω9
↓	Δ6	↓	Δ6	↓
18:4ω3		18:3ω6		18:2ω9
↓	CE	↓	CE	↓
20:4ω3		20:3ω6		20:2ω9
↓	Δ5	↓	Δ5	↓
20:5ω3		20:4ω6		20:3ω9
↓	CE	↓	CE	↓
24:5ω3 ← 22:5ω3		22:4ω6 → *24:4ω6*		22:3ω9
↓ *Δ6*	?Δ4	↓	↓ *Δ6*	
24:6ω3 → 22:6ω3		22:5ω6 ← *24:5ω6*		

Figure 2. Desaturation and chain elongation reactions of dietary and endogenously synthesised FA. Δx: Δx-desaturase; CE: chain elongation; ?Δ4: probably composed of three reactions, i.e. chain elongation, Δ6-desaturation and chain shortening.

The conventional view is that the Δ4-desaturation does not involve another specific desaturase, but that it is composed of an elongation, then Δ6-desaturation, followed by chain shortening via the β-oxidation pathway. The last step most likely taking place in peroxisomes [16,17]. An alternative hypothesis proposes two independent desaturation – elongation pathways: a mitochondrial system that synthesises DHA and 22:5ω6, and a microsomal system that is able to synthesise only up to 22:5ω3 and 22:5ω6 [18-20]. In this view, 24:6ω3 and 24:5ω6 are considered to be dead-end elongation product of their respective precursors. Very recently a Δ4-desaturase enzyme has been identified in a common type of marine microheterotroph [21].

1.1.4.3 *Interaction between ω3, ω6 and ω9 fatty acids*

Because ω3 and ω6FA compete for the same desaturation enzymes, alterations of the ALA/LA ratio will affect the composition of their long chain metabolites [22-24]. Clark *et al*. [25] observed the highest EPA levels in infants fed the lowest amount of LA in a study in which term infants were fed formulas with different ALA/LA ratios. Similarly Jensen *et al.* [26] found the highest AA levels in children fed the lowest amount of ALA. Since there is no definitive proof for different Δ6-desaturase enzymes, it implies that 24:5ω3 and 24:4ω6 also compete with ALA, LA and 18:1ω9 for Δ6-desaturation. Consequently, high intakes of ALA and/or LA could have inhibitory effects on endogenous DHA synthesis [26,27]. Indeed Mantzioris *et al.* [28] observed an inverse relationship between ALA intake and DHA levels in different blood compartments of healthy humans. During EFA deficiency (EFAD), desaturation of 18:1ω9 becomes less inhibited by ALA and LA, allowing synthesis of 20:3ω9 (Mead acid). Therefore 20:3ω9 has been widely used as a marker for EFAD [1,4,29].

1.1.4.4 *β-Oxidation*

Next to their role as structural components of cell membranes or as precursors of eicosanoids, PUFA are an efficient source of energy. β-Oxidation to H_2O and CO_2 takes place in the mitochondria and depends upon the presence of carnitine, because long chain FA (C12-C18) can cross mitochondrial membranes only in the form of acyl-carnitine [4].

1.2. Nutritional aspects of essential fatty acids

1.2.1. Introduction

Since EFA cannot be synthesised by the human body LA and ALA need to be derived from the diet. The long chain metabolites, LCPUFA, can be synthesised from their precursors, but only to a limited extent [30], and this process may not be optimal in newborns and in several illnesses [31-33]. In other words, natural sources of LCPUFA may become important at certain circumstances, which are referred to as a state of 'conditional essentiality'. Human milk is the principal source of EFA and LCPUFA for babies. The PUFA content of breastmilk depends mainly on the diet, although it also varies according to time postpartum, gestational age, parity and maternal diseases [34-36]. Like for other nutrients several studies have been undertaken to provide guidelines for daily PUFA intake regarding optimal growth, neurodevelopment and health [37-39]. There are several reasons for the difficulty to determine a minimum requirement for EFA and LCPUFA. The human body can convert parent EFA to LCPUFA, which is on its turn dependent on the relative amounts of the different FA. Secondly, there are no documented plasma or erythrocyte (RBC) FA concentrations representing a biochemical deficiency and finally there are no clinical tests to establish a functional EFAD [37].

1.2.2. Dietary sources

1.2.2.1 *ω3 Fatty acids*

ALA is available from green leafy vegetables, nuts and some vegetable oils such as canola (rapeseed) and soybean oils. Extremely high ALA contents are encountered in perilla (beefsteak plant), linseed (flaxseed) and black currant seed oils. EPA and DHA are found in fatty fish and fish oil (FO) [2,38,40-43]. The most widely used ω3LCPUFA supplements are derived from marine oils. High intake of EPA may reduce AA incorporation into lipids by competition. Reduction of AA in favour of EPA modulates inflammatory reactions in diseases, such as rheumatoid arthritis and cardiovascular disease [44-46]. It is, however, considered undesirable in neonates, since high EPA intake from FO in preterms may be at the basis of the correlation between the diminished first year growth and low AA status found by Carlson *et al*. [47]. Single cell DHA oils from algae and fungi, which contain almost no EPA, have recently become available [48]. Egg yolk PL has been used as a source of both DHA and AA. The AA and DHA contents in egg PL mimic those found in breastmilk of western women [37,38,49].

1.2.2.2 *ω6 Fatty acids*

LA is found in seeds of most plants, except for coconut, cocoa and palm. AA is present in substantial amounts in meat, eggs and certain seafoods [2,50,51]. Single cell oils can contain up to 50% AA, and have been used in several studies to elevate AA levels [48,52]. Evening primrose, borage and black currant seed oils are high in γ-linolenic acid (18:3ω6) and have been used as alternative sources to increase AA levels, however with little effect [3,42,53-55].

1.2.3. Human milk

Human milk contains the full range of PUFA, including small amounts of the whole series of ω3 and ω6LCPUFA [34,35]. For many babies this will be the only source of dietary LCPUFA, since until recently formula milks did not contain LCPUFA [56,57]. Even during weaning breastmilk will be the most important LCPUFA source, because most weaning foods contain only small amounts of egg, meat or fish [58,59]. Human milk may also be an important source of EFA in the so-called 'developing countries', since in those countries oils are often used in only small amounts for the preparation of weaning food [60-62]. The FA composition of human milk is strongly dependent on maternal diet and to a smaller extent to time postpartum, gestational age, parity and some diseases [34-36].

The FA in human milk derive from the diet, biosynthesis in the mammary gland, or mobilisation from tissue stores. The contributions of these sources are estimated at 29, 11 and 59%, respectively [63,64]. Only a small proportion of milk AA originates from chain elongation/desaturation of LA, and the majority of milk LA and AA (70 and 90%, respectively) does not derive directly from the diet [65,66]. Palmitic acid (16:0) and oleic acid (18:1ω9) are the quantitatively most important fractions, together accounting for 35-70% of total FA. DHA and AA account usually for only less than 1% [34,35]. (see Appendix 1. 'Breastmilk fatty acid composition in different populations'). More than 200 FA have been identified in human milk, including *trans*-FA and cyclic monomers [35]. The milk FA composition is not influenced by the sampling method, is the same for both breasts and does not change much during a nursing or during the day [67-71]. Therefore it is relatively easy to collect a milk sample with a representative FA composition. Only in marginally nourished women, or in women consuming diets extremely high in carbohydrates or fat this may be more difficult, since a postprandial response on milk FA composition has been noted [72,73]. Also the ingestion of fish or FO affects milk FA composition within several hours [74].

1.2.3.1 *Maternal diet*

It has been known for many years that the FA composition can be altered by changes in caloric balance, carbohydrate and FA intake [72,75-77]. During energy equilibrium dietary FA are rapidly transferred to milk lipids, whereas in a negative energy balance milk FA composition resembles that of adipose tissue [75,78].

1.2.3.1.(a) Fatty acid intake

Comparison data from different communities reveals that the dietary FA composition becomes reflected by the FA composition of breastmilk. Milk LA is high among women with high intakes of fat mainly from vegetable origin, such as in some Asian or African countries, or in vegetarians [68,78-81]. Their milk LA is significantly correlated with intake of vegetable oils or LA [80,82]. Relatively low amounts of LA have been found in milk of women on low-fat diets and women consuming diets with predominantly animal fat [76,83]. Over the last 20 years the average breastmilk LA content of women from western societies has increased, probably reflecting dietary changes [84,85]. Oleic acid is higher in milk from women consuming a Mediterranean diet that is rich in olive oil (high in 18:1ω9) [71,86]. DHA levels are much higher in milk of women with high intakes of marine foods [86-90]. Although the milk AA content does not seem to be so much influenced by diet and

is remarkably similar in omnivores, vegetarians and vegans [80,81,89,91] higher levels of AA were reported in milk from Egyptian, Nigerian and Chinese women, as compared to milk from women living in western countries [92-94]. Within China, milk AA differed slightly between 5 distinct geographic regions with different dietary patterns [93]. However, in view of the sizeable difference in AA intakes, the differences in milk AA were marginal. Chen *et al*. [93] suggested that the lower AA levels in western countries compared to China may be due to higher intakes of *trans*-FA in western countries, since these are known to inhibit EFA desaturation and elongation.

