ESSENTIAL FATTY ACID DEFICIENCY IN MALNOURISHED CHILDREN

Erythrocyte and breastmilk fatty acid compositions in different populations

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ESSENTIAL FATTY ACID DEFICIENCY IN MALNOURISHED CHILDREN

Erythrocyte and breastmilk fatty acid compositions in different populations

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Bestijg de trein nooit zonder uw valies met dromen,
dan vindt ge in elke stad behoorlijk onderkomen.

Jan van Nijlen

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Anneke Smit

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Foreword

In a country like The Netherlands most people have heard about essential fatty acids or polyunsaturated fatty acids (‘meervoudig onverzadigde vetzuren'). Reports in magazines focussing on young and expectant parents mention the importance of fish consumption and breastfeeding for the well being of the baby. In magazines directed at elderly people fish or fish oil consumption is promoted to prevent cardiovascular diseases and also neurological disorders like Parkinson's disease and dementia. In some of these articles even ω3 fatty acids are mentioned as the nutrient that transmits the beneficial effects. However, most of the investigations presented in this thesis do not address the western situation. Data for the present studies were collected in non-western countries like Pakistan and Palestine in which most of the inhabitants have never heard about fatty acids and are not interested in the subject because of other priorities. Clearly, carrying out research in such countries has different challenges compared to the challenges one deals with ‘back home’. For example the poor compliance, which does not merely find its origin in different attitude but the more in less favorable weather conditions (too hot, too wet), and the large number of holidays, especially in areas populated by people with different religions. Another difficulty relates to ‘product reliability’: The locally purchased soybean-oil for one of the intervention studies appeared to be of unknown origin according to analysis in Groningen, displaying a different fatty acid composition than anticipated.

To put the obtained data on the red blood cell and breastmilk fatty acid compositions into a wider context, they were added to the many data that were already collected in other parts of the world by other investigators from the same research group. This thesis describes both studies that are based on data obtained from individual countries and those that are based on the compiled data sets. It thereby provides information about differences and similarities between different populations regarding the various fatty acids in red blood cells and breastmilk.

Actually, neither the topic of fatty acids, nor the places where the data have been collected were intentionally chosen. If the author had not met Prof. Boersma who was interested in fatty acids and if her husband had not found a job in those specific places, the title of this thesis could as well have been: ‘Polyamine status of African people’. The similarity between these subjects is that both are about nutrition in less privileged societies. I sincerely hope that the underlying work of this thesis will already have had and will also in the future have beneficial effects on this topic. I therefore dedicate this work to the local people who might have benefited directly from my local cooking and breastfeeding demonstrations (not presented in this thesis), and to the researchers and policymakers who are invited to pay more attention to the importance of essential fatty acids in malnutrition.

List of abbreviations

AA	arachidonic acid, 20:4ω6
ALA	α-linolenic acid, 18:3ω3
CE	cholesterol esters
DHA	docosahexaenoic acid, 22:6ω3
EFA	essential fatty acid(s)
EFAD	EFA deficiency
en%	% of energy intake
EPA	eicosapentaenoic acid, 20:5ω3
FA	fatty acid(s)
FFA	free FA
LA	linoleic acid, 18:2ω6
LBW	low-birth-weight
LCPUFA	long chain polyunsaturated FA (containing ≥20 carbon atoms and ≥3 double bonds)
LCP	LCPUFA
LT	leukotrienes
LTB_4	leukotriene B_4
MCSAFA	medium chain SAFA
MUFA	monounsaturated FA
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PG	prostaglandins
PGE_2	prostaglandin E_2
PL	phospholipids
PUFA	polyunsaturated fatty acid(s), EFA + LCPUFA
RBC	erythrocyte(s)
SAFA	saturated FA
TG	triglycerides
TPN	total parenteral nutrition
TSH	thyroid stimulating hormone
TX	thromboxanes
TXB_2	thromboxane B_2
VLDL	very low density lipoprotein
%FA	% of total FA

Thesis background

Many studies have shown that essential fatty acids (EFA) and their long-chain metabolites (long-chain polyunsaturated fatty acids, abbreviated LCPUFA) play important roles in processes like growth and development. EFA and LCPUFA are either from the ω3 series (e.g. α-linolenic acid, ALA; docosahexaenoic acid, DHA) or ω6 series (e.g. linoleic acid, LA; arachidonic acid, AA). They are mainly derived from vegetable oils, animal products (e.g. meat, eggs and fish) and breastmilk. Intake of these products may be low in malnourished children and these children may consequently be expected to have marginal or even deficient EFA and/or LCPUFA status. The aim of the thesis was to contribute to insight into EFA status of malnourished children and its causes, giving special attention to the role of breastfeeding. For this we concentrated on the fatty acid (FA) compositions of erythrocytes (RBC) and breastmilk from various populations in different parts of the world.

In the North of Pakistan we conducted several studies to investigate whether:

- Malnourished Pakistani children suffer from EFA and/or LCPUFA deficiency (Chapter 2.1, 2.2)
- Their low DHA status is caused by low milk DHA contents of their mothers (Chapter 3.1)
- Their low DHA status could be improved by fish oil supplementation (Chapter 2.3)

In Jerusalem we carried out a supplementation study in lactating women to investigate whether:

- The FA composition of human milk would be affected by supplementation of AA and DHA (Chapter 3.2)

The studies based on the compiled data set of human milk FA were designed to:

- Estimate the biological variation of the human milk FA composition (Chapter 3.3)
- Compare the human milk FA composition with guidelines for formula (Chapter 3.4)

The study based on the compiled data set of RBC FA was aimed at:

- The establishment of cut-off values for biochemical EFA and ω3-deficiencies (Chapter 4.1)

Chapter 1 General introduction

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.

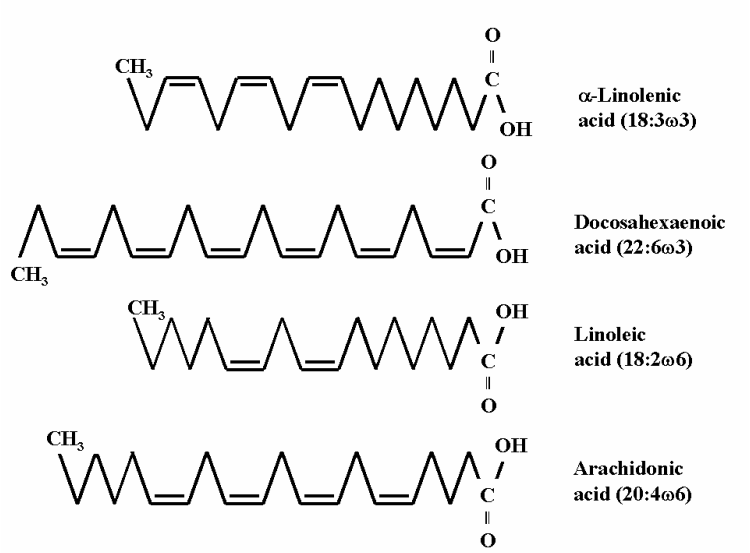

Figure 1. Structural formulas for α-linolenic acids (18:3ω3), docosahexaenoic acid (22:6ω3), linoleic acid (18:2ω6) and arachidonic acid (20:4ω6). The first number gives the number of carbon atoms, the second gives the number of double bonds. ω3 and ω6 indicate the posotion of the first double bond.

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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Submitted in modified form

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

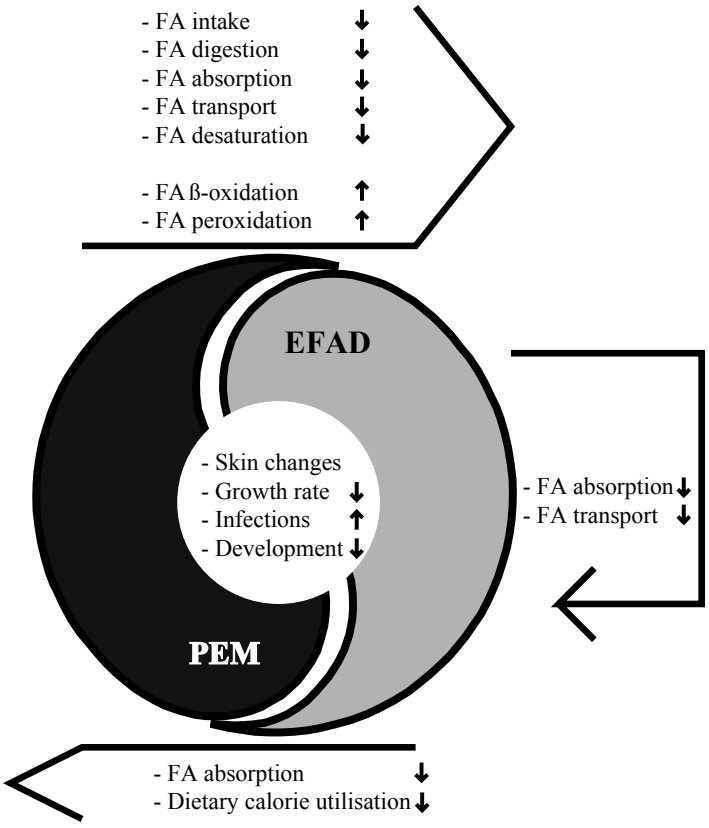

Figure 3. The PEM-EFAD vicious cycle. PEM causes EFAD because of reduced EFA supply (low intake, digestion, absorption and transport), decreased EFA desaturation and high EFA expenditure (β-oxidation and peroxidation). EFAD perpetuates itself by decreasing FA absorption and transport. EFAD negatively 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.