Several supplementation studies have been performed to study the effects of dietary FA on the milk FA composition more in detail. Providing women with a diet high in PUFA, mainly LA, resulted in high LA levels in milk [75,95-97]. More recently the focus has been on the possibility to increase DHA levels in breastmilk. Harris *et al.* [98] and Henderson *et al.* [74] supplemented women with 5-47 g FO per day for total periods between 8 and 28 days. Helland *et al*. [99] supplemented lactating women with up to 10 ml cod liver oil for 2 weeks. The supplements raised both milk EPA and DHA significantly. Milk DHA increased within 8 hours after supplementation and reached steady state levels within one week [74]. Because of concerns of possible adverse effects of high milk EPA levels, FO with low amounts of EPA, DHA oil from algae and DHA-rich eggs [100-102] have been used in later research. Makrides *et al.* [100] supplemented women with different DHA doses (ranging from 0.2 to 1.3 g DHA/day) for almost 12 weeks and observed a strong dose-dependent effect on breastmilk DHA. In addition, they found a strong correlation between the DHA content of maternal plasma PL and that of milk lipids. Jensen *et al.* [102], who supplemented women with different sources of DHA for 6 weeks, has also observed this correlation. The increase in milk DHA was also reflected in the infant's plasma and RBC PL [102,103]. There appears to be only a minimal effect of dietary DHA on milk AA levels. We [104] supplemented lactating women with either AA (300 mg), or AA plus ω3LCPUFA (110 mg EPA, 400 mg DHA) for one week. Supplementation with AA alone had no effect on breastmilk AA, but tended to reduce EPA and DHA levels, whereas the combination of AA, EPA and DHA tended to increase both milk AA and ω3LCPUFA contents.

1.2.3.1.(b) Carbohydrate intake

Dietary intervention studies by Insull *et al*. in 1958 [75] and Read *et al*. in 1964 [72] have shown that a diet high in carbohydrate and low in fat (or no fat) leads to increased production of *de novo* synthesised lauric acid (12:0) and myristic acid (14:0). Similarly, comparison of different populations showed higher levels of 12:0 and 14:0 in milk from women with a relatively high carbohydrate/low fat intake in countries like Egypt, Nigeria, Tanzania, Mexico and the Caribbean Region, compared to western countries [76,87,91,96].

Because of the strong influence of diet on the milk FA composition it could be expected that women on low fat diets could produce milk that contains insufficient EFA [75,76,105,106]. Moreover, in marginally nourished women both the secreted milk volume and its fat content may be lower than in well-fed mothers [107,108]. The children of these women could therefore be at risk for EFAD [34,105].

1.2.3.2 *Duration of lactation*

The human milk FA composition changes as lactation progresses. FA of the earliest colostrum are derived almost entirely from extra-mammary sources, explaining high levels of 16:0, 18:0 and 18:1ω9 [69,87,109]. Within a few days the proportions of *de novo* synthesised 12:0 and 14:0 start to increase, probably reflecting maturation of the mammary gland [69,71,80,87,109,110]. LCPUFA are high in colostrum and decrease gradually [69,71,80,87,93,110-112]. Makrides *et al*. [84] observed a decrease of DHA till 16 weeks of lactation, while most ω6LCPUFA continued to decrease till 30 weeks. Milk LA and ALA increase during the first month of lactation [69,87,93,112]. These changes have led to the notion that the increase of precursors and the decrease of LCPUFA could reflect adaptation to the improving desaturase activity of the newborn [69].

1.2.3.3 *Parity*

Finley *et al*.[80] have found a positive correlation between milk 12:0 and 14:0 contents and the number of children in American women with 1-4 children. However, Prentice *et al.* [78] found the proportion of *de novo* synthesised FA significantly reduced in marginally nourished Gambian women with 10 children or more compared to primiparous women. Neither of them observed significant changes of ω3LCPUFA with number of children.

1.2.4. Requirements and recommendations

1.2.4.1 *Prenatal*

Since PUFA are structural components of every cell membrane, it is not surprising that the rapidly developing foetus has a very high PUFA demand. This is especially the case during the last trimester of pregnancy due to rapid synthesis of vascular and neural tissues. The two major FA in brain and retina are DHA and AA, and the rate of their accretion increases as gestation progresses [1,113-116]. It has been estimated that the foetus accumulates around 400 mg/kg/day ω6FA and 50 mg/kg/day ω3FA during the 3rd trimester [117].

1.2.4.2 *Newborns*

The ω3 and ω6LCPUFA contents in brain increase up to at least 2 years of age [113]. Next to ω3 and ω6LCPUFA there is after birth also a high demand for ω9FA, because ω9FA are high in myelin, which is formed very rapidly in the early postnatal period [113,114,118,119]. Crawford *et al*. [120] tend to stress the importance of AA in relation to its role in endothelial integrity. AA is a major component of the inner membrane of the endothelial cell, and the endothelium will grow to become the largest organ.

To cover these high LCPUFA demands the newborn infant is dependent on body stores, conversion of parent EFA to LCPUFA and intake of pre-formed LCPUFA from human milk. Most classical formulas contain LA and ALA, but no LCPUFA [56,57]. Current recommendations for EFA in term infant formulas (in % of total FA [%FA]) vary between 8-10 %FA for LA and 1.5-1.75 %FA for ALA [39,121]. LCPUFA, especially DHA, supply might be important for newborns, because their desaturation activity is probably not

adapted to the high LCPUFA need [31,122-124], and also because incorporation in brain seems to occur more efficiently from orally administered DHA and AA than from DHA and AA that is synthesised from its precursors [113,125-127]. Whether LCPUFA are conditionally essential for term infants is still under investigation. Several investigators argue that to date there is insufficient support for the addition of LCPUFA to formulas for term infants, by lack of evidence showing any long-term effects of DHA intake on global development [121,128,129]. This view is however not supported by all [39]. Significant functional advantages have on the other hand been shown for LCPUFA enrichment of formulas for preterm infants [130-132] (see also paragraph 1.3.3 'Effects on neurological development'). Requirements of pre-term infants are higher because of low body pools at birth, rapid growth rate, use of ALA and LA for energy, and the high incidence of pathological conditions that may interfere with substrate turnover [56,120,122,133]. Current recommendations for preterm and term babies have been made in the lower and upper range of human milk, i.e. 0.35-0.50 %FA for AA and 0.20-0.35 %FA for DHA [39,134].

1.2.4.3 *Infants and children*

The EFA requirement of infants and children are presumably higher than for adults because of the need for structural lipid synthesis associated with growth [1]. The estimated daily LA requirements range from 1 to 4.5 % of energy intake (en%) [1]. Holman *et al.* [135] calculated the minimal ALA requirement at 0.54 en% for a 7-year-old girl. Bjerve *et al.* [136], reporting on another 7-year old girl, estimated the optimal ω3FA requirement at 1.1-1.2 en%. A critical period with regard to LCPUFA supply may be the weaning period, especially in formula fed children, since most weaning foods provide only small amounts of LCPUFA [58,59].

1.2.4.4 *Adults*

The minimal daily requirements for LA and ALA for adults have been estimated at 1-3 en% and 0.2-0.3 en%, respectively [1,137]. Bjerve *et al*. [137] calculated minimal daily requirement for ω3LCPUFA of 0.1-0.2 en%. Yet dietary recommendations for ω3FA are higher than the proposed minimal requirements and vary considerably between countries. Summarising the different guidelines the intake of ALA (if specified) should be around 1 en%, ω3LCPUFA 0.2-0.5 en% and total ω3FA 0.4-1.5 en% [38,39]. The recommended ω6/ω3 ratio ranges from 10:1 to 2:1 [38]. It has been pointed out that the ω3FA target will be difficult to meet. It could be achieved for example by including around 4 fatty fish meals per week along with ≈22-32 g/day of a vegetable oil that is relatively rich in ALA, like soybean, canola and flaxseed oils [38]. For pregnant and lactating women some recommend a DHA minimum intake of 300 mg/day [39], whereas others feel that it is premature to recommend specific LCPUFA intakes for these groups [134].

1.3. (Patho)physiology of essential fatty acids

1.3.1. Introduction

Since the functions of EFA are apparent in every organ, it is not surprising that a deficiency can become manifest in many different ways. The first clinical symptoms of EFAD have been described in rats by the well-known studies of Burr and Burr in 1929 [138,139]. They observed reduced growth rate, scaly condition of the skin and decreased fertility in rats on a fat-free diet. Thirty years later, Hansen *et al.* [140] were the first to describe EFAD in humans. They observed unsatisfactory growth rates and dryness of the skin in many infants on low LA intakes. EFAD has been most extensively described in subjects on fat-free total parenteral nutrition (TPN) [141-147]. For example, O'Neill *et al.* [142] reported on 28 patients, ranging from newborns to 66 years old, who received fat-free TPN. LA levels fell rapidly, followed by AA. In most of the patients the 20:3ω9/20:4ω6 ratio (a biochemical marker for EFAD) had increased after a few weeks above the 0.4 criterion [148], followed approximately one week later by clinical signs of a scaly and thin skin, and hair loss. In addition to these classical EFAD symptoms, many other biological and behavioural changes have been documented [149-151]. Subjects especially at risk for EFAD are those with low EFA intakes like in malnutrition (see section 1.4 'Essential fatty acid deficiency in malnourished children'), and anorexia nervosa [152] and/or severe fat malabsorption [153].

The essentiality of ALA in humans was recognised in 1982 by Holman and co-workers [135]. They observed neurological abnormalities in an ALA deficient, 7-year old girl on TPN. After including ALA in the TPN the symptoms gradually disappeared. Since then Bjerve *et al.* have reported several cases of ALA deficiency exhibiting skin changes and growth retardation [136,137,154]. Although DHA is not an EFA, it is nowadays widely considered to be (conditionally) essential in the pre- and early postnatal periods of at least preterm infants, because at this stage of development synthesis from DHA precursors do not seem to cover the infants' high needs. (Pre)term infants are therefore partly dependent on DHA intake from either breastmilk or formula [31,155,156]. The effects of ω3LCPUFA on visual and mental development have been extensively studied to arrive at the conclusion that ω3LCPUFA play important roles during development [6,128-131].

Human populations exhibit broad ranges of both ω3 and ω6FA and their ratio, showing that life permits large variations in EFA status [157]. PUFA status also changes during lifetime [158]. This may, e.g. be derived from Appendix 2, showing the 'Erythrocyte fatty acid compositions in different populations'. It does, however, not mean that all PUFA levels are equally beneficial. Also under 'normal' circumstances the various PUFA levels may be related to e.g. pre- and postnatal growth, neurological functioning and cardiovascular diseases, as described more in detail in the following sections.

1.3.2. Prenatal period

1.3.2.1 *Maternal-neonatal relationships*

Maternal FA metabolism is crucial for foetal growth and development, and the foetus is completely dependent on the mother for its EFA supply. This is also primarily the case for

LCPUFA accumulation. Although it is generally accepted that foetal conversion of parent EFA to LCPUFA does occur, this process is most probably insufficient to meet the very high needs [126,159,160]. Indeed, there appears to be a strong correlation between maternal and foetal PUFA status, as measured at birth [81,161-165]. Supplementation with LCPUFA during pregnancy has been shown to increase newborn LCPUFA status [166-168]. Because stronger relationships between maternal and neonatal plasma PL levels have been observed for ω3FA, compared to ω6FA, some kind of foetal autonomy for AA compared to DHA status has been proposed [161,167]. This could be explained by the fact that DHA synthesis probably requires two rate-limiting Δ6-desaturation steps, whereas AA synthesis requires only one [127].

1.3.2.2 *Transplacental transport*

Albumin-bound FFA in the maternal circulation and those liberated by lipoprotein lipase from circulating TG within the placenta are the major sources for FA transport across the placenta [1]. Yet, the processes of uptake, transport and release by the placenta are different for the various FA. Levels of LCPUFA are higher in the foetal circulation (cord blood) compared to the maternal circulation, whereas levels of ALA and LA are lower [159,165,169-174]. Crawford *et al.* [175] observed progressively diminishing ALA and LA levels and increasing ω3 and ω6LCPUFA levels in the phosphoglycerides from the maternal liver to the placenta, foetal liver and finally foetal brain. This sequence, which explains the high content of LCPUFA in the brain, was referred to as 'biomagnification'. The mechanism for preferential LCPUFA transfer is as yet unknown. The involvement of α-fetoprotein has been suggested [169,173], while more recently a major role of FA binding proteins has been proposed [176].

1.3.2.3 *Maternal polyunsaturated fatty acid status*

Circulating plasma concentrations of all FA increase during pregnancy, but reduction of maternal EFA and DHA status from early pregnancy to delivery seems to be a general phenomenon, as measured from the gradually declining (Σω3+Σω6)/(Σω7+Σω9) and increasing 22:5ω6/22:4ω6 ratios, respectively [161,164]. However, the proportion of DHA itself in plasma PL increases continuously from pre-pregnancy through 18 weeks, after which a slight decline occurs. Also plasma PL AA increases from early pregnancy, but subsequently declines to reach below pre-pregnancy levels at term delivery [164,177]. Larger decreases in AA, DHA, ω6 and ω3LCPUFA during the course of the pregnancy were observed in mothers of heavier babies, suggesting that maternal-to-fetal transfer of EFA might be a limiting factor in determining neonatal EFA status [165]. Comparison between pregnant and non-pregnant women has shown that all PUFA, except 22:5ω6 (an indicator for DHA deficiency) were lower in the pregnant women [178]. Furthermore, the absolute and relative amounts of DHA in maternal plasma PL were significantly lower in multigravidae compared with primigravidae [179].

1.3.2.4 *Effects on intrauterine growth and duration of gestation*

Low placental weight is associated with lower plasma concentrations of AA and DHA in preterm newborns [127]. Both AA and DHA levels in preterm infants are related to birth

weight, head circumference and length [180-182]. Similarly, in 3 pairs of twins (born at 32, 39 and 40 weeks of pregnancy) the heaviest child contained the highest plasma TG LCPUFA percentages [173]. Crawford *et al.* [183] observed a correlation between maternal EFA intake and birth weight in a group of low-birth-weight (LBW) babies. They also observed higher maternal and cord blood AA and DHA levels in relation to higher placental weight, birth weight and larger head circumference. It was proposed that low EFA intake would be expected to retard placental growth and hence lead to foetal growth retardation, since EFA play an important role in placental growth and function through both their membrane structural and 'eicosanoid-blood-flow' roles. However, in term infants negative relationships between AA, DHA and LA in cord blood and birth weight have been found, whereas 20:3ω6 or 20:3ω6/18:2ω6 were positively correlated with birth weight [165,168]. Negative correlations of cord vessel AA and DHA with anthropometric parameters in term babies were also found by Tjoonk *et al*. [184], but do not exclude the existence of a positive relationship between LCPUFA status and lean body mass. This relation might become confounded near term because of the rapidly growing, LCPUFA-poor, adipose tissue compartment in the last weeks of pregnancy.

The duration of gestation has been correlated with plasma DHA in preterm babies [181]. Among term infants Olsen *et al.* [185] observed a prolonged gestation in women supplemented with FO compared with olive oil, but found in a later study no correlation between ω3FA intake at 30 weeks of gestation and length of gestation in a population-based study [186]. In term Dutch newborns gestational age was negatively related to LA and ω6LCPUFA in cord plasma PL, and positively to EPA, DHA and ω3LCPUFA [165]. Tjoonk *et al*. [184] found positive relationships between cord vessel AA and DHA contents and duration of gestation in term Dutch babies.

1.3.3. Neonatal period

1.3.3.1 *Neonatal polyunsaturated fatty acid status*

As noted in the previous section, at birth plasma and RBC levels of AA and DHA are higher than maternal levels, while ALA and LA are lower. Next to high ω3 and ω6LCPUFA levels, also high levels of 20:3ω9 have been observed in the newborn [158,169,171,174,183,187,188]. Already in 1966 Pikaar and Fernandes [188] raised the question whether these high 20:3ω9 levels were caused by a high rate of desaturation in the foetus, because of its great need for AA and DHA. Indeed several studies show that desaturation takes place in the foetus and preterm infant [27,126,159,189]. Recently Uauy *et al*. [126] showed that LCPUFA formation from deuterated precursors occurs as early as 26 weeks of gestation, and is even more active in preterm compared to term infants. However, high levels of 20:3ω9 are more likely to be explained by an imbalance between the precursors ALA, LA and 18:1ω9, or by accumulation of maternal 20:3ω9 in de foetus due to biomagnification.

Postnatal LCPUFA status is very much dependent on the diet. Breastfed infants have higher DHA and AA levels, compared with formula fed counterparts [53,190-202]. These differences can already be observed as early as 5 days after delivery [191,201,203]. Similarly, the differences in human milk PUFA levels are reflected by the RBC PUFA composition of the breastfed infant [81,95,103,106]. Independent from feeding regimen, ω3

and ω6LCPUFA levels in most blood lipid fractions decrease during the first months of life, although to a larger extent in the formula-fed infants [55,124,133,158,187,192,194, 199-201,203,204]. Also the high postnatal 20:3ω9 levels decrease [158,187,188]. On the other hand LA levels increase [55,124,158,187,199-201,204,205], and by the age of around 4 months the child has developed a more or less adult FA pattern [158] (see also Appendix 2 'Erythrocyte fatty acid composition in different populations').

The absolute amounts of DHA and AA in brain continue to increase until at least 2 years of age [113], although their accumulation is different in various lipid fractions [119]. Lower DHA levels are reported in the cortex of formula fed compared to breastfed infants, while AA levels in the cortex were independent from the diet [125,206,207]. Farquharson *et al*. [207] noted that a reduction in brain DHA is usually compensated for by 22:5ω6. Since in early infancy Δ4-desaturation is not optimal, DHA may initially be replaced by less unsaturated ω6LCPUFA.