References

1. Innis SM. Essential fatty acids in growth and development. Prog Lipid Res 1991;30:39-101
2. Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 1991;54:438-63
3. Horrobin DF. Gamma linolenic acid: An intermediate in essential fatty acid metabolism with potential as an ethical pharmaceutical and as a food. Rev Cont Pharmacother 1990;1:1-45
4. Linscheer WG, Vergroesen J. Lipids. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea and Febiger.1994:47-88
5. Budowski P, Crawford M. α-linoleic acid as a regulator of the metabolism of arachidonic acid: dietary implications of the ratio n-6/n-3 fatty acids. Proc Nutr Soc 1985;44:221-9
6. Uauy R, Mena P. Lipids and neurodevelopment. Nutr Rev 2001;59S:S34-48
7. Clarke SD, Jump DB. Polyunsaturated fatty acid regulation of hepatic gene transcription. Lipids 1996;31:S7-11
8. Simopoulos AP. The role of fatty acids in gene expression: Health implications. Ann Nutr Metab 1996;40:303-11
9. Price PT, Nelson CM, Clarke SD. Omega-3 polyunsaturated fatty acid regulation of gene expression. Curr Opin Lipidol 2000;11:3-7
10. Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am J Clin Nutr 1990;52:1-28
11. Brenner RR. Regulatory function of delta 6 desaturase -- key enzyme of polyunsaturated fatty acid synthesis. Adv Exp Med Biol 1977;83:85-101
12. Narce M, Poisson JP, Belleville J, Chanussot B. Time-course effects of protein malnutrition on hepatic fatty acids Δ6 and Δ5 desaturation in the growing rat. Br J Nutr 1988;60:389-402
13. De Tomas ME, Mercuri O, Rodrigo A. Effects of dietary protein and EFA deficiency on liver delta 5, delta 6 and delta 9 desaturase activities in the early developing rat. J Nutr 1980;110:595-9
14. Johnson SB, Kramer TR, Briske-Anderson M, Holman RT. Fatty acid pattern of tissue phospholipids in copper and iron deficiencies. Lipids 1989;24:141-5
15. Innis SM. Essential fatty acids in infant nutrition: lessons and limitations from animal studies in relation to studies on infant fatty acid requirement. Am J Clin Nutr 2000;71:S238-44
16. Mohammed BS, Sankarappa S, Geiger M, Sprecher H. Reevaluation of the pathway for the metabolism of 7,10,13,16-docosatetraenoic acid to 4,7,10,13,16-docosapentaenoic acid in rat liver. Arch Biochem Biophys 1995;317:179-184
17. Voss A, Reinhart M, Sankarappa S, Sprecher H. The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. J Biol Chem 1991;266:19995-20000
18. Infante JP, Huszagh VA. On the molecular etiology of decreased arachidonic (20:4n-6), docosapentaenoic (22:5n-6) and docosahexaenoic (22:6n-3) acids in Zellweger syndrome and other peroxisomal disorders. Mol Cell Biochem 1997;168:101-15
19. Infante JP. A function for the vitamin E metabolite α-tocopherol quinone as an essential enzyme cofactor for the mitchondrial fatty acid desaturases. FEBS letters 1999;446:1-5
20. Infante JP, Huszagh VA. Analysis of the putive role of 24-carbon polyunsaturated fatty acids in the biosynthesis of docosapentaenoic (22:5n-6) and docosahexaeonic (22:6n-3) acids. FEBS letters 1998;431:1-6
21. Qui X, Hong H, MacKenzie SL. Identification of a Δ4 fatty acid desaturase from *Thraustochytrium* sp. Involved in the biosynthesis of docosahexaenoic acid by heterologous expression in *Saccharomyces cerevisiae* and *brassica juncea*. J Biol Chem 2001;276:31561-6

22. Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adults. Biochim Biophys Acta 1994;1213:277-88
23. Boyle FG, Yuhas RJ, Goldberg K, Lien EL. Interaction of n-3 long-chain polyunsaturated fatty acids with n-6 fatty acids in suckled rat pups. Lipids 1998;33:243-50
24. Lands WEM. Dose-response relationship for ω3/ω6 effects. World Rev Nutr Diet 1991;66:177-94
25. Clark KJ, Makrides M, Neumann MA, Gibson RA. Determination of the optimal ratio of linoleic acid to α-linoleic acid in infant formulas. J Pediatr 1992;120:S151-8
26. Jensen CL, Chen H, Fraley K, *et al.* Biochemical effects of dietary linoleic/α-linolenic acid ratio in term infants. Lipids 1996;31:107-13
27. Sauerwald TU, Hachey DL, Jensen CL, *et al*. Intermediates in endogenous synthesis of C22:6ω3 and C20:4ω6 by term and preterm infants. Pediatr Res 1997;41:183-7
28. Mantzioris E, James MJ, Gibson RA, Cleland LG. Differences exist in the relationship between dietary linoleic and α-linolenic acids and their respective long-chain metabolites. Am J Clin Nutr 1995;61:320-4
29. Fulco AJ, Mead JF. Metabolism of essential fatty acids. VIII. Origins of 5,8,11-eicosatrienoic acid in the fat-deficient rat. J Biol Chem 1959;234:1411-6
30. Crawford MA. Commentary on the workshop statement. Prostaglandins Leukot Essent Fatty Acids 2000;63:131-4
31. Cunnane SC, Francescutti V, Brenna JT. Docosahexaenoate requirement and infant development. Nutrition 1999;15:801-2
32. Holman RT, Johnson S. Changes in essential fatty acid profile of serum phospholipids in human disease. Prog Lipid Res 1981;20:67-73
33. Martinez M. Severe deficiency of docosahexaenoic acid in peroxisomal disorders: a defect of Δ4 desaturation? Neurology 1990;40:1292-8
34. Jensen RG. Lipids in human milk- Composition and fatsoluble vitamins. In: Lebenthal E, ed. Textbook of gastroenterology and nutrition in infancy. 2nd ed. New York: Raven Press. 1989:157-208
35. Jensen RG. Lipids in human milk. Lipids 1999;34:1243-71
36. Neville MC, Picciano MD. Regulation of milk lipid secretion and composition. Annu Rev Nutr 1997;17:159-84
37. Gibson RA, Makrides M. n-3 Polyunsaturated fatty acid requirements of term infants. Am J Clin Nutr 2000;71:S251-5
38. Kris-Etherton PM, Shaffer Taylor D, Yu-Poth S, *et al*. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 2000;71:S179-88
39. Simopoulos AP, Leaf A, Salem Jr N. Workshop statement on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids 2000;63:119-121
40. Mantzioris E, James MJ, Gibson RA, Cleland L. Dietary substitution with an α-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. Am J Clin Nutr 1994;59:1304-9
41. Hunter JE. n-3 fatty acids from vegetable oils. Am J Clin Nutr 1990;51:809-14
42. Okuyama H, Sakai K. Effects of dietary oils with extreme ω3/ω6 ratios - Selective incorporation and differential catabolism. World Rev Nutr Diet 1991;66:195-204
43. Wu D, Meydani M, Leka LS, *et al*. Effect of dietary supplementation with black currant seed oil on the immune response of healthy elderly subjects. Am J Clin Nutr 1999;70:536-43
44. Kremer JM, Jubiz W, Michalek A, *et al*. Fish-oil fatty acid supplementation in active rheumatoid arthritis. A double-blinded, controlled, crossover study. Ann Intern Med 1987;1064:497-503
45. van der Tempel H, Tulleken JE, Limburg PC, *et al*. Effects of fish oil supplementation in rheumatoid arthritis. Ann Rheum Dis 1990;49:76-80
46. Horrobin DF. Abnormal membrane concentrations of 20 and 22-carbon essential fatty acids: A common link between risk factors and coronary and peripheral vascular disease? Prostaglandins Leukot Essent Fatty Acids 1995;53:385-96
47. Carlson SE, Werkman SH, Peeples JM, *et al*. Arachidonic acid status correlates with first year growth in preterm infants. Proc Natl Acad Sci USA 1993;90:1073-7
48. Innis SM, Hansen JW. Plasma fatty acid responses, metabolic effects, and safety of microalgal and fungal oils rich in arachidonic and docosahexaenoic acids in healthy adults. Am J Clin Nutr 1996;64:159-67
49. Auestad N, Halter R, Hall RT, *et al*. Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked, randomized, parallel, prospective, multivariate study. Pediatrics 2001;108:372-81
50. Taber L, Chiu C, Whelan J. Assessment of the arachidonic acid content in foods commonly consumed in the American diet. Lipids 1998;33:1151-7
51. Sinclair AJ, O'Dea K, Dunstan G, *et al*. Effects on plasma lipids and fatty acid composition of very low fat diets enriched with fish or kangaroo meat. Lipids 1987;22:523-9

52. Nelson GJ, Schmidt PC, Bartolini G, *et al*. The effect of dietary arachidonic acid on plasma lipoprotein distributions, apoproteins, blood lipid levels, and tissue fatty acid composition in humans. Lipids 1997;32:427-33
53. Jørgensen MH, Hølmer G, Lund P, *et al.* Effect of formula supplemented with docosahexaenoic acid and γ-linolenic acid on fatty acid status and visual acuity in term infants. J Pediatr Gastroenterol Nutr 1998;26:412-21
54. Woltil HA, van Beusekom CM, Schaafsma A, Okken A, Muskiet FAJ. Does supplementation of formula with evening primrose and fish oils augment long chain polyunsaturated fatty acid status of low birthweight infants to that of breast-fed counterparts? Prostaglandins Leukot Essent Fatty Acids 1999;60:199-208
55. Makrides M, Neumann MA, Simmer K, Gibson RA. Erythrocyte fatty acids of term infants fed either breast milk, standard formula, or formula supplemented with long-chain polyunsaturates. Lipids 1995;30:941-8
56. Decsi T, Koletzko B. Polyunsaturated fatty acids in infant nutrition. Acta Paediatr Suppl 1994;395:31-7
57. Huisman M, van Beusekom CM, Lanting CI, *et al.* Triglycerides, fatty acids, sterols, mono- and disaccharides and sugar alcohols in human milk and current types of infant formula milk. Eur J Clin Nutr 1996;50:255-60
58. Giovanni M, Agostoni C, Riva E. Fat needs of term infants and fat content of milk formulae. Acta Paediatr 1994;S402:59-62
59. Jackson KA, Gibson RA. Weaning foods cannot replace breastmilk as sources of long-chain polyunsaturated fatty acids. Am J Clin Nutr 1989;50:980-2
60. Walker AF. The contribution of weaning foods to protein-energy malnutrition. Nutr Res Rev 1990;3:25-47
61. Muskiet FAJ, Offringa PJ, Boersma ER. Lipid content and fatty acid composition of human milk in relation to developing countries. In Boersma ER *et al*, eds. A holistic approach to perinatal care and the prevention of handicap. Erven B. van der Kamp Publishers, Groningen. 1988:294-305
62. Prentice AM, Alison AP. Fat and energy needs of children in developing countries. Am J Clin Nutr 2000;72:1253-65
63. Thompson BJ, Smith S. Biosynthesis of fatty acids by lactating human breast epithelial cells: An evaluation of the contribution to the overall composition of human milk fat. Pediatr Res 1985;19:139-43
64. Hachey DL, Thomas MR, Emken EA, *et al*. Human lactation: maternal transfer of dietary triglycerides labeled with stable isotopes. J Lipid Res 1987;28:1185-92
65. Demmelmair H, Baumheuer M, Koletzko B, *et al*. Metabolism of U^{13}C-labelled linoleic acid in lactating woman. J Lipid Res 1998;39:1389-96
66. Del Prado M, Villalpando S, Elizondo A, *et al.*. Contribution of dietary and newly formed arachidonic acid to human milk lipids in women eating a low-fat diet. Am J Clin Nutr 2001;74:242-7
67. Gibson RA, Kneebone GM. Effect of sampling on fatty acid composition of human colostrum. J Nutr 1980;110:1671-5
68. Budowski P, Druckmann H, Kaplan B, Merlob P. Mature milk from Israeli mothers is rich in polyunsaturated fatty acids. World Rev Nutr Diet 1994;75:105-8
69. Hall B. Uniformity of human milk. Am J Clin Nutr 1979;32:304-12
70. Harzer G, Haug M, Dieterich I, Gentner PR. Changing patterns of human milk lipids in the course of the lactation and during the day. Am J Clin Nutr 1983:37;612-21
71. Serra G, Marletta A, Bonacci W, *et al*. Fatty acid composition of human milk in Italy. Biol Neonate 1997;72:1-8
72. Read WWC, Lutz PG, Tashjian A. Human milk lipids. III. Short-term effects of dietary carbohydrate and fat. Am J Clin Nutr 1965;17:184-7
73. Villalpando S, Del Prado-Manriquez M, Stafford J, Delgado G. Diurnal variations in the fatty acid composition of milk fat from marginally nourished women. Arch Med Res 1995;25:S139-43
74. Henderson RA, Jensen RG, Lammi-Keefe CJ, *et al*. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 1992;27:901-7
75. Insull W, Hirsch J, James T, Ahrens EH. The fatty acids of human milk. II. Alterations produced by manipulation of caloric balance and exchange of dietary fats. J Clin Invest 1959;39:443-50
76. Read WWC, Lutz PG, Tashjian A. Human milk lipids. II. The influence of dietary carbohydrates and fat on the fatty acids of mature milk. Am J Clin Nutr 1965;17:180-3
77. Söderhjelm L. Fat absorption studies VII. Polyunsaturated fatty acids in human milk and their variation with dietary fat. Acta Soc Med Upsal 1953;58:244-251
78. Prentice A, Jarjou LM, Drury PJ, *et al*. Breast-milk fatty acids of rural Gambian mothers: effects of diet and maternal parity. J Pediatr Gastroenterol Nutr 1989;8:486-90

79. Kneebone GM, Kneebone R, Gibson RA. Fatty acid composition of breast milk from three racial groups from Penang, Malaysia. Am J Clin Nutr 1985;41:765-9
80. Finley DA, Lonnerdal B, Dewey KG, Grivetti LE. Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians. Am J Clin Nutr 1985;41:787-800
81. Sanders TAB, Reddy S. The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant. J Pediatr 1992;120:S71-7
82. Vuori E, Kiuru K, Makinen SM, *et al*. Maternal diet and fatty acid pattern of breast milk. Acta Paediatr Scand 1982;71:959-63
83. Underwood BA, Hepner R, Abdullah H. Protein, lipid, and fatty acids of human milk from Pakistani women during prolonged periods of lactation. Am J Clin Nutr 1970;23:400-7
84. Makrides M, Simmer K, Neumann M, Gibson R. Changes in the polyunsaturated fatty acids of breast milk from mothers of full-term infants over 30 wk of lactation. Am J Clin Nutr 1995;61:1231-3
85. Sanders TAB. Polyunsaturated fatty acids in the food chain in Europe. Am J Clin Nutr 2000;71:S176-8
86. de la Presa-Owens S, Lopez-Sabater MC, Rivero-Urgell M. Fatty acid composition of human milk in Spain. J Pediatr Gastroenterol Nutr 1996;22:180-5
87. Boersma ER, Offringa PJ, Muskiet FAJ, *et al*. Vitamin E, lipid fractions, and fatty acid composition of colostrum, transitional milk, and mature milk: an international comparative study. Am J Clin Nutr 1991;53:1197-1204
88. Innis SM, Kuhnlein HV. Long-chain n-3 fatty acids in breast milk of Inuit women consuming traditional foods. Early Human Dev 1988;18:185-9
89. Koletzko B, Thiel I, Abiodun PO. The fatty acid composition of human milk in Europe and Africa. J Pediatr 1992;120:S62-70
90. Chulei R, Xiaofang L, Hongseng M, *et al*. Milk composition in women from five different regions of China: The great diversity of milk fatty acids. J Nutr 1995;125:2993-80
91. Villalpando S, Butte NF, Flores-Huerta SF, Thotathuchery M. Qualitative analysis of human milk produced by women consuming a maize-predominant diet typical of rural Mexico. Ann Nutr Metab 1998;42:23-32
92. Borschel MW, Elkin RG, Kirksey A, *et al*. Fatty acid composition of mature human milk in Egyptian and American women. Am J Clin Nutr 1986;44:330-5
93. Chen ZY, Kwan KY, Tong KK, *et al*. Breast milk fatty acid composition: A comparative study between Hong Kong and Chongqing Chinese. Lipids 1997;32:1061-7
94. Koletzko B, Thiel I, Abiodun PO. Fatty acid composition of mature human milk in Nigeria. Z Ernährungswiss 1991;30:289-97
95. Mellies MJ, Ishikawa TT, Gartside PS, *et al*. Effects of varying maternal dietary fatty acids in lactating women and their infants. Am J Clin Nutr 1979;32:299-302
96. Harzer G, Dieterich I, Haug M. Effects of the diet on the composition of human milk. Ann Nutr Metab 1984;28:231-9
97. Potter JM, Nestel PJ. The effects of dietary fatty acids and cholesterol on the milk lipids of lactating women and the plasma cholesterol of breast-fed infants. Am J Clin Nutr 1976;29:54-60
98. Harris WS, Connor WE, Lindsey S. Will dietary ω-3 fatty acids change the composition of human milk? Am J Clin Nutr 1984;40:780-5
99. Helland IB, Saarem K, Saugstad OD, Drevon CA. Fatty acid composition in maternal milk and plasma during supplementation with cod liver oil. Eur J Clin Nutr 1998;52:839-45
100. Makrides M, Neumann MA, Gibson RA. Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. Eur J Clin Nutr 1996;50:352-7
101. Cherian G, Sim JS. Changes in the breast milk fatty acids and plasma lipids of nursing mothers following consumption of n-3 polyunsaturated fatty acid enriched eggs. Nutrition 1996;12:8-12
102. Jensen CL, Maude M, Anderson RE, Heird W. Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. Am J Clin Nutr 2000;71:S292-9
103. Gibson RA, Neumann MA, Makrides M. Effect of increasing breastmilk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. Eur J Clin Nutr 1997;51:578-84
104. Smit EN, Koopmann M, Boersma ER, Muskiet FAJ. Effect of supplementation of arachidonic acid (AA) or a combination of AA plus docosahexaenoic acid on breastmilk fatty acid composition. Prostaglandins Leukot Essent Fatty Acids 2000;62:335-40
105. Glew RH, Omene JA, Vignetti S, *et al*. Fatty acid composition of breastmilk of Nigerian women. Nutr Res 1995;15:477-89
106. Smit EN, Oelen EA, Seerat E, *et al*. Breastmilk docosahexaenoic acid (DHA) correlates with DHA status of malnourished infants. Arch Dis Child 2000;6:493-4

107. Rana IA, Gilani M, Jafri S. Nutritive value of human milk and nutritional status of wives of army personnel of low income from Rawalpindi. JPMA 1990;40:109-12
108. Jelliffe DB, Jelliffe EFP. The volume and composition of human milk in poorly nourished communities. A review. Am J Clin Nutr 1978;31:492-515
109. Read WWC, Sarrif A. Human milk lipids. I. Changes in fatty acid composition of early colostrum. Am J Clin Nutr 1965;17:177-9
110. Rueda R, Ramirez M, Garcia-Salmeron JL, Maldonado J, Gil A. Gestational age and origin of human milk influence total lipid and fatty acid contents. Ann Nutr Metab 1998;42:12-22
111. Xiang M, Lei S, Li T, Zetterstrom R. Composition of long chain polyunsaturated fatty acids in human milk and growth of young infants in rural areas of northern China. Acta Paediatr 1999;88:126-31
112. Yu G, Duchén K, Björkstén B. Fatty acid composition in colostrum and mature milk from non-atopic and atopic mothers during the first 6 months of lactation. Acta Paediatr 1998;87:729-36
113. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129-38
114. Crawford MA, Hassam AG, Stevens PA: Essential fatty acid requirements in pregnancy and lactation with special reference to brain development. Prog Lipid Res 1981;20:31-40
115. Ballabriga A. Essential fatty acids and human tissue composition. An overview. Acta Paediatr 1994;S402:63-8
116. Clandinin MT, Chappel JE, Leong S, *et al*. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Hum Dev 1980;4:121-9
117. Clandinin MT, Chappell JE, Heim T, *et al*. Fatty acid utilization in perinatal de novo synthesis of tissues. Earl Hum Dev 1981;5:355-66
118. Clandinin MT, Chappel JE, Leong S, *et al*. Extrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Hum Dev 1980;4:131-8
119. Martinez M, Mougan I. Fatty acid composition of human brain phospholipids during normal development. J Neurochem 1998;71:2528-33
120. Crawford MA, Costeloe K, Ghebremeskel K, *et al*. Are deficits of arachidonic and docosahexaenoic acids responsible for the neural and vascular complications of preterm babies? Am J Clin Nutr 1997;66S:1032S-41S
121. Life Sciences Research Office, American Society for Nutritional Sciences. Assessment of nutrient requirements for infant formulas. J Nutr. 1998;128S:S2059-2293
122. Innis SM. n-3 fatty acid requirements of the newborn. Lipids 1992;27:879-85
123. Salem N Jr, Wegher B, Mena P, Uauy R. Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. Proc Natl Acad Sci USA 1996;93:49-54
124. Koletzko B, Decsi T, Demmelmair H. Arachidonic acid supply and metabolism in human infants born at full term. Lipids 1996;31:79-83
125. Makrides M, Neumann MA, Byard RW, *et al*. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. Am J Clin Nutr 1994;60:189-94
126. Uauy R, Mena P, Wegher B, *et al*. Long chain polyunsaturated fatty acid formation in neonates: Effect of gestational age and intrauterine growth. Pediatr Res 2000;47:127-35
127. Crawford MA. Placental delivery of arachidonic and docosahexaenoic acids: implications for the lipid nutrition of preterm infants. Am J Clin Nutr 2000;71:S275-84
128. Simmer K. Longchain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database Syst Rev 2000;(2):CD000376
129. SanGiovanni JP, Berkey CS, Dwyer JT, Colditz GA. Dietary essential fatty acids, long-chain polyunsaturated fatty acids and visual resolution acuity in healthy fullterm infants: a systematic review. Early Hum Dev 2000;57:165-88
130. Simmer K. Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev 2000;(2):CD000375
131. SanGiovanni JP, Parra-Cabrera S, Colditz GA, *et al*. Meta-analysis dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. Pediatrics 2000;105:1292-8
132. Uauy R, Hoffman DR. Essential fat requirements of preterm infants. Am J Clin Nutr 2000;71:S245-50
133. Innis SM. Plasma and red blood cell fatty acid values as indexes of essential fatty acids in the developing organs of infants fed with milk or formulas. J Pediatr 1992;120:S78-86
134. Koletzko B, Agostoni C, Carlson SE, *et al*. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. Report of workshop. Acta Paediatr 2001;90:460-4
135. Holman RT, Johnson SB, Hatch TF. A case of human linolenic acid deficiency involving neurological abnormalities. Am J Clin Nutr 1982;35:617-23