1.3.3.2 *Polyunsaturated fatty acid supplementation*

Many studies are performed to augment LCPUFA status of formula fed infants to reach levels of breastfed counterparts. FO, high in DHA and EPA, has been used to improve the infants' ω3LCPUFA status [53,191,199,208-210]. This regimen might, however, result in a concomitant decrease in AA levels. EPA-poor FO, single cell DHA/AA+DHA oil, and DHA+AA from egg PL have subsequently been used to counter-act this adverse effect [49,192-194,199,200,211-213]. Also the effects of a combination of LCPUFA supplements with evening primrose or borage oil (high in 18:3ω6) have been investigated [53-55,124]. Taken together these studies show that addition of DHA and/or AA to infant formula does indeed increase the infants' DHA and/or AA levels in various compartments to levels similar or even beyond those of breastfed infants. Addition of 18:3ω6 did not augment AA status to that of breastfed infants. The effect of LCPUFA supplementation is however dependent on the levels of the other FA in the formula. Innis *et al.* [193] observed a higher blood lipid DHA response to dietary DHA in infants fed 20% LA and 2.4% ALA, compared with 32% LA and 4.9% ALA. They suggested that this might be caused by reduced Δ6-desaturation, due to the higher absolute amounts of LA and/or ALA. Another possibility could be competition between LA, ALA, and 24:5ω3, the latter being an intermediate in the conversion of 22:5ω3 to DHA.

The alternative strategy to improve LCPUFA status has been to decrease the formula LA/ALA ratio, usually by using ALA-rich oils, like rapeseed (canola), linseed (flaxseed) or soybean oils [25,26,201,203,208,214]. Studies in term children have shown that lowering the LA/ALA ratio from as high as 44 [26] to as low as 3.2 [25] resulted in an increase in DHA levels. DHA levels did, however, not reach those of breastfed infants. The largest effect may be expected when the LA/ALA ratio is decreased to below 6/1 [203]. Nevertheless, lowering of the LA/ALA ratios should be done with caution, because feeding the lowest ratios could reduce AA status of formula fed infants even further [25]. Studies in preterm infants showed different results. Billeaud and co-workers [214] have reported that an LA/ALA ratio of around 6 could efficiently maintain DHA levels of premature new-borns at 37 postconceptional weeks in RBC, but not in plasma. Hoffman *et al.* [208] showed similar effects at 36 postconceptional weeks on RBC and plasma DHA using a formula with an LA/ALA ratio of around 7. However, by 57 weeks the 2.8% ALA in the

formula was insufficient to maintain DHA levels in plasma and RBC lipids at levels found in infants fed human milk or formula with LCPUFA. Innis *et al*. [215] observed no differences in DHA status between LBW infants fed either their mother's expressed breastmilk or a formula containing 2% ALA and 20% LA at day 28.

1.3.3.3 *Maternal postpartum polyunsaturated fatty acid status*

After delivery maternal PUFA status normalises slowly [178,216,217]. Holman found six weeks postpartum levels of most LCPUFA still to be as low as during pregnancy [178]. Makrides *et al.* [216] observed an even further reduction in plasma PL DHA in breastfeeding mothers till week 12 and Al *et al*. [164] found still decreased DHA levels at 6 months post delivery. By that time AA had returned to early pregnancy levels. In contrast to observations by Holman and Makrides who observed only a small difference in DHA status between lactating and non-lactating women, Otto *et al*. [217] found DHA to be lower in breastfeeding women. DHA decreased more in women with a longer lactation period. ω6LCPUFA levels were similar for lactating and non-lactating women. One year postpartum maternal DHA status was not different from nonpregnant women. Yet, mothers had lower DHA status compared to nulliparas [218].

1.3.3.4 *Effects on neurological development*

Since DHA levels are high in the retina and brain it is not entirely surprising that low levels of dietary ω3FA during development could cause functional changes. Over the last few years the effects of LCPUFA status on neurodevelopment during infancy have been extensively reviewed [6,37,128-132,155,219-222]. These papers show that preterm and LBW infants receiving LCPUFA supplemented formula have improved visual function, and score better on the Bayley mental and psychomotor developmental indices, suggesting that neurodevelopment of formula fed preterm and/or LBW infants benefit from augmentation of their ω3LCPUFA status. Yet, no *long-term* benefits have been demonstrated for preterm infants receiving formula supplemented with LCPUFA [130,131].

Whether the above also applies to babies born at term is still controversial. Some LCPUFA supplementation studies in formula fed full-term infants clearly show improvement of visual and cognitive functions, while others fail to do so [reviews (see above),49,53,103, 196,211-213,223-226]. In a unique study in *breastfed* children, in which a range of DHA levels was achieved by supplementing the diet of the mother with DHA, Gibson *et al*. [103] investigated whether infant DHA status at 12 weeks of age was related to neurodevelopment. Since breastfed children have higher levels of DHA and score higher on mental development tests than children receiving unsupplemented formula [224,227-234], it is interesting to note that, even in these breastfed children, they observed a correlation between DHA status at 12 weeks and Bayley mental development index at 1 year. However, this correlation was not evident at 2 years. A more recent study by Agostoni *et al*. [235] did not find an association between either AA or DHA in breastmilk at different points in time with 12-months mental development index in breastfed infants. However, the FA status of the infants was not examined. Yet, another study [236] showed a positive correlation between the mother's antenatal DHA status and the infant's stereoacuity score at the age of 3.5 years. There is some evidence that certain infants may, while others may not, benefit from LCPUFA supplementation. Willats *et al.* [237] observed that

unsupplemented infants with a poorer attention at 3 months had reduced two-step problem-solving ability at 9 months, while infants with a better attention at 3 months scored the same as the LCPUFA-supplemented and breastfed groups. These findings suggest that infants showing evidence of impaired attention control may have enhanced information processing because of LCPUFA supplementation. Also social economic status (SES) and health could interact with the influence of DHA status on behaviour. Poor DHA status may have little, or no, effect on development of healthy or high-SES babies, but may contribute to developmental risk in sick or low-SES infants [221].

1.3.3.5 *Effects on growth*

In 1960 Hansen *et al.* [140] reported a study including 428 children on different diets. The study showed unsatisfactory growth rates for many of the infants on low LA intakes. Whether growth was directly related to LA, or to one of its metabolites was, however, not established. Carlson *et al*. reported some 30 years later that marine oil supplemented very LBW preterm infants had impaired growth in the first year of life compared to a formula fed control group, which was correlated with AA status [47,238]. Another study in preterm infants did however not report adverse effects of FO supplementation on growth [208]. Woltil *et al*. [239] observed in LBW infants no correlation between AA status and growth on day 42, but parameters of postnatal brain growth were related to DHA status.

The majority of studies in term infants addressing the relation between LCPUFA status and growth found no between-group differences [49,53,196,203,211,212,240,241]. Only Jensen *et al*. [242] reported significantly lower body weight at 120 days in infants fed with a high (3.2%) ALA formula, compared to infants fed 0.4% ALA. Across groups, weight at 120 days was positively correlated with plasma PL AA, 22:4ω6 and 22:5ω6, while no correlations with ω3FA were observed. Two studies by Makrides *et al*. [203,241], varying either ALA or DHA intake, showed no difference on growth between different treatment groups. However, post hoc regressions in the LCPUFA study demonstrated a small negative association between DHA status at 16 weeks of age and weight at 1 and 2 years. In both studies breastfed infants had lower weight and length gains compared to the formula fed infants. They concluded that mimicking DHA and AA status of breastfed children does not result in a comparable growth pattern [241]. Reviews based on all randomised trials of LCPUFA supplemented formula conclude that LCPUFA supplements do not influence growth of either preterm or term children [128,130].

1.3.4. Childhood

1.3.4.1 *Polyunsaturated fatty acid levels of infants and children*

PUFA levels of children will be discussed in the next section (‘Adulthood’), since adult levels are already reached around the age of 4-6 months for most EFA and LCPUFA [158,187,204]. Only for AA and DHA it seems to take longer than half a year to achieve adult levels [187,204]. DHA levels were still lower in 10-15 years old teenagers compared with 20-26 years old adults, while AA had reached adult levels already in the 1-5 years old children [204]. Whether these differences are caused by age or diet has not been established as yet.

1.3.4.2 *Neurological effects*

Some relations between PUFA status and neurological effects have been reported. Holman *et al.* [135] described a case of ALA deficiency involving neurological abnormalities in a 6 years old girl. Stevens *et al.* [243] reported that boys with attention-deficit hyperactivity disorder (ADHD) had lower blood concentrations of e.g. DHA. They also noted that a greater number of behavioural problems and lower overall academic scores were found in boys with lower ω3FA status [244]. Stordy [245] described improvement of motor skills in a group of 15 dyspraxic children after supplementation with DHA, AA and 18:3ω6.

1.3.4.3 *Effects on growth*

There are to our knowledge no data available on the relations between PUFA status, growth, weight and length in healthy children. In malnourished children Decsi *et al*. [246] found a positive correlation between body weight and AA and DHA. Bjerve *et al*. [136] observed that a daily supplement of linseed and cod liver oils induced rapid growth in a 7-years old girl with ω3FA deficiency.

1.3.5. Adulthood

1.3.5.1 *Polyunsaturated fatty acid levels of adults*

Plasma and RBC PUFA levels are very much dependent on dietary intake [197,247]. This seems to be especially the case for the ω3LCPUFA. Blood levels of EPA and DHA are much higher in communities with high seafood intakes, compared to other regions [10]. Vegans, who do not consume animal products, have, on the other hand, low levels of ω3LCPUFA [248,249]. Many studies have shown that supplementation with fish or FO results in an increase in blood ω3LCPUFA levels, usually resulting in a concomitant AA decrease [10,99,100,250-252]. AA is less dependent on diet, although somewhat lower levels have been found in vegans (no dietary AA) compared to omnivores [248,253]. AA supplementation studies are scarce, probably because of suggested harmful effects of high AA levels [254]. Daily amounts of 6 g [253] and 1.7 g [52] resulted in increased AA levels. The latter study also measured ω3 levels, which appeared to be little affected.

1.3.5.2 *Neurological effects*

LCPUFA, especially DHA, may affect brain functions in adults. Holman [157] described ω3FA deficiency in patients with neuropathy, while in an interesting review article Yoshida *et al.* [255] report on low DHA levels in patient suffering from schizophrenia, depression, dementia, Parkinsonism and other behavioural disorders. They describe that in some of the cases ω3FA supplementation had positive effects on the neurological symptoms.

1.3.5.3 *Other effects*

The most extensively investigated effects of LCPUFA are those of ω3FA in relation to coronary heart disease and hypertension [2,10,256-258]. Moreover, ω3FA play a role in the modulation of inflammatory and immune reactions, in the treatment of cancer and diabetes

[2] and are probably involved in skin changes other than those observed in ω6-deficiency [137]. These effects are most probably related to the function of EPA as precursor of eicosanoids and its interaction with eicosanoids originating from the ω6FA [2,10,259]. For example, high incidence of cardiovascular disease, cancer and diabetes in Israel have been associated with the high intake of LA in that country [260]. Recently it has been shown that ω3FA supplementation caused an accumulation not only of ω3FA, but also of ω6FA, suggesting that ω3FA are required for a normal metabolism and incorporation of FA into membrane lipids [261].

1.4. Essential fatty acid deficiency in malnourished children

A review

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1.4.1. Introduction

'Three quarters of the children who die world-wide of malnutrition-related causes are mildly to moderately malnourished and betray no outward signs of problems' [quoted from *The State of the World's Children 1998 Unicef report*]. Anaemia, vitamin A and iodine deficiency are often encountered in malnutrition, but a shortage of EFA and its metabolites may also be involved. For example, a dry skin and impairment of the immune system are clinical symptoms of both malnutrition and EFAD [4,262]. EFAD is in the strict sense of the word defined as deficiency of LA, ALA, or both. However, in practice it mostly refers to deficiency of the parent EFA and their long chain metabolites, and in that way EFAD will also be used in this paper. Low EFA and LCPUFA levels could obviously originate from a low fat intake, but may also have other causes, like disturbed lipid metabolism and higher utilisation. Protein-energy malnutrition (PEM) may lead to the clinical syndrome of kwashiorkor or marasmus, or a combination (marasmic kwashiorkor). All are characterised by weight deficit, while oedema and fatty liver are special features of kwashiorkor [262-264]. Because of the partly different aetiology of the two and the higher prevalence of marasmus, we will focus in this manuscript mainly on marasmic children. We will however often refer to PEM in general, since in many of the cited studies the distinction between marasmus and kwashiorkor was not made.

In this part of the general introduction we will review available data on the EFA status of malnourished, mostly marasmic, children. Attention is paid to the biochemical and clinical features of EFAD in PEM. The data are finally aggregated to a model to indicate the relationship and interaction of PEM and EFAD. Possibilities of intervention and nutritional recommendations are also addressed. Although the emphasis is on malnourished children in developing countries, current concepts may also apply to more prosperous populations, since malnutrition is neither confined to children nor to developing countries. Symptoms of malnutrition in western countries are notably encountered in seriously ill paediatric and elderly patients, in which some authors estimate the prevalence of malnutrition at 25 and 40 percent, respectively [265,266].

1.4.2. Do malnourished children suffer from biochemical essential fatty acid deficiency?

Several papers have been published on EFAD in marasmic children from non-western countries [246,267-277]. An overview is given in Table 2. Unfortunately comparison of studies is difficult, because of small sample sizes [269,271,276], inappropriate age-matching of controls [267,268,269,271,277] and origination of controls from a western country [246]. Grade and type of malnutrition vary widely among the different studies and are not always adequately specified [267,269]. Most of the studies in which the distinction between kwashiorkor and marasmus was made report differences in blood FA composition between the two [268,271,273,274,276]. Only Koletzko *et al*. [270] did not find this difference. In one study [246] 19 out of 35 malnourished children were HIV infected, which by itself may affect FA metabolism [278]. Another factor that complicates comparison is FA measurements in different blood compartments or lipid classes. Wolff *et*

Table 2. Comparison of the characteristics of malnourished children and controls in various studies concerning the effect of malnutrition on fatty acid status.

Study	Patients				Controls			
	n	Age	Nutritional status	Country	n	Age	Nutritional status	Country
Holman 1981 [267]	40	2-24 m	Low weight for age	Argentina	48	1-48 m	Adequate	Argentina
Wolff 1984 [268]	44	1-27 m	Gr 3; k:11, m:22, mk:11	Peru	11	?	Recovered gr 3	Peru
Chen 1985 [269]	10	5 m-6 y	Low weight for height	Honduras	20	4-6 y	Healthy	Honduras
Koletzko 1986 [270]	17	5-24 m	Maln; k:9, m:8	Benin	8	5-23 m	Adequate	Benin
Vajreswari 1990 [271]	10	1-4 y	Maln; k:6, m:4	India	17	1-4 y	Adequate	India
Marin 1991 [272]	26	2-5 m	Maln; gr 1:13 gr 2:6, gr 3:7	Argentina	24	2-5 m	Adequate	Argentina
Leichsenring 1992 [273]	18	6-42 m	Severe maln; k:8, m:10	Sudan	20	12-60 m	Adequate	Sudan
Decsi 1995 [246]	35	9-43 m	Severe maln; HIV-:16, +:19	Rumania	25	1-5 y	Adequate	Germany
Leichsenring 1995 [274]	44	8-36 m	Severe maln; k:12, m:32	Nigeria	23	8-40 m	Adequate	Nigeria
Smit 1997 [275]	67	4-56 m	Maln; gr 2:47, gr 3:21	Pakistan	26	2-60 m	Adequate	Pakistan
Franco 1999 [276]	15	2-42 m	Gr 3; k:5, m:5, mk:5	Brazil	8	3-22 m	Adequate	Brazil
S Houssaini 1999 [277]	29	23±14 m	Maln; mild:12, severe:17	Morocco	15	16±14 m	Healthy Adequate	Morocco

Age: range or mean ± SD; Gr: grade of malnutrition; k: kwashiorkor; m: marasmus; mk: marasmic kwashiorkor; Maln: malnourished. Studies carried out in children explicitly classified as kwashiorkor are not listed.

al. [268] found that plasma 18:1ω9, LA and AA were significantly correlated with their respective erythrocyte (RBC) levels, whereas Leichsenring *et al*. [274] observed inconsistent differences in the FA compositions of lipid fractions in plasma and RBC. For example, LA was reduced in plasma cholesterol esters (CE) of children with PEM, while no differences in LA levels were found in other lipid fractions (RBC phosphatidylethanolamine [PE], phosphatidylcholine [PC] and total plasma PL). The underlying discrepancy may derive from selective FA incorporation into different lipid classes [273]. Moreover, analytical techniques differ among the various studies. Some authors make use of capillary gas chromatography [246,270,273-275,277], which has a much higher separating potential compared with the packed column gas chromatography used by others [267-269, 271,272]. Finally, not all studies present the complete list of FA, with some showing the major ones [273,274,276], and others merely the ω6FA [268,272].

1.4.2.1 *ω3 Fatty acids*

No significant differences are found for ALA between malnourished children and controls in any of the studies. However, most studies reported a certain decrease of DHA. Only Holman *et al*. [267] found a significant increase in ω3FA in serum CE and TG. They explained these increases, which were accompanied by elevated ω9FA, by a compensatory mechanism for the drastic ω6FA decrease. On the other hand Decsi *et al*. [246] found in Rumanians a more pronounced depletion of ω3LCPUFA compared to those of the ω6FA, which could possibly derive from a lower dietary intake, as compared to German controls. We [275] observed no significant differences in RBC DHA of malnourished and adequately nourished children in Pakistan, probably because of the generally low dietary DHA intake in the North of Pakistan. In malnourished breastfed children RBC DHA was associated with DHA levels in the milk of their mothers [106].