136. Bjerve KS, Thoresen L, Borsting S. Linseed and cod liver oil induce rapid growth in a 7-year-old girl with n-3 fatty acid deficiency. J Parent Enteral Nutr 1988;12:521-5
137. Bjerve KS, Lovold-Mostad I, Thoresen L. Alpha-linolenic acid deficiency in patients on long-term gastric-tube feeding: an estimation of linolenic acid and long-chain unsaturated n-3 fatty acid requirement in man. Am J Clin Nutr 1987;45:66-77
138. Burr GO, Burr MM. A new deficiency disease produced by the rigid exclusion of fat from the diet. J Biol Chem 1929;82:345-67
139. Burr GO, Burr MM. On the nature and role of the fatty acids essential in nutrition. J Biol Chem 1930;86:587-621
140. Hansen EA, Wiese HF, Boelsche AN, *et al*. Role of linoleic acid in infant nutrition. Pediatrics 1963;31:S171-92
141. Wene JD, Connor WE, DenBesten L. The development of essential fatty acid deficiency in healthy men fed fat-free diets intravenously and orally. J Clin Invest 1975;56:127-34
142. O'Neill JA, Caldwell MD, Meng HC. Essential fatty acid deficiency in surgical patients. Ann Surg 1977;185:535-41
143. Paulsrud JR, Pensler L, Whitten CF, *et al*. Essential fatty acid deficiency in infants induced by fat-free intravenous feeding. Am J Clin Nutr 1972:25;897-904
144. Collins RD, Sinclair AJ, Royle JP, *et al*. Plasma lipids in human linoleic deficiency. Nutr Metab 1971;13:150-67
145. White HB, Turner MD, Turner AC, Miller RC. Blood lipid alterations in infants receiving intravenous fat-free alimentation. J Pediatr 1973;83:305-13
146. Riella MC, Broviac JW, Wells M, Scribner BH. Essential fatty acid deficiency in human adults during total parenteral nutrition. Ann Inter Med 1975;83:786-9
147. Richardson TJ, Sgoutas D. Essential fatty acid deficiency in four adult patients during total parenteral nutrition. Am J Clin Nutr 1975;28:258-63
148. Holman RT. The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. J Nutr 1960;70:405-10
149. Wainwright PE. Do essential fatty acids play a role in brain and behavioral development? Neurosci Biobehav Rev 1992;16:193-205
150. Salem N, Ward GR. Are ω3 fatty acids essential nutrients for mammals? World Rev Nutr Diet 1993;72:128-47
151. Uauy R, Birch E, Birch D, Peirano P. Visual and brain function measurements in studies of n-3 fatty acid requirements of infants. J Pediatr 1992;120:S168-80
152. Langan SM, Farrell PM. Vitamin E, vitamin A and essential fatty acid status of patients hospitalized for anorexia nervosa. Am J Clin Nutr 1985;41:1054-60
153. Jeppesen PB, Christensen MS, Hoy CE, Mortensen PB. Essential fatty acid deficiency in patients with severe fat malabsorption. Am J Clin Nutr 1997;65:837-43
154. Bjerve KS, Fischer S, Alme K. Alpha-linolenic acid deficiency in man: effect of ethyl linoleate on plasma and erythrocyte fatty acid composition and biosynthesis of prostanoids. Am J Clin Nutr 1987;46:570-6
155. Hamosh M, Salem N Jr. Long-chain polyunsaturated fatty acids. Biol Neonate 1998;74:106-20
156. Makrides M, Neumann MA, Gibson RA. Is dietary docosahexaenoic acid essential for term infants? Lipids 1996;31:115-9
157. Holman RT. The slow discovery of the importance of omega 3 essential fatty acids in human health. J Nutr 1998;128:S427-33
158. Holman RT, Smythe L, Johnson S. Effect of sex and age on fatty acid composition of human serum lipids. Am J Clin Nutr 1979;32:2390-9
159. Chambaz J, Ravel D, Manier MC, *et al*. Essential fatty acids interconversion in the human fetal liver. Biol Neonate 1985;47:136-40
160. Poisson JP, Dupuy RP, Sarda P, *et al*. Evidence that liver microsomes of human neonates desaturate essential fatty acids. Biochem Biophys Acta 1993;1167:109-13
161. Otto SJ, van Houwelingen AC, Antal M, *et al*. Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. Eur J Clin Nutr 1997;51:232-42
162. Chen HW, Lii CK, Ou CC, *et al*. Plasma vitamins A and E and red blood cell fatty acid profile in newborns and their mothers. Eur J Clin Nutr 1996;50:556-9
163. Al MDM, van Houwelingen AC, Badart-Smook A, Hornstra G. Some aspects of neonatal essential fatty acid status are altered by linoleic acid supplementation during pregnancy. J Nutr 1995:125;2822-30
164. Al MDM, van Houwelingen AC, Kester ADM, *et al*. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. Br J Nutr 1995;74:55-68
165. Rump P, Mensink RP, Kester DM, Hornstra G. Essential fatty acid composition of plasma phospholipids and birth weight: a study in term neonates. Am J Clin Nutr 2001;73:797-806

166. Connor WE, Lowensohn R, Hatcher L. Increased docosahexaenoic acid levels in human newborn infants by administration of sardines and fishoil during pregnancy. Lipids 31;1996:S183-7
167. Al MDM, van Houwelingen AC, Hornstra G. Long-chain polyunsaturated fatty acids, pregnancy and pregnancy outcome. Am J Clin Nutr 2000;71:S285-91
168. Velzing-Aarts FV, van der Klis FRM, van der Dijs FPL, *et al.* Effect of three low-dose fish oil supplements, administered during pregnancy, on neonatal long-chain polyunsaturated fatty acid status at birth. Prostaglandins Leukot Essent Fatty Acids 2001;65:51-7
169. van Beusekom CM, Nijeboer HJ, van der Veere CN, *et al*. Indicators of long chain polyunsaturated fatty acid status of exclusively breastfed infants at delivery and after 20-22 days. Early Hum Dev 1993;32:207-18
170. Ruyle M, Conner WE, Anderson GJ, Lowensohn RI. Placental transfer of essential fatty acids in humans: Venous-arterial difference for docosahexaenoic acid in fetal umbilical erythrocytes. Proc Natl Acad Sci USA 1990;7902-6
171. Al MDM, Hornstra G, van der Schouw YT, *et al*. Biochemical EFA status of mothers and their neonates after normal pregnancy. Early Hum Dev 1990;24:239-48
172. Koletzko B, Müller J. Cis- and trans isomeric fatty acids in plasma lipids of newborn infants and their mothers. Biol Neonate 1990;57:172-8
173. Hoving EB, van Beusekom CM, Nijeboer HJ, Muskiet FAJ. Gestational age dependency of essential fatty acids in cord plasma cholesterol esters and triglycerides. Pediatr Res 1994;35:461-9
174. Olegard R, Svennerholm L. Fatty acid composition of plasma and red cell phosphoglycerides in full term infants and their mothers. Acta Paediatr Scand 1970;59:637-47
175. Crawford MA, Hassam AG, Williams G. Essential fatty acids and fetal brain growth. Lancet 1976;1:452-3
176. Dutta-Roy AK. Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta. Am J Clin Nutr 2000;71:S315-22
177. Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Changes in the maternal essential fatty acid profile during early pregnancy and the relation of the profile to diet. Am J Clin Nutr 2001;73:302-7
178. Holman RT, Johnson SB, Ogburn PL. Deficiency of essential fatty acids and membrane fluidity during pregnancy and lactation. Proc Natl Acad Sci USA 1991;88:4835-9
179. MDM Al, van Houwelingen AC, Hornstra G. Relation between birth order and the maternal and neonatal docosahexaenoic acid status. Eur J Clin Nutr 1997;51:548-53
180. Koletzko B, Braun M. Arachidonic acid and early human growth: Is there a relation? Ann Nutr Metab 1991;35:128-31
181. Leaf AA, Leighfield MJ, Costeloe KL, Crawford MA. Long chain polyunsaturated fatty acids and fetal growth. Early Hum Dev 1992;30:183-91
182. Foreman-van Drongelen MMHP, van Houwelingen AC, Hasaart THM, *et al*. Long-chain polyunsaturated fatty acids in preterm infants: status at birth and its influence on postnatal levels. J Pediatr 1995;126:611-8
183. Crawford MA, Doyle W, Drury P, *et al*. n-6 and n-3 fatty acids during early human development. J Intern Med Suppl 1989;225:159-69
184. Tjoonk HM, Wildeman JAL, Bakker RR, *et al*. Cord vessel arachidonic- and docosahexaenoic acid contents of healthy term infants at birth: negative correlations with anthropometry and positive correlations with gestational age. Abstract no 45. J Pediatr Gastroenterol Nutr 2001:32:404
185. Olsen SF, Sorensen JD, Secher NJ, *et al*. Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. Lancet 1992;339:1003-7
186. Olsen FS, Hansen SH, Secher JN, *et al*. Gestation length and birth weight in relation to intake of marine n-3 fatty acids. Br J Nutr 1995;73:397-404
187. Laryea M, Cieslicki P, Diekmann E, Wendel U. Age-dependent fatty acid composition of erythrocyte membrane phospholipids in healthy children. Z Ernahrungswiss 1990;29:284-94
188. Pikaar NA, Fernandes J. Influence of different types of dietary fat on the fatty acid composition of some serum lipid fractions in infants and children. Am J Clin Nutr 1966;19:194-204
189. Rodriguez A, Sarda P, Nessman C, *et al*. Fatty acid desaturase activities and polyunsaturated fatty acid composition in human liver between the seventeenth and thirty-sixth gestational weeks. Am J Obstet Gynecol 1998;179:1063-70
190. Olegard R, Svennerholm L. Effects of diet on fatty acid composition of plasma and red cell phosphoglycerides in three-month-old infants. Acta Paediat Scand 1971;60:505-11
191. Makrides M, Neumann M, Simmer K, *et al*. Are long-chain polyunsaturated fatty acids essential nutrients in infancy? Lancet 1995;345:1463-8

192. Carlson SE, Ford AJ, Werkman SH, *et al*. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. Pediatr Res 1996;39:882-8
193. Innis S, Auestad N, Siegman JS. Blood lipid docosahexaenoic and arachidonic acid in term gestation infants fed formulas with high docosahexaenoic acid, low eicosapentaenoic acid fish oil. Lipids 1996;31:617-25
194. Kohn G, Sawatzki G, Van Biervliet JP. Long-chain polyunsaturated fatty acids in infant nutrition. Eur J Clin Nutr 1994;48:S2:S1-7
195. Decsi T, Thiel I, Koletzko B. Essential fatty acids in full term infants fed breast milk or formula. Arch Dis Child 1995;72:F23-8
196. Innis SM, Akrabawi SS, Diersen-Schade DA, *et al*. Visual acuity and blood lipids in term infants fed human milk or formulae. Lipids 1997;32:63-72
197. Putnam JC, Carlson SE, DeVoe PW, Barness LA. The effect of dietary fatty acids on the fatty acid composition of erythrocyte phosphatidylcholine and phosphatidylethanolamine in human infants. Am J Clin Nutr 1982;36:106-14
198. Sanders TAB, Naismith DJ. A Comparison of the influence of breast-feeding and bottle-feeding on the fatty acid composition of the erythrocytes. Br J Nutr 1979;41:619-23
199. Maurage C, Guesnet P, Pinault M, *et al*. Effect of two types of fish oil supplementation on plasma and erythrocyte phospholipids in formula-fed term infants. Biol Neonate 1998;74:416-29
200. Bondia-Martinez E, Lopez-Sabater MC, Castellote-Bargallo AI, *et al*. Fatty acid composition of plasma and erythrocytes in term infants fed human milk and formulae with and without docosahexaeonic and arachidonoic acids from egg yolk lecithin. Earl Hum Dev 1998;53:109-19
201. Ponder DL, Innis SM, Benson JD, Siegman JS. Docosahexaenoic acid status of term infants fed breast milk or infant formula containing soy oil or corn oil. Pediatr Res 1992;32:683-8
202. De-Lucchi C, Faus PMJ, Periago JL, Gil A. Influences of diet and postnatal age on the lipid composition of red blood cell membrane in newborn infants. Ann Nutr Metab 1988;32:231-9
203. Makrides M, Neumann MA, Jeffrey B, *et al*. A randomized trial of different ratios of linoleic to α-linolenic acid in the diet of term infants: effects on visual function and growth. Am J Clin Nutr 2000;71:120-9
204. Decsi T, Koletzko B. Fatty acid composition of plasma lipid classes in healthy subjects from birth to young adulthood. Eur J Pediatr 1994;153:520-5
205. Boersma ER. Changes in fatty acid composition of body fat before and after birth in Tanzania: An international comparative study. Br Med J 1979;1:850-3
206. Farquharson J, Cockburn F, Patrick WA, *et al*. Infant cerebral cortex phospholipid fatty-acid composition and diet. Lancet 1992;340:810-3
207. Farquharson J, Jamieson EC, Abbasi KA, *et al*. Effect of diet on the fatty acid composition of the major phospholipids of infant cerebral cortex. Arch Dis Child 1995;72:198-203
208. Hoffman DR, Uauy R. Essentiality of dietary ω3 fatty acids for premature infants: plasma and red blood cell fatty acid composition. Lipids 1992;27:886-95
209. Carlson SE, Cooke RJ, Rhodes PG, *et al*. Effect of vegetable and marine oils in preterm infant formulas on blood arachidonic and docosahexaenoic acids. J Pediatr 1992;120:159-67
210. Carlson SE, Rhodes PG, Rao VS, Goldgar DE. Effect of fish oil supplementation on the n-3 fatty acid content of red blood cell membranes in preterm infants. Pediatr Res 1987;21:507-10
211. Auestad N, Montalto MB, Hall RT, *et al*. Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. Pediatr Res 1997;41:1-10
212. Birch EE, Hoffman DR, Uauy R, *et al*. Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatr Res 1998;44:201-9
213. Birch EE, Garfield S, Hoffman DR, *et al*. A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Dev Med Child Neurol 2000;42:174-81
214. Billeaud C, Bougle D, Sarda P, *et al*. Effects of preterm infant formula supplementation with α-linolenic acid with a linoleate/α-linolenate ratio of 6: a multicentric study. Eur J Clin Nutr 1997;51:520-6
215. Innis SM, Foote KD, MacKinnon JM, King DJ. Plasma and red blood cell fatty acids of low-birth-weight infants fed their mother's expressed breast milk or preterm-infant formula. Am J Clin Nutr 1990;51:994-1000
216. Makrides M, Gibson RA. Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. Am J Clin Nutr 2000;71:S307-11

217. Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Comparison of the peripartum and postpartum phospholipid polyunsaturated fatty acid profiles of lactating and nonlactating women. Am J Clin Nutr 2001;73:1074-9
218. van den Ham EC, van Houwelingen AC, Hornstra G. Evaluation of the relation between n-3 and n-6 fatty acid status and parity in nonpregnant women from the Netherlands. Am J Clin Nutr 2001;73:622-7
219. Gibson RA, Makrides M. The role of long chain polyunsaturated fatty acids (LCPUFA) in neonatal nutrition. Acta Paediatr 1998;87:1017-22
220. Carlson SE. Long-chain polyunsaturated fatty acids and development of human infants. Acta Paediatr Suppl 1999;430:72-7
221. Carlson SE, Neuringer M. Polyunsaturated fatty acid status and neurodevelopment: A summary and critical analysis of the literature. Lipids 1999;34:171-8
222. Gibson RA, Makrides M. Polyunsaturated fatty acids and infant visual development: A summary of the visual development literature and critical appraisal of randomized clinical trials. Lipids 1999;34:179-84
223. Willats P, Forsyth JS, DiModugno MK, *et al*. Effects of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. Lancet 1998;352:688-91
224. Makrides MM, Neumann MA, Simmer K, Gibson RA. A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: A randomized, controlled trial. Pediatrics 2000;105:32-8
225. Scott DT, Janowsky JS, Carroll RE, *et al*. Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? Pediatrics 1998;102:E59
226. Lucas A, Stafford R, Abbott R, *et al*. Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant-formula milk: a randomised trial. Lancet 1999;354:1948-54
227. Florey C du V, Leech AM, Blackhall A. Infant feeding and mental and motor development at 18 months of age in first born singletons. Int J Epidemiol 1995;24:S21-6
228. Lanting CI, Fidler V, Huisman M, *et al*. Neurological differences between 9-year-old fed breastmilk or formula-milk as babies. Lancet 1994;344:1319-22
229. Agostoni C, Trojan S, Belli R, *et al*. Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: The role of long-chain polyunsaturated fatty acids. Pediatr Res 1995;38:262-6
230. De Andraca I, Uauy R. Breastfeeding and optimal mental development. World Rev Nutr Diet 1995;78:1-27
231. Anderson JW, Johnstone BM, Remely DT. Breastfeeding and cognitive development: A meta-analysis. Am J Clin Nutr 1999;70:525-35
232. Angelsen NK, Vik T, Jacobsen G, Bakketeig LS. Breast feeding and cognitive development at age 1 and 5 years. Arch Dis Child 2001;85:183-8
233. Uauy R, Peirano P. Breast is best: human milk is the optimal food for brain development. Am J Clin Nutr 1999;70:433-4
234. Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992;339:261-4
235. Agostoni C, Marangoni F, Lammardo AM, *et al*. Breastfeeding duration, milk fat composition and developmental indices at 1 year of life among breastfed infants. Prostaglandins Leukot Essent Fatty Acids 2001;64:105-9
236. Williams C, Birch EE, Emmett PM, Northstone K, and the ALSPAC study team. Stereoacuity at age 3.5 y in children born full-term is associated with prenatal and postnatal dietary factors: a report from a population-based cohort study. Am J Clin Nutr 2001;73:316-22
237. Willats P, Forsyth JS, DiModugno MK, *et al*. Influence of long-chain polyunsaturated fatty acids on infant cognitive function. Lipids 1998;33:973-80
238. Carlson SE, Cooke RJ, Werkman SH, Tolley EA. First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula. Lipids 1992;27:901-7
239. Woltil HA, van Beusekom CM, Schaafsma A, *et al*. Long-chain polyunsaturated fatty acid status and early growth of low birth weight infants. Eur J Pediatr 1998;157:146-52
240. Decsi T, Koletzko B. Growth, fatty acid composition of plasma lipid classes, and plasma retinol and α-tocopherol concentrations in full-term infants fed formula enriched with ω-6 and ω-3 long-chain polyunsaturated fatty acids. Acta Paediatr 1995;84:725-32
241. Makrides M, Neumann MA, Simmer K, Gibson RA. Dietary long-chain polyunsaturated fatty acids do not influence growth of term infants: A randomized clinical trial. Pediatrics 1999;104:468-75
242. Jensen CL, Prager TC, Fraley JK, *et al*. Effect of dietary linoleic/alpha-linolenic acid ratio on growth and visual function of term infants. J Pediatr 1997;131:200-9
243. Stevens LJ, Zentall SS, Deck JL, *et al*. Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. Am J Clin Nutr 1995;62:761-8

244. Stevens LJ, Zentall SS, Abate ML, *et al*. Omega-3 fatty acids in boys with behavior, learning and health problems. Physiol Behav 1996;59:915-20
245. Stordy BJ. Dark adaptation, motor skills, docosahexaenoic acid, and dyslexia. Am J Clin Nutr 2000;71:S323-6
246. Decsi T, Zaknun D, Zaknun J, *et al*. Long-chain polyunsaturated fatty acids in children with severe protein-energy malnutrition with and without human immunodeficiency virus-1 infection. Am J Clin Nutr 1995;62:1283-8
247. Dougherty RM, Galli C, Ferro-Luzzi A, Iacono JM. Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. Am J Clin Nutr 1987;45:443-55
248. Fokkema MR, Brouwer DAJ, Hasperhoven MR, *et al*. Polyunsaturated fatty acid status of Dutch vegans and omnivores. Prostaglandins Leukot Essent Fatty Acids 2000;63:279-85
249. Ägren JJ, Törmälä M, Nenonen MT, Hänninen OO. Fatty acid composition of erythrocyte, platelet, and serum lipids in strict vegans. Lipids 1995;30:365-9
250. Rodriguez-Palmero M, Lopez-Sabater MC, Castelote-Bargallo AI, de la Torre-Boronat MC, *et al*. Administration of low doses of fish oil derived N-3 fatty acids to elderly subjects. Eur J Clin Nutr 1997;51:554-60
251. Popp-Snijders C, Schouten JA, de Jong AP, van der Veen EA. Effect of dietary cod-liver oil on the lipid composition of human erythrocyte membranes. Scand J Clin Lab Invest 1984;44:39-46
252. Vognild E, Elvevoll EO, Brox J, *et al*. Effects of dietary marine oils and olive oil on fatty acid composition, platelet membrane fluidity, platelet responses, and serum lipids in healthy humans. Lipids 1998;33:427-36
253. Phinney SD, Odin RS, Johnson SB, Holman RT. Reduced arachidonate in serum phospholipids and cholesteryl esters associated with vegetarian diets in humans. Am J Clin Nutr 1990;51:385-92
254. Seyberth HW, Oelz O, Kennedy T, *et al*. Increased arachidonate in lipids after administration to man: effects on prostaglandin biosynthesis. Clin Pharmacol Ther 1975;18:521-9
255. Yoshida S, Sato A, Okuyama H. Pathophysiological effects of dietary essential fatty acid balance on neural systems. Jpn J Pharmacol 1998;77:11-22
256. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 1985;312:1205-9
257. Nestel PJ. Fish oil and cardiovascular disease: lipids and arterial function. Am J Clin Nutr 2000;71:S228-31
258. de Lorgeril M, Renaud S, Mamelle N, *et al*. Mediterranean alpha-linoleic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454-9
259. Sammon AM. Dietary linoleic acid, immune inhibition and disease. Postgrad Med J 1999;75:129-32
260. Yam D, Eliraz A, Berry EM. Diet and disease-The Israeli paradox: Possible dangers of a high omega-6 polyunsaturated fatty acid diet. Isr J Med Sci 1996;32:1134-43
261. Bjerve KS, Fischer S, Wammer F, Egeland T. α-Linolenic acid and long-chain ω-3 fatty acid supplementation in three patients with ω-3 fatty acid deficiency: effect on lymphocyte function, plasma and red cell lipids, and prostanoid formation. Am J Clin Nutr 1989;49:290-300
262. Torun B, Chew F. Protein-energy malnutrition. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea and Febiger.1994:950-76
263. Waterlow JC, ed. Protein-energy malnutrition. London: Edward Arnold, 1992
264. Jackson AA, Golden MHN, Pereira SM. Protein energy malnutrition: Kwashiorkor and marasmic kwashiorkor. Physiopathology. In: Clinical nutrition of the young child, pp. 133-42. Raven Press, New York. 1986
265. Hendricks KM, Duggan C, Gallagher L, *et al*. Malnutrition in hospitalized pediatric patients. Current prevalence. Arch Pediatr Adolesc Med 1995:149;1118-22
266. Allison SP, Rawlongs J, Field J, *et al*. Nutrition in the elderly hospital patient Nottingham studies. J Nutr Health Aging 2000:4;54-7
267. Holman RT, Johnson SB, Mercuri O, *et al*. Essential fatty acid deficiency in malnourished children. Am J Clin Nutr 1981;34:1534-9
268. Wolff JA, Margolis JA, Bujdoso-Wolff K, *et al*. Plasma and red blood cell fatty acid composition in children with protein-calorie malnutrition. Ped Res 1984;18:162-7
269. Chen SCH, Dickerman MD. Iron, thyroid hormone and essential fatty acid status of Honduran preschoolers. Nutr Res 1985;5:21-30
270. Koletzko B. Abiodun PO, Larya MD, Bremer HJ. Fatty acid composition of plasma in Nigerian children with protein-energy malnutrition. Eur J Pediatr 1986;145:109-15
271. Vajreswari A, Narayanareddy K, Srinivasa Rao P. Fatty acid composition of erythrocyte membrane lipid obtained from children suffering from kwashiorkor and marasmus. Metabolism 1990;39:779-82