1.4.2.2 *ω6 Fatty acids*

The picture concerning ω6FA seems quite unequivocal, since both LA and its metabolites are found to be decreased in malnutrition. However, to which extent varies between studies. Wolff *et al*. [268] observed the most profound reduction of ω6FA, with plasma LA in marasmic children being only one-third of that in controls. In most studies LA was less reduced than its desaturation-elongation products, which may be due to diminished desaturation capacity (see below). Wolff *et al*. [268] did not observe lower 20:3ω6 and AA in malnourished children, which may be explained by a selection bias. The controls in Wolffs' study had recently recovered from third degree malnutrition, following hospitalisation for at least 1 month. Koletzko *et al*. [270] found AA levels of children in the recovery phase (14 days after the first sample) to be even more reduced than at the time of admission, whereas LA was already increasing. Leichsenring *et al*. [273] note that although ω6FA were reduced in malnourished Sudanese children compared to controls, they were still in the normal range of well nourished children living elsewhere in the world.

1.4.2.3 *ω9 Fatty acids*

The non-essential ω9FA are increased in malnutrition. All studies that provide data on 18:1ω9 found this FA to be significantly elevated. Also 20:3ω9 was higher, although in

most cases not to a significant extent [246,269,273,275]. As described previously ω9FA compensate for the decrease of particularly ω6FA, and in some cases ω3FA.

In summary, malnourished children suffer from biochemical EFAD, as demonstrated by investigation of their plasma and RBC FA status. The data show low LA, often low AA and DHA and high 18:1ω9 and 20:3ω9.

1.4.3. Could some of the clinical symptoms in protein energy manutrution be explained by essential fatty acid deficiency?

EFAD and PEM have several clinical symptoms in common. A dry and scaly skin, hair loss, reduced growth rate, increased susceptibility to infections, shortened RBC survival, changes in the structure and function of organs like heart, liver and gastrointestinal tract, and transient impaired cognitive, visual and motor skill development are observed in both EFAD and PEM [4,140,262-264,279-283]. There is some evidence that some of these symptoms can indeed partly be explained by the roles of EFA in membrane structure and in the biosynthesis of regulatory molecules such as eicosanoids [3,4].

Skin changes can possibly be ascribed to deficiency of LA *per se*, or to the lower levels of the PG precursors 20:3ω6 and AA [3,4,140]. Recent studies indicate that EFA regulate cell adhesion by modifying the expression of cell adhesion molecules, suggesting that EFAD induces pathological features in the skin [284]. The higher infection rate as observed in PEM could be a result of the depressed immune system caused by reduced PG precursor levels [3,285,286], increased permeability of the skin and the gastrointestinal tract due to EFAD [4,284,287], or both. PG production does not seem to be directly related to absolute FA levels but rather to the relative amounts of the different FA, particularly the ratio between ω3 and ω6FA [3,285,286]. The mechanisms underlying the positive effects of one or more of the FA LA, AA and DHA on growth [47,140,180,181,239,246] are not very well understood. PGE_2, a cyclooxygenase metabolite of AA, is most probably involved, possibly through its direct growth promoting effects, its effects on growth-related early gene expression, or its effects on calcium metabolism [288,289]. Inefficient use of dietary calories in EFAD may play an additional role [290-292]. The influence of EFA status on neurological development has attracted much attention over the last two decades and has recently been extensively reviewed [6,221,222] (See also section 1.3.3). The brain and the central nervous system are very rich in AA and DHA, where they affect membrane enzymes, ion channels, signal transduction and neural network systems [1,6,255,293]. However, most of the mechanisms by which EFA status modulates the functions of brain cells and their networks remain as yet unclear [221,255]. Many trials with LCPUFA supplemented preterm infants have shown significant, though transient, functional advantages, such as better visual functions and higher psychomotor development scores [130,131]. Benefits for full-term infants remain controversial [6,128,129]. The first results from a study on visual function and LCPUFA supply of malnourished children have recently been published. Marin *et al*. [294] found a correlation between DHA in RBC PL and visual function in a group of malnourished babies (1,5-3 months of age) who received breastmilk, LCPUFA supplemented formula or regular formula. The latency time of the breastfed children was significantly shorter compared with counterparts receiving regular formula, showing that also during malnutrition breastfeeding exhibits functional advantages. It should be noted that, apart from EFAD, mental development in PEM may

also be affected by deficiencies of other nutrients. Examples are deficiency of protein itself and micro-nutrients deficiencies that often accompany PEM like those of zinc, iron, copper, calcium, iodine and various vitamins [263,282,283]. PEM coincides often with a poor socio-economic and psychological environment, which by themselves may affect neurological functioning [283]. It seems therefore almost impossible to determine the specific effects of EFAD on neurological parameters in malnourished children.

In summary, some of the clinical symptoms in PEM like skin changes, impaired resistance to infections, impaired growth rate, and disturbed development may in part derive from EFAD.

1.4.4. Why do malnourished children suffer from essential fatty acid deficiency?

It might be too simple to ascribe EFAD in malnutrition to reduced intake only. Altered gastrointestinal handling (digestion, absorption, transport), altered FA biosynthesis and metabolism, and altered energy utilisation and peroxidation might also be involved.

1.4.4.1 *Intake*

Vegetable oils are the main source of parent EFA. LA is found in the seeds of most plants and ALA in green leafy vegetables and soybeans. LCPUFA are mostly derived from animal products. Meat and eggs are rich sources of AA, and fish is the most important source of EPA and DHA. However, the intake of LCPUFA is very small (<5%) compared to that of its precursors [2,4]. As FA levels in tissues are highly influenced by the dietary FA composition [197] it seems reasonable to assume that the low ω3 and ω6FA blood contents are caused by low intakes of these FA. Although in none of the previously mentioned studies an accurate nutritional survey was performed, most investigators attribute the encountered low blood LA levels to low LA intake [267,268,270,275,276], while the low RBC DHA levels observed in the North of Pakistan were ascribed to minimal fish consumption [275]. Other studies ascribe the low levels of LCPUFA to impaired conversion of parent EFA to LCPUFA, rather than to a diminished intake of its precursors [246,271,274]. A low fat intake may also negatively affect the status of the fat-soluble vitamins A, D and E, which on its turn could impair LCPUFA status, as will be discussed later. Moreover, a low fat intake is often accompanied by a high carbohydrate intake, which has been reported to enhance the nutritional needs for EFA [11,295].

1.4.4.2 *Digestion and absorption*

In malnutrition the process of digestion and uptake of lipids is impaired. Gastric acid secretion was found to be reduced in malnourished children, which may contribute to bacterial overgrowth in the upper gut [296-298]. This may cause bacterial degradation of bile salts, reduced micellular solubilisation and result in impaired intestinal fat absorption [299-301]. Also bile production appears to be decreased [262,300]. Since biliary PC production seems to be an important source of intestinal EFA supply [302], a reduced bile production could further impair EFA status. Moreover, during episodes of diarrhoea, which are often encountered in malnourished children, bile salts will be lost in the faeces [300]. Intestinal digestion may further be hampered by decreased production of lipase [263,301].

Finally, structural changes of the small intestinal epithelium characterised by flattening of the villi [263,264,299,303,304], occurring more severely in kwashiorkor than marasmus [303], will affect intestinal absorptive capacity. Diarrhoea, accompanied by an increase of the bacterial overgrowth, might even further aggravate intestinal absorption [301].

In addition, there is some evidence that EFAD itself may impair lipid digestion and absorption. Some animal models have shown that EFA stimulate bile flow and bile acid output and subsequently influence intestinal uptake rates [287,302,305-307]. Moreover, the small intestine of malnourished piglets fed LCPUFA supplemented formula recovered more completely from the histologically demonstrable lesions and biochemical alterations, compared with piglets fed LCPUFA-unsupplemented formula [308]. Since both EFAD and PEM cause flattening of the villi [263,264,287,299,304,306], it could be speculated that the changes observed in PEM are partly caused by EFAD. This notion is supported by several animal studies showing that the FA composition of the enterocyte responds rapidly to dietary changes, including malnutrition and FA intake [304,307,309].

1.4.4.3 *Transport*

Like gastrointestinal FA absorption, also FA transport, either across the enterocyte or between the various organs, may be affected by EFAD itself. Chylomicron assembly and secretion seem to be decreased in EFAD rats [302], and both total very low density lipoprotein (VLDL) concentration and VLDL-FA composition was affected by an ALA deficient diet [310].