272. Marin MC, De Tomas ME, Mercuri O, *et al*. Interrelationship between protein-energy malnutrition and essential fatty acid deficiency in nursing infants. Am J Clin Nutr 1991;53:466-8
273. Leichsenring M, Ahmed HM, Laryea MD, *et al*. Polyunsaturated and essential fatty acids in malnourished children. Nutr Res 1992;12:595-603
274. Leichsenring M, Sütterlin N, Less S, *et al*. Polyunsaturated fatty acids in erythrocyte and plasma lipids of children with severe protein-energy malnutrition. Acta Paediatr 1995;84:516-20
275. Smit EN, Dijkstra JM, Schnater TA, *et al*. Effects of malnutrition on the fatty acid composition and plasma vitamin E levels of Pakistani children. Acta Paediatr 1997;86:690-5
276. Franco VHM, Hotta JKS, Jorge SM, dos Santos JE. Plasma fatty acids in children with grade III protein energy malnutrition in its different clinical forms: marasmus, marasmic kwashiorkor and kwashiorkor. J Trop Pediatr 1999;45:71-5
277. Squali Houssaini FZ, Foulon T, Iraqi MR, *et al*. Lipids, lipoproteins and fatty acids during infantile marasmus in the Fes area of Morocco. Biomed Pharmacother 1999;53:278-83
278. Begin ME, Manku MS, Horrobin DF. Plasma fatty acid levels in patients with acquired immune deficiency syndrome and in controls. Prostaglandins Leukot Essent Fatty Acids 1989;37:135-7
279. Scrimshaw NS, SanGiovanni JP. Synergism of nutrition, infection, and immunity: an overview. Am J Clin Nutr 1997;66:464S-477S
280. Fondu P, Mozes N, Neve P, *et al*. The erythrocyte membrane disturbances in protein-energy malnutrition: Nature and machanisms. Br J Haematol 1980;44:605-18
281. Karlsson I, Svennerholm L. Biochemical development of rat forebrains in severe protein and essential fatty acid deficiencies. J Neurochem 1978;31:657-62
282. Morley R, Lucas A. Nutrition and cognitive development. Br M Bull 1997;53:123-34
283. Simeon DT, Grantham-McGregor SM. Nutritional deficiencies and children's behaviour and mental development. Nutr Res Rev 1990;3:1-24
284. Jiang WG, Eynard AR, Mansel RE. The patology of essential fatty acid deficiency: is it cell adhesion mediated? Med Hypotheses 2000;55:257-62
285. Horrobin DF, Manku MS, Huang YS. Effects of essential fatty acids on prostaglandin biosynthesis. Biomed Biochim Acta 1984;43:S114-20
286. Sellmayer A, Koletzko B. Long-chain polyunsaturated fatty acids and eicosanoids in infants-physiological and pathophyisiological aspects and open questions. Lipids 1999;34:199-205
287. Christon R, Meslin JC, Thevenoux J, *et al*. Effects of a low dietary linoleic acid level on intestinal morphology and enterocyte brush border membrane lipid composition. Reprod Nutr Dev 1991;31:691-701
288. Sellmayer A, Danesch U, Weber PC. Effects of polyunsaturated fatty acids on growth related early gene expression and cell growth. Lipids 1996;31:S37-40
289. Kruger MC, Horrobin DF. Calcium metabolism, osteoporosis and essential fatty acids: a review. Prog Lipid Res. 1997;36:131-51
290. Panos TC, Finerty JC, Wall RL. Increased metabolism in fat deficiency. Proc Soc Exp Biol Med 1956;93:581-4
291. Naismith DJ. The role of dietary fat in the utilization of protein. II. The essential fatty acids. J Nutr 1962;77:381-6
292. Adam DJD, Hansen AE, Wiese HF. Essential fatty acids in infant nutrition. II Effect of linoleic acid on caloric intake. J Nutr 1958;66:555-64
293. Fernstrom JD. Effects of dietary polyunsaturated fatty acids on neuronal function. Lipids 1999;34:161-9
294. Marin MC, Rey GE, Pedersoli LC, *et al*. Dietary long-chain fatty acids and visual response in malnourished nursing infants. Prostaglandins Leukot Essent Fatty Acids 2000;63:385-90
295. Bird MI, Williams MA. Triacylglycerol secretion in rats: effects of essential fatty acids and influence of dietary sucrose, glucose or fructose. J Nutr 1982;112:2267-78
296. Gracey M, Cullity GJ, Suharjono, Sunoto. The stomach in malnutrition. Arch Dis Child 1977;52:325-7
297. Gracey M, Suharjono, Sunoto, Stone DE. Microbial contamination of the gut: another feature of malnutrition. Am J Clin Nutr 1973;26:1170-4
298. Mata LJ, Jiménez F, Cordón M, *et al*. Gastrointestinal flora of children with protein-calorie malnutrition. Am J Clin Nutr 1972;25:118-26
299. Gracey MS. Nutrition, bacteria and the gut. Br Med Bull 1981;37:71-5
300. Suskind RM. Gastrointestinal changes in the malnourished child. Pediatr Clin North Am 1975;22:873-83
301. Viteri FE, Schneider RE. Gastrointestinal alterations in protein-calorie malnutrition. Med Clin North Am 1974;58:1487-1505
302. Minich DM, Vonk RJ, Verkade HJ. Intestinal absorption of essential fatty acids under physiological and essential fatty acid-defecient conditions. J Lipid Res 1997;38:1709-21

303. Brunser O. Effects of malnutrition on intestinal structure and function in children. Clin Gastroenterol 1977;6:341-53
304. Lopez-Pedrosa JM, Torres MI, Fernandez MI, *et al*. Severe malnutrition alters lipid composition and fatty acid profile of small intestine in newborn piglets. J Nutr 1998;128:224-33
305. Alessandri JM, Arfi TS, Thevenoux J, Léger CL. Diet-induced alterations of lipids during cell differentiation in the small intestine of growing rats: effect of an essential fatty acid deficiency. J Pediatr Gastroenterol Nutr 1990;10:504-15
306. Snipes RL. The effects of essential fatty acid deficiency on the ultrastructure and functional capacity of the jejunal epithelium. Lab Invest 1968;18:179-89
307. Dodge JA. Dietary fats and gastrointestinal function. Eur J Clin Nutr 1994;48:S2:S8-16
308. Lopez-Pedrosa JM, Ramirez M, Torres MI, Gil A. Dietary phospholipids rich in long-chain polyunsaturated fatty acids improve the repair of small intestine in previously malnourished piglets. J Nutr 1999;129:1149-55
309. Garg ML, Keelan M, Thomson ABR, Clandinin MT. Desaturation of linoleic acid in the small bowel is increased by short-term fasting and by dietary content of linoleic acid. Biochim Biophys Acta 1992;1126:17-25
310. Bouziane M, Prost J, Belleville J. Changes in serum and lipoprotein fatty acids of growing rats fed protein-deficient diets with low or adequate linolenic acid concentrations. J Nutr 1992;122:2037-46
311. Bouziane M, Prost J, Belleville J. Dietary protein deficiency affects n-3 and n-6 polyunsaturated fatty acids hepatic storage and very low density lipoprotein transport in rats on different diets. Lipids 1994;29:265-72
312. Khan L, Bamji MS. Plasma carnitine levels in children with protein-calorie malnutrition before and after rehabilitation. Clin Chim Acta 1977;75:163-6
313. Guyton AC. Lipid metabolism. In:textbook of medical physiology. 8th ed. Philadelphia: W.B. Saunders Company. 1991:754-64
314. Brasel JA. Endocrine adaptation to malnutrition. Pediatr Res 1980;12:1299-303
315. Clugston GA, Hetzel BS. Iodine. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea and Febiger.1994:252-63
316. Becker DJ. The endocrine responses to protein calorie malnutrition. Ann Rev Nutr 1983;3:187-212
317. Becker K, Botticher D, Leichsenring M. Antioxidant vitamins in malnourished Nigerian children. Internat J Vit Nutr Res 1994;64:306-10
318. Mathias PM. Vitamin E status of children recovering from severe malnutrition. Proc Nutr Soc 1983;41:143A
319. Alam SQ, Alam BS. Microsomal fatty acid desaturase activities in vitamin A-deficient rat liver. Biochim Biophys Acta 1985;833:175-7
320. James WPT, Coore HG. Persistent impairment of insulin secretion and glucose tolerance after malnutrition. Am J Clin Nutr 1970;23:386-9
321. Decsi T, Molnar D, Koletzko B. The effect of under- and overnutrition on essential fatty acid metabolism in childhood. Eur J Clin Nutr 1998;52:541-8
322. Goel R, Misra PK, Seth TD. Study of plasma copper in protein energy malnutrition. Indian Pediatr 1980;17:869-73
323. Caddell JL. Magnesium deficiency in protein-calorie malnutrition: a follow-up study. Ann N Y Acad Sci. 1969;162:874-90
324. Huang YS, Cunnane SC, Horrobin DF. Effect of different dietary proteins on plasma and liver fatty acid compositions in growing rats. Proc Soc Exp Biol Med 1986;181:399-403
325. Sugiyama K, Kumazawa A, Zhou H, Saeki S. Dietary methionine level affects linoleic acid metabolism through phosphatidylethanolamine N-methylation in rats. Lipids 1998;33:235-42
326. Butzner DJ, Brockway PD, Meddings JB. Effects of malnutrition on microvillus membrane glucose transport and physical properties. Am J Physiol 1990;259:G940-6
327. Smuts CM, Tichelaar HY, Van Jaarsveld PJ, *et al*. The effect of iron fortification on the fatty acid composition of plasma and erythrocyte membranes in primary school children with and without iron deficiency. Prostaglandins Leukot Essent Fatty Acids 1995;52:59-67
328. Tichelaar HY, Smuts CM, Gross R, *et al*. The effect of dietary deficiency on the fatty acid composition of plasma and erythrocyte membrane phospholipids in the rat. Prostaglandins Leukot Essent Fatty Acids 1997;56:229-33
329. Stangl GI, Kirchgessner M. Different degrees of moderate deficiency modulate lipid metabolism of rats. Lipids 1998;33:889-95
330. Cunnane SC, McAdoo KR. Iron intake influences essential fatty acid and lipid composition of rat plasma and erythrocytes. J Nutr 1987;117:1514-9

331. Ayala S, Brenner RR. Essential fatty acid status in zinc deficiency. Effect on lipid and fatty acid composition, desaturation activity and structure of microsomal membranes of rat liver and testes. Acta Physiol Lat Am 1983;33:193-204
332. Cunnane SC, Horrobin DF. Probable role of zinc in the mobilization of dihomo-γ-linolenic acid and in the desaturation of linoleic acid. Prog Lipid Res 1981;20:835-7
333. Hamilton RM, Gillespie CT, Cook HW. Relationship between levels of essential fatty acids and zinc in plasma of cystic fibrosis patients. Lipids 1981;16:374-6
334. Cabre E, Periago JL, Mingorance MD, *et al*. Factors related to the plasma fatty acid profile in healthy subjects, with special reference to antioxidant micronutrient status: a multivariate analysis. Am J Clin Nutr 1992;55:831-7
335. Cunnane SC, McAdoo KR, Prohaska JR. Lipid and fatty acid composition of organs from copper-deficient mice. J Nutr 1986;116:1248-56
336. Lawrence CB, Davies NT, Mills CF, Nicol F. Studies on the effects of copper deficiency on rat liver mitochondria. I. Changes in mitochondrial composition. Biochim Biophys Acta 1985;809:351-61
337. Mahfouz MM, Kummerow FA. Effect of magnesium deficiency on delta 6 desaturase activity and fatty acid composition of rat liver microsomes. Lipids 1989;24:727-32
338. Soma M, Cunnane SC, Horrobin DF, *et al*. Effects of low magnesium diet on the vascular prostaglandin and fatty acid metabolism in rats. Prostaglandins 1988;36:431-41
339. Cunnane SC, Soma M, McAdoo KR, Horrobin DF. Magnesium deficiency in the rat increases tissue levels of docosahexaenoic acid. J Nutr 1985;115:1498-1503
340. Galland L. Impaired essential fatty acid metabolism in latent tetany. Magnesium 1985;4:333-8
341. Levine RJ, Olson RE. Blood selenium in Thai children with protein-calorie malnutrition. Proc Soc Exp Biol Med 1970;134:1030-4
342. Infante JP. Vitamin E and selenium participation in fatty acid desaturation. A proposal for an enzymatic function of these nutrients. Mol Cell Biochem. 1986;69:93-108
343. Huang YS, McAdoo KR, Mitchell J, Horrobin DF. Effects of calcium deprivation on n-6 fatty acid metabolism in growing rats. Biochem Med Metab Biol. 1988;40:61-7
344. Parsons HG, O'Loughlin EV, Forbes D, *et al*. Supplemental calories improve essential fatty acid deficiency in cystic fibrosis patients. Pediatr Res 1988;24:353-6
345. Olson AL, Nelson SE, Rebouche CJ. Low carnitine intake and altered lipid metabolism in infants. Am J Clin Nutr 1989;49:624-8
346. Kerr DS, Stevens MC, Robinson HM. Fasting metabolism in infants. I. Effect of severe undernutrition on energy and protein utilization. Metabolism 1978;27:411-35
347. Infante JP, Huszagh VA. Secondary carnitine deficiency and impaired docosahexaeonic (22:6n-3) acid synthesis: a common denominator in the pathophysiology of diseases of oxidative phosphorylation and β-oxidation. FEBS letters 2000;468:1-5
348. Chow CK. Oxidative damage in the red cells of vitamin E-deficient rats. Free Rad Res Comms 1992;16:247-58
349. van Gastel C, van den Berg D, de Gier J, van Deenen LLM. Some lipid characteristics of normal red blood cells of different age. Br J Haematol 1965;11:193-9
350. Redmond HP, Shou J, Kelly CJ, Schreiber S, Miller E, Leon P, Daly JM. Immunosuppressive mechanisms in protein-calorie malnutrition. Surgery 1991;110:311-7
351. Redmond HP, Gallagher HJ, Shou J, Daly JM. Antigen presentation in protein-energy malnutrition. Cell Immunol 1995;163:80-7
352. Skerrett SJ, Henderson WR, Martin TR. Alveolar macrophage function in rats with severe protein calorie malnutrition. J Immunol 1990;144:1052-61
353. Golden MHN. Marasmus and kwashiorkor. In: Dickerson JWT, Lee MA, eds. Nutrition in the clinical management of disease. 2nd ed. Edward Arnold, London. 1988:88-109
354. Smit EN, Oelen EA, Seerat E, *et al*. Fish oil supplementation improves docosahexaenoic acid status of malnourished infants. Arch Dis Child 2000;5:366-9

Chapter 2 Essential fatty acid status in malnutrition

2.1. Effects of malnutrition on the erythrocyte fatty acid composition and plasma vitamin E levels of Pakistani children

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Abstract

Erythrocyte fatty acids and plasma vitamin E concentrations were determined in 47 grade 2 and 21 grade 3 malnourished Pakistani children (ages 4-56 months). Data were compared with those of 26 age and sex matched apparently healthy controls. Evaluation with three statistical approaches revealed that both grade 2 and grade 3 malnourished children had decreased erythrocyte ω6 fatty acids and to a lesser extent decreased ω3 fatty acids. These decreases were compensated for by increased ω9 fatty acids. Grade 2 patients had lower plasma vitamin E concentrations. We conclude that malnourished Pakistani children have low essential fatty acid status, notably those of the ω6 series. The combination of low erythrocyte 22:6ω3 and a low 22:5ω6/22:4ω6 ratio in grade 2 patients suggests low Δ4-desaturation activity, which may be due to impaired peroxisomal β-oxidation.

2.1.1. Introduction

In the Punjab area in Pakistan, 5% of infant mortality is caused by malnutrition and diarrhoea [1], and only 46% of children is, according to WHO standards, adequately nourished [2]. A one day survey in the paediatric out-patient department of the Federal Government Services Hospital Islamabad showed that only 38% of the children was in a satisfactory nutritional state [3].

In malnutrition lipid metabolism is disturbed [4-9]. Malnourished children have reduced dietary intake, and poor digestion and absorption of lipids [10], which may cause essential fatty acid (EFA) deficiency. In addition, changes in the hepatic chain elongation and desaturation enzyme systems may lead to shortage of long chain polyunsaturated fatty acids (LCPUFA) [4-7,9-12] of the ω6- and ω3-series that derive from the two parent EFAs linoleic- (18:2ω6) and α-linolenic (18:3ω3) acids, respectively. LCPUFAs are structural components of cellular membranes and precursors of eicosanoids (Prostaglandins, thromboxanes and leukotrienes) [11,13-15]. A deficiency may lead to permanent damage, especially during the vulnerable period of rapid cell growth in the brain, which takes place before and after birth up to the age of about 18 months [6,11,16-18]. EFA deficiency may be worsened by low vitamin E levels, causing an increased oxidative decomposition of LCPUFA [4].

EFA deficiency may occur as either a combined ω3 and ω6 deficiency or an isolated ω3 deficiency [11]. Previous studies among malnourished children in Nigeria [4], Peru [5], Argentine [6,7] and Romania, including children suffering from human immunodeficiency virus [9], showed a deficiency of 18:2ω6 and its derivates in plasma [4-7,9] or erythrocytes (RBC) [5,6]. In other studies from Nigeria [12] and India [8], only some RBC and plasma LCPUFAω6 were decreased, whereas the RBC 18:2ω6 content was unaffected [12] or even slightly increased [8]. The ω3 fatty acids in plasma phospholipids were found to be either slightly increased [7] or decreased [4,9].

We investigated the EFA status of malnourished children in Pakistan. Fatty acids were measured in RBC and compared with data from well nourished age and sex matched controls. We additionally studied their vitamin E status, as derived from the plasma vitamin E concentration.

2.1.2. Patients and methods

2.1.2.1 *Patients, controls, blood sampling and questionnaire*

Patients and controls were recruited from the Nutrition Rehabilitation Center of the Paediatric Department, Federal Government Services Hospital, Islamabad. They were classified according to local growth-charts, provided by WHO. Severe (grade 3) and moderate (grade 2) malnutrition were defined as a weight-for-age below the mean-3SD and mean-2SD, respectively, using the data from the United States National Center for Health Statistics (NCHS) as reference. Accordingly, the finally selected group was composed of 47 moderately and 21 severely malnourished children (1 kwashiorkor and 20 marasmus), and 26 controls. EDTA anticoagulated blood (maximum 2.5 ml) was taken in an undefined metabolic state and anthropometric, demographic and socioeconomic data were collected. Characteristics of the study groups are given in Table 1. Diarrhoea was defined as 3 or more loose stools per day. Informed consent was obtained from all mothers. The study conformed to local ethical standards and the Helsinki declaration of 1975 as revised in 1989.

2.1.2.2 *Sample processing and analyses*

Blood samples were immediately cooled on ice and counted by means of a Sysmex counter. Within 24 hours, the samples were centrifuged at 800 g for 10 minutes in a cooled centrifuge. Plasma was centrifuged again at 1600 g for 10 min. The buffy coat was removed and the RBC washed three times with isotonic saline. The RBC were finally resuspended to a hematocrit of about 50% and counted again. For the analysis of RBC fatty acids, 200 µl of this suspension was transferred to a 15 ml teflon-stoppered tube, containing 1 mg butylated hydroxytoluene (antioxidant) and 50.0 µg margaric acid (17:0; internal quantification standard). For the analysis of plasma vitamin E, 200 µl EDTA-plasma was transferred to a tube that contained 0.5 ml antioxidant solution A (25 mmol potassium EDTA and 910 mmol vitamin C/l water), 1.3 ml antioxidant solution B (110 mmol pyrogallol and 250 mmol butylated hydroxytoluene/l methanol) and 1.7 ml methanol. All samples were stored at -20°C and transported to The Netherlands in dry ice. RBC fatty

Table 1. Characteristics of the study groups.

	Controls (n=26)	Grade 2 patients (n=47)	Grade 3 patients (n=21)
Children			
Age (months)	13 (2-60)	18 (4-56)	16 (8-30)
Gender (M/F)	14/12	22/25	12/9
Weight (kg)	8.8 (3.8-17.0)	7.3 (3.3-11.0)[a]	6.0 (3.5-7.8)[a,c]
Hb (g/l)	101 (58-132)	98 (40-132)	87 (51-106)[a,b]
RBC (10^{12}/l)	4.7 (2.9-5.2)	4.4 (1.4-5.9)	4.3 (3.1-5.5)
WBC (10^{9}/l)	9.8 (4.7-19.3)	10.4 (3.5-16.5)	11.0 (6.2-27.8)
HCT	0.32 (0.24-0.40)	0.31 (0.15-0.39)	0.30 (0.20-0.45)
MCV (fl)	71 (52-100)	72 (54-113)	68 (55-98)
MCH (pg)	22 (14-31)	23 (13-32)	20 (14-25)
MCHC (g/l)	313 (247-341)	307 (231-342)	272 (232-327)[a,c]
Diarrhoea (%)	19	59	57
Cough and cold (%)	50	59	57
Skin lesions (n)	0	1	2
Breastfeeding (%)	76[1]	52[2]	60[3]
Exclusive breastfeeding (months)[#]	4[4] (0-23)	5[5] (0-26)	6[6] (0.5-26)
Breastfeeding (months)[#]	9[7] (2-23.5)	13[8] (0.3-42)	15[9] (0.5-30)
Weaning food			
Buffalo milk (%)	40	65	65
Goat milk (%)	4	2	10
Formula (%)	0	7	0
Porridge (%)	52	91	50
Other (%)	52	65	25
Mothers			
Age (years)	25 (18-40)	25 (18-40)	25 (18-50)
Illiteracy (%)	45[10]	77[11]	81[12]
Living in urban areas (%)	36[10]	57[11]	30[12]

Data represent median (range), unless other indicated.
[#] At moment of blood collection.
[1],n=25; [2], n=46; [3], n=20; [4], n=24; [5], n=34; [6], n=18; [7], n=21; [8], n=29; [9], n=16; [10], n=16; [11], n=30; [12], n=11.
[a] Significantly different from controls at $p<0.01$; [b] Significantly different from grade 2 at $p<0.05$; [c] Significantly different from grade 2 at $p<0.01$. Abbreviations: Hb = haemoglobin, RBC = red blood cell count, WBC = white blood cell count, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration.

acids were determined as their methyl esters by capillary gas chromatography with flame ionization detection [19]. Data were expressed in mol/100 mol fatty acids. Vitamin E (i.e. α- and γ-tocopherol) was quantified by high-performance liquid chromatography with ultraviolet detection [20,21], using tocol as internal standard. Total vitamin E is the sum of α- and γ-tocopherol. Because of insufficient sample volumes vitamin E could not be measured in all samples.