Protein malnutrition diminishes VLDL levels and alters VLDL composition in rats. Bouziane *et al*. [310,311] have shown that after 28 days on a low protein diet VLDL contained less protein, PL and TG. Moreover, LA and AA were decreased in VLDL PL and TG, together decreasing EFA availability. Plasma free FA (FFA) are transported in the form of complexes with albumin [4]. Plasma albumin levels in PEM are low [262,263,268,277,280,312], which may theoretically affect FFA transport capacity. However, the binding capacity of albumin for FFA can increase ten times if the need for FA transport is elevated [313]. Hydrolysis of TG from chylomicrons and VLDL is catalysed by lipoprotein lipase, of which the activity is affected by many factors. Insulin has a stimulating effect, while glucagon and thyroid stimulating hormone (TSH) repress lipoprotein lipase activity [4]. Therefore, low insulin levels as often-encountered in PEM [262,263,314], may lower the release of FFA from circulating TG. Iodine deficiency, which is common in developing countries, may aggravate this effect, since it lowers thyroxin levels and subsequently raises TSH [315]. However, several studies have shown that during malnutrition, especially marasmus [316], TSH levels are either normal or low, despite low thyroxin levels [262,264,269,314,316]. The few available data on glucagon levels during malnutrition are contradictory. Both reduced [264,316] and increased [262] levels have been reported. Also FA uptake (re-esterification) and release (lipolysis) from adipose tissue is regulated by insulin. Low insulin levels reduce re-esterification and increase lipolysis [262], which contributes to maintenance of energy homeostasis in PEM. Moreover, higher levels of growth hormone, as often observed in PEM [262-264,314,316], stimulate lipolysis, together resulting in an increased concentration of circulating FFA. Catecholamines also stimulate lipolysis [4], but data on catecholamines levels in PEM are scanty and conflicting [262,314,316]. Taken together, it seems that FA transport might be altered in PEM and that this may have a negative impact on EFA transport. Interpretation of

current data in terms of EFA fluxes is, however, difficult, since responses to hormonal stimuli may be altered in PEM. Consequently, the levels of the circulating hormones may not always explain metabolic and endocrine changes [262].

1.4.4.4 *Biosynthesis and metabolism*

1.4.4.4.(a) De novo synthesis and Δ9-desaturation

When the fat content of the diet is low, rates of FA synthesis in the liver increases. *De novo* synthesis yields mainly palmitic acid (16:0) and stearic acid (18:0), which are desaturated by Δ9-desaturase to the monounsaturated FA (MUFA) palmitoleic acid (16:1ω7) and oleic acid (18:1ω9). LA limits 18:1ω9 synthesis by inhibiting 18:0 desaturation [4,11]. The high levels of MUFA as found in malnutrition, and the increase of Δ9-desaturation activity [267,277] may thus be explained by low fat intake. Vitamin A deficiency, as often observed in PEM [262-264,317,318], may also contribute to higher 18:1ω9 levels, since Alam *et al*. [319] observed an increase of Δ9-desaturase activity in liver microsomes of vitamin A deficient rats, while Δ6-desaturase activity was not affected.

1.4.4.4.(b) Desaturation

Impaired desaturation activity, as interpreted from the FA composition, is a common feature in PEM. Several investigators [270,271,273] found a significantly decreased AA/LA ratio, which reflects the sum of Δ6- and Δ5-desaturation and elongation. Marin *et al*. [272] found a reduced ratio of (sumω6 minus LA)/LA in malnourished children. Wolff *et al*. [268], however, found the AA/LA ratio to be increased, as they observed no difference in AA levels between malnourished children and controls. An explanation for this discrepancy has been mentioned before: controls in the latter study were recently recovered malnourished children, who might still have an altered EFA status, e.g. as a result of a decreased Δ6-desaturase activity [270]. The Δ6-desaturase activity might be impaired for months, for example due to low insulin levels. Insulin is known to augment Δ6-desaturase activity [11], and the low insulin levels in PEM persist for a while after recovery [320]. Reports concerning Δ5- and Δ4-desaturase activities are rather inconsistent. Deducted from the plasma PL 20:4ω6/20:3ω6 ratio, Δ5-desaturase activity was reduced in malnourished children in one study [246], but increased in another [273]. The first study observed decreasing activity with progressing stages of HIV infection [246]. Holman *et al*. [267] and Koletzko *et al*. [270] also found inconsistencies concerning Δ5- and Δ4-desaturation, while we [275] suggested reduced Δ4-desaturation. The final step in the desaturation-elongation chain is considered to proceed by initial elongation, followed by a Δ6-desaturation and a final chain shortening by peroxisomal β-oxidation (Figure 2). We suggested that reduced Δ4-desaturation could derive from impaired peroxisomal β-oxidation, since no concomitant changes in Δ6-desaturation and elongation were observed. Yet, another explanation could be competition for Δ6-desaturase between ALA and LA on the one hand and 24:5ω3 and 24:4ω6 on the other, which could turn out to be in favour of the parent EFA [321].

Factors that are known to decrease Δ6-desaturase activity are the already mentioned low insulin levels, and also deficiency of protein and minerals such as iron, zinc, copper and magnesium, which are often associated with malnutrition [263,264,269,322,323]. Dietary

protein deficiency has been shown to decrease the AA/LA ratio (a marker for Δ6- plus Δ5-desaturase activity) in rat serum and VLDL [310], and to reduce Δ6- and Δ5-desaturase activity in the liver of young rats [12,13]. Huang *et al*. [324] found that FA desaturation was decreased in rats fed plant protein compared to a casein-fed group, suggesting that it is unlikely that protein deficiency *per se* was responsible for the reduced AA/LA ratio, but that the low lysine/arginine ratio of plant protein could play a role. The notion that plant proteins may affect desaturation is supported by a study conducted by Sugiyama *et al*. [325] who observed that dietary methionine, which is also low in plant protein, stimulates conversion of LA to AA. They also showed an increase of the PC/PE ratio of liver microsomes. Because there seems to be a positive relationship between the activity of Δ6- and Δ5-desaturase and the PC/PE ratio, they proposed that methionine affects the metabolism of LA through alteration of the PC/PE ratio of liver microsomes in rats. Since the dietary protein of malnourished children will mainly be of vegetable origin, the same mechanism could possibly be operational in malnutrition. Butzner *et al*. [326] found a decreased PC/PE ratio in the microvillus PL of malnourished rabbits, which may theoretically negatively affect desaturation activity in intestinal microsomes. However, oppose to this finding, Fondu *et al*. [280] observed a higher PC/PE ratio in the RBC membrane of malnourished children. There appears to be a relationship between iron and lipid metabolism [14,327-330]. Higher LA accompanied by lower AA has been observed in plasma and liver PL of rats consuming an iron deficient diet. This suggests an adverse effect of iron deficiency on Δ6-desaturase activity [14,329,330]. In iron deficient young children Tichelaar *et al*. [327] have shown that iron fortification increased ω3LCPUFA. This observation could, however, not be substantiated in iron deficient rats [328]. They concluded that dietary iron deficiency affected the incorporation of LA in plasma PL, but that Δ6-desaturase activity was not affected. Several reports describe an impaired conversion of LA to AA in zinc deficient rats [331,332]. Human studies report a positive correlation between zinc levels on the one hand and AA and 20:3ω6 on the other in plasma of cystic fibrosis patients [333]. In healthy subjects zinc showed an inverse relationship with ω3LCPUFA [334]. The authors suggested that because of the higher affinity of Δ6-desaturase for ω3FA compared to ω6FA the conversion of ALA to its long chain metabolites was increased when the activity of this enzyme was reduced, resulting in relatively higher amounts of ω3LCPUFA. The effects of copper deficiency on Δ5- and Δ6-desaturase have not been thoroughly investigated and the results are inconsistent [14,335,336]. Cunnane *et al*. [335] found lower 20:3ω6 and 20:3ω6/20:4ω6 in several organs of copper deficient mice, suggesting either increased Δ5-desaturation or increased 20:3ω6 utilisation. Lawrence *et al*. [336] observed no substantial changes in mitochondrial FA composition in copper deficient rats, while Johnson *et al*. [14] observed significantly lower AA and total ω6 metabolites in liver PL of copper-deficient rats, when compared to rats fed a copper-excess diet. A deficiency of another mineral, magnesium, resulted in a decrease of the Δ6-desaturase activity in liver microsomes of rats [337]. However, in two other studies LCPUFA and DHA were higher in the low-magnesium group as compared to controls [338,339]. Humans with latent tetany and low magnesium levels exhibited impaired LA desaturation, as concluded from their higher LA and lower ω6LCPUFA [340].

The activity of Δ6-desaturase may also be affected by other factors that are altered in PEM. A relatively high carbohydrate intake and increased circulating epinephrine and glucocorticoids seem to depress Δ6-desaturase activity [11,262,263]. Low selenium and

vitamin E levels [263,280,341] may not only affect EFA status by providing protection against peroxidation (see below), but may also impair FA desaturation [342]. Moreover desaturase activities are affected by the FA composition itself in a complicated manner. The FA composition of the diet, the amounts of product and precursor and the ratio between saturated FA, ω3 and ω6FA all have their own impact [3,11,22-24,309]

1.4.4.4.(c) Elongation

Reports on elongation, the other alternating step in the parent EFA conversion, are inconsistent. Two studies [270,275] found no effect, whereas Holman *et al*. [267] found a significant rise in the sum of elongation products in serum CE and TG of malnourished children. Koletzko *et al*. [270] observed a significant reduction in the 18:3ω6/20:3ω6 ratio in plasma TG, also pointing to increased elongation activity. Yet, another possible explanation for the higher levels of the elongation products like 20:2ω6, 22:4ω6, 22:4ω3 and also EPA in PEM as observed in some studies [246,267], was recently brought up by Decsi *et al*. [321]. They proposed that the reduced precursor/product ratios are caused by augmented retroconversion rather than by reduced elongation. However, reduced elongation could, based on animal studies, have been expected. Calcium deficient rats showed impaired 18:3ω6 elongation [343], and calcium deficiency is highly prevalent among malnourished children [263].

1.4.4.5 *β-Oxidation and peroxidation*

Since FA constitute a calorie dense source of energy it seems likely that ALA, LA and probably also LCPUFA will be used for energy generation during energy shortage [263,269,344]. β-Oxidation takes place in the mitochondria in the presence of carnitine, because long chain FA (C12-C18) merely cross mitochondrial membranes in the form of acyl-carnitines [4]. In malnutrition both intake and biosynthesis of carnitine appear to be low, which may theoretically affect β-oxidation [312,345]. Yet, it has been shown that severely wasted infants were able to derive virtually all of their energy needs from fat [346].

EFAD seems to impair dietary calorie utilisation [290-292]. This may derive from structural changes of mitochondrial membranes, causing disturbed mitochondrial energy metabolism [347]. Incorporation of FA in membranes is increased during PEM. Fondu *et al*. [280] observed a higher uptake of radioactive LA in RBC membranes of PEM patients *in vitro*, which they contributed to accelerated FA turnover. This could be explained by increased membrane peroxidation, possibly because of a deficiency of the synergistically acting antioxidants vitamin E and selenium [342,348]. In a study among healthy adults selenium was directly associated with relative amounts of EFA and ω6LCPUFA [334]. Indeed low levels of these antioxidants, as well as reduced RBC life span, have been observed in malnutrition [263,275,317,318,341]. Rapid RBC turnover results in a high number of young RBC (e.g. reticulocytes), which are characterised by relatively low LA content [349]. This is likely to be an important cause of the reduced RBC LA in PEM. Also higher AA turnover has been suggested [271]. It could be expected that the demand for eicosanoids and prostanoids is elevated, since infections often occur in PEM. However, whether the production of eicosanoids and prostanoids is increased in PEM has, to our knowledge, not been investigated in humans. In a study in mice, PGE_2 production was

enhanced above control values in a low protein dietary group at 3 weeks, but significantly decreased compared with controls at 8 weeks [350,351]. In another study, malnourished rats alveolar macrophages exhibited an enhanced release of PGE_2 and TXB_2 and an impaired production of LTB_4 [352]. The authors mention that these changes were not due to substrate deficiency, since uptake and membrane content of AA was not different from controls, but that the altered eicosanoid production could be caused by the lack of a cofactor like calcium or selenium.

In summary, the available data on the interaction between PEM and EFAD can be put into perspective as depicted in Figure 3. It seems clear that in PEM on the one hand EFA supply (i.e. the resultant of intake, digestion, absorption and transport) is reduced, while on the other hand EFA expenditure (i.e. β-oxidation and peroxidation) is increased. These two factors together lead to low parent EFA and LCPUFA status. Impaired desaturation also attributes to decreased LCPUFA status and may find its origin in deficiencies of protein, probably specific amino acids, and micro-nutrients that are involved in desaturation activity, either as cofactors or otherwise. EFAD will in its turn negatively affect EFA status by causing decreased lipid absorption and transport of FA and possibly other nutrients. In addition, EFAD aggravates PEM by impairing lipid absorption and dietary calorie utilisation, altogether resulting in a vicious cycle.

1.4.5. Intervention

To break through the PEM-EFAD vicious cycle may seem easy by the simple inclusion of EFA rich food in the rehabilitation diet of the malnourished child. However, attention should be paid to adequate amounts of anti-oxidants [353], while also the balance between ω3 and ω6FA should be taken into consideration [259]. Moreover, without a sufficient supply of certain micro-nutrients, EFA metabolism may remain hampered. To our knowledge there are no studies in which PUFA were administered to malnourished children and in which the children were subsequently both biochemically and clinically monitored. Only some data on plasma and RBC FA status of recovering children have been reported. Koletzko *et al*. [270] studied the plasma FA composition of 8 recovering malnourished children during hospital treatment with a high-calorie and high-protein diet (including maize porridge, milk, eggs, beans, fish, meat and vegetable oils). They found a slight improvement of EFA status after 14 days treatment. We [354] supplemented malnourished children with 500 mg fish oil daily for 9 weeks, next to the usual nutritional advice. The intervention resulted in a 50% increase of RBC DHA and ω3LCPUFA, without affecting RBC ω6LCPUFA. The supplement was apparently well absorbed and not exclusively used as a source of energy.

1.4.6. Conclusions and recommendations

We conclude that biochemical EFAD is prevalent in PEM and characterised by low LA, often low AA and DHA and high 18:1ω9 and 20:3ω9. Some of the clinical symptoms in PEM notably skin changes, impaired resistance to infections, impaired growth rate and disturbed development may partly be explained by EFAD. Factors in PEM that may cause EFAD include low EFA intake, poor lipid digestion, absorption and transport, impaired desaturation and augmented β-oxidation and peroxidation. EFAD may perpetuate itself by decreased FA absorption and transport. In addition, EFAD negatively affects PEM by

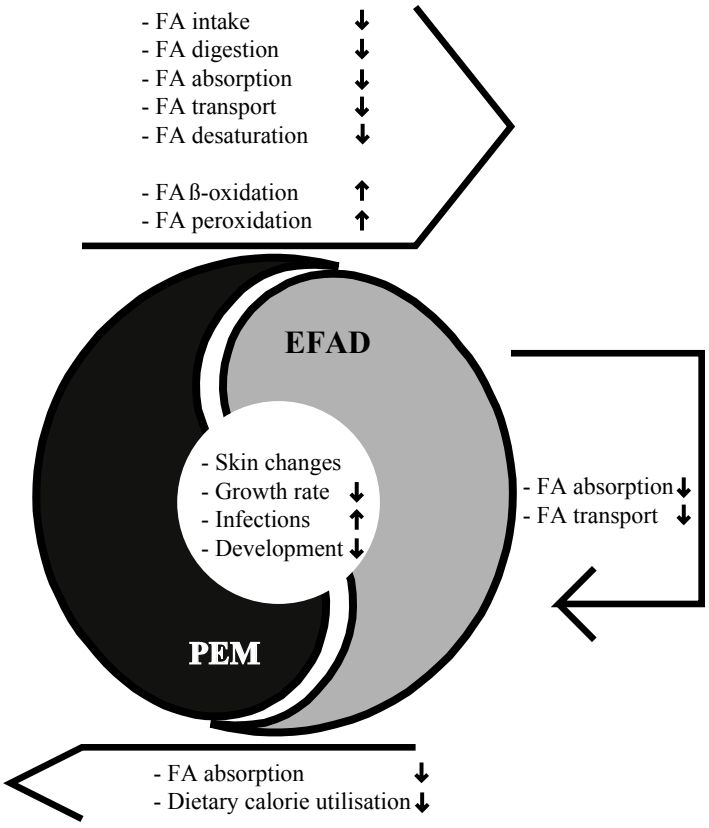

Figure 3. The PEM-EFAD vicious cycle. PEM causes EFAD because of reduced EFA supply (low intake, digestion, absorption and transport), decreased EFA desaturation and high EFA expenditure (β-oxidation and peroxidation). EFAD perpetuates itself by decreasing FA absorption and transport. EFAD negatively affects PEM by causing impaired lipid absorption and dietary calorie utilisation, resulting in a vicious cycle.

causing impaired lipid absorption and dietary calorie utilisation, altogether resulting in a vicious cycle. To improve EFA status of malnourished children, nutrition rehabilitation programs should pay more attention to the intake of EFA and cofactors that play roles in EFA bioavailability and metabolism. Micro-nutrients that may need special attention in connection with EFA are iron, zinc, selenium and vitamin E. The first two because of their role in FA desaturation and the latter in their capacities as a cofactor of enzymatic radical detoxification and anti-oxidant, respectively.

Locally available vegetable oils, such as corn, sunflower and peanut oils, could be used to improve the child's LA status. However, to ensure a balance between ω3 and ω6FA it would be advisable to enhance ALA status as well. Therefore soybean oil would be a better alternative, since it contains both LA and ALA. As conversion of parent EFA to LCPUFA is usually impaired in PEM, LCPUFA supplementation seems advisable, especially during rapid rehabilitation. Fish, eggs and meat are rich sources of DHA and AA, respectively.

Unfortunately these supplements are often expensive and may therefore not be suitable to be included into the diet of malnourished children in developing countries on a large scale. Human milk is an important source of LA, ALA and LCPUFA, although their levels may be low in milk of marginally nourished women. Breastfeeding should therefore not only be encouraged for its anti-infective, anti-conceptive, psychological and developmental properties, but also because for some children human milk will be the only LCPUFA source. Since malnourished children often have marginally nourished mothers, future efforts should preferably aim at improvement of the EFA status of lactating women and, ideally, both lactating and pregnant women.

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Chapter 2 Essential fatty acid status in malnutrition