2.1.2.3 *Data evaluation and statistics*

RBC fatty acid data were evaluated in three ways. Data of grade 2 and 3 patients were compared with those of controls using analysis of variance (ANOVA) and the Mann-Whitney U tests [22]. P ≤0.05 (after correction with the Bonferroni inequality rule for type-I errors) was considered significant. Data were also evaluated by stepwise logistic regression [23], which identifies RBC fatty acids that contribute significantly (p ≤0.05) to the separation of the control group with the grade 2 and grade 3 patients, respectively. Initially the following RBC fatty acids were used for logistic regression: 18:3ω3, 20:5ω3, 22:5ω3, 22:6ω3, 18:2ω6, 20:4ω6, 22:4ω6, 22:5ω6, 20:3ω9 and 22:3ω9. Finally we established 95% confidence intervals for each of the RBC fatty acids of the control group by established methods [24]. After counting the number of grade 2 and grade 3 patients with RBC data above or below these 95% confidence limits of the control group, we prepared 2x2 contingency tables. From these we calculated the odds ratios and their 95% confidence intervals [25]. Between-group differences in plasma vitamin E concentrations were compared with the Mann-Whitney U test at p ≤0.05 (after correction with the Bonferroni inequality rule). All statistics were evaluated with Systat (Systat 6.0 for Windows, SPSS Inc, Chicago, Illinois).

2.1.3. Results

Anthropometric, clinical and nutritional data of the malnourished children and controls are given in Table 1. Controls were more likely to be breastfed than malnourished children. The mean duration of breastfeeding was lower in controls compared to the malnourished children. Apart from breastmilk buffalo milk is the main source of milk substitute. Formula was seldomly given. Porridge ('suji', 'dalia', 'kitcherie', mixtures of cereals or rice and pulses with milk and/or water and sometimes oil or ghee) was commonly used as weaning food. Other types of food were given less often to the grade 3 malnourished children. These included vegetables, cereals, pulses and meat. Meat consumption was nevertheless very low. Eggs, fish and fruit were rarely eaten. The amount of oil or ghee used was low. There were no relevant differences in the feeding practices between the urban and rural population.

Selected RBC fatty acids of grade 2 and 3 malnourished children and controls are given in Table 2. Using the Mann-Whitney U test, the following fatty acids were found to be significantly decreased: 22:6ω3 (grade 2), PUFAω6 (grades 2 and 3), and PUFA (grades 2 and 3). Increased RBC fatty acids in grades 2 and 3 were noted for: 18:1ω9, 20:1ω9, 24:1ω9 (grade 3 only), sum ω9 and MUFA. Grade 3 patients had lower RBC 18:2ω6 and higher 24:1ω9, compared with grade 2 counterparts. In addition, grade 2 patients had lower plasma total vitamin E, compared with controls.

Stepwise logistic regression analysis identified 20:5ω3, 22:6ω3, 18:2ω6 and 22:5ω6 as RBC fatty acids that contributed to the separation between grade 2 patients and controls. RBC 22:5ω6 contributed to separation between grade 3 patients and controls. Each of these RBC fatty acids were decreased.

Table 2. Erythrocyte fatty acid compositions of apparently healthy controls and grade 2 and 3 malnourished children.

Fatty acids (mol %)	Controls (n=26)	Grade 2 patients (n=47)	Grade 3 patients (n=21)
18:3ω3	0.17 (0.07-0.35)	0.18 (0.08-0.33)	0.21 (0.10-1.20)
20:5ω3	0.24 (0.13-0.94)	0.28 (0.06-1.12)	0.28 (0.10-1.41)
22:5ω3	1.40 (0.62-2.91)	1.44 (0.41-2.73)	1.49 (0.85-3.86)
22:6ω3	2.90 (1.72-4.04)	2.36 (1.13-3.63)[a]	2.51 (1.11-5.20)
sumω3	4.80 (4.50-6.12)	4.50 (1.85-6.77)	4.46 (3.23-8.36)
LCPUFAω3	4.58 (3.37-5.85)	4.29 (1.55-6.50)	4.33 (3.00-7.89)
18:2ω6	8.83 (5.67-13.62)	8.52 (5.05-13.53)	6.71 (4.06-11.20)[a,c]
20:2ω6	0.25 (0.12-0.48)	0.21 (0.08-0.49)	0.23 (0.08-1.05)
20:3ω6	1.60 (1.25-2.36)	1.52 (0.85-2.16)	1.49 (1.02-2.18)
20:4ω6	14.68 (12.34-16.94)	14.40 (5.91-16.69)	13.81 (7.52-17.41)
22:4ω6	3.05 (2.13-3.85)	2.81 (1.27-4.24)	2.76 (1.00-4.02)
22:5ω6	1.08 (0.78-2.16)	0.97 (0.30-1.45)	0.97 (0.50-1.68)
sumω6	29.97 (23.49-32.46)	28.65 (16.15-34.46)[a]	27.66 (14.23-23.38)[a]
LCPUFAω6	20.80 (17.27-23.00)	20.31 (8.83-24.41)	19.43 (10.18-23.36)
18:1ω9	10.86 (9.44-14.72)	11.76 (8.69-15.43)[a]	12.80 (9.38-18.02)[b]
20:1ω9	0.22 (0.12-0.34)	0.25 (0.15-0.39)[a]	0.27 (0.17-1.14)[a]
20:3ω9	0.37 (0.14-1.32)	0.39 (0.12-2.59)	0.48 (0.26-2.24)
22:3ω9	0.15 (0.10-0.53)	0.18 (0.05-0.80)	0.21 (0.09-0.80)
24:1ω9	3.48 (2.39-4.53)	3.77 (2.57-4.92)	4.33 (3.13-5.27)[a,c]
sumω9	15.04 (13.33-20.36)	16.18 (12.15-22.78)[a]	17.54 (14.10-25.45)[b]
PUFA	35.29 (31.37-37.27)	33.83 (18.27-38.79)[b]	33.70 (19.33-37.29)[a]
MUFA	16.67 (14.10-19.60)	17.66 (13.71-21.52)[a]	19.07 (15.21-24.94)[b]
18:2ω6/20:4ω6	0.60 (0.42-0.98)	0.60 (0.37-1.24)	0.52 (0.30-0.83)
20:3ω9/20:4ω6	0.03 (0.01-0.10)	0.03 (0.01-0.22)	0.04 (0.02-0.20)
22:6ω3/22:5ω3	2.05 (0.64-6.07)	1.52 (0.53-3.11)	1.70 (0.59-3.94)
22:5ω6/22:4ω6	0.37 (0.26-0.71)	0.33 (0.20-0.62)	0.37 (0.27-0.56)
Total vitamin E[1]	13.3 (4.3-18.6)[2]	9.2 (0.4-19.2)[3,a]	13.8 (2.5-19.4)[4]
α-tocopherol[1]	12.1 (3.9-18.0)[2]	9.0 (0.4-19.2)[3]	13.8 (2.5-19.4)[4]
γ-tocopherol[1]	0.5 (0.0-1.5)[2]	0.3 (0.0-1.2)[3]	0.0 (0.0-0.9)[4]

Data represent median (range), in mol/100 mol fatty acids.
[1], µmol/l; [2], n=17; [3], n=34; [4], n=9; [a], significantly different from control at $p<0.05$; [b], significantly different from control at $p<0.01$; [c], significantly different from grade 2 at $p<0.05$.
Abbreviations: PUFA, polyunsaturated fatty acids; LCPUFA, PUFA with 20 or more carbon atoms; MUFA, monounsaturated fatty acids.

Table 3. Odds ratio's (95 % confidential intervals) for erythrocyte fatty acids.

Fatty acids	Grade 2 patients	Grade 3 patients	
	Decreased	Decreased	Increased
18:2ω6		11.87 (1.31-585.2)	
22:4ω6		9.55 (1.01-477.9)	
sumω6		11.87 (1.31-585.2)	
LCPUFAω6		9.55 (1.01-477.9)	
18:1ω9			14.53 (1.66-709.1)
24:1ω9			9.55 (1.01-477.9)
sumω9			21.21 (2.50-1024)
PUFA		14.53 (1.66-709.1)	
MUFA			21.21 (2.50-1024)
22:5ω6/22:4ω6	8.39 (1.11-380.8)		

The data indicate odds ratios and their 95% confidence intervals for RBC fatty acids that showed statistically significant increases or decreases relative to the 95% confidence limits of the control group. For abbreviations see legend of Table 2.

Table 3 shows the odds-ratios calculated for the number of grade 2 and 3 patients that had RBC fatty acid results above or below the 95% confidence interval of the control group. Decreases of RBC 22:5ω6/22:4ω6 were noted in grade 2 and decreases of 18:2ω6, 22:4ω6, sumω6, LCPUFAω6 and PUFA in grade 3. Grade 3 patients had increased 18:1ω9, 24:1ω9, sumω9 and MUFA.

2.1.4. Discussion

We investigated the RBC fatty acid composition of 68 (47 grade 2, 21 grade 3) malnourished Pakistani children and 26 controls. Clinical characteristics of the study population (Table 1) are comparable with those of a national survey, conducted in 1991. In Pakistan, almost 65% of the children under the age of five years suffer from anaemia (Hb <100 g/l) and about 50% from symptoms of the gastro-intestinal or respiratory tract, suggesting an intercurrent infection [2]. We found a higher incidence of symptoms of gastrointestinal infections in the malnourished group, which may support the importance of other factors than merely reduced dietary intake in the etiology of malnutrition. Infection may cause loss of appetite, increased metabolism/catabolism and is often accompanied by impaired absorption [10].

Data were analyzed with different statistical methods. Taken together, they showed decreased RBC ω6 and, to a lesser extent, ω3 fatty acids, which were compensated for by increased ω9 fatty acids. Although the frequently used 20:3ω9/20:4ω6 ratio for establishment of EFA deficiency did not show statistically significant increases, the encountered substitution of EFA for the, non-essential, ω9 fatty acids indicates at least low EFA status. This substitution may be caused by the preference of the Δ6-desaturase enzyme to accept 18:1ω9 as a substrate, under the condition of low 18:2ω6 and 18:3ω3 [4,11,15].

Several factors may contribute to EFA deficiency in malnourished children. Inadequate dietary intake, malabsorption, oxidative decomposition of PUFAs, low desaturation activity

and rapid turnover of RBCs may all be partly responsible for low EFA levels. It is generally conceived that malnourished children consume most of their body lipid stores, which cannot be compensated for by an adequate dietary intake [4,10]. Low EFA intake seems likely in all groups. Buffalo milk and goat milk are low in EFAs. The porridge given during weaning will provide carbohydrates and proteins, but little fat. The low consumption of oil or ghee will also not supply much EFAs, particularly no EFAs of the ω3 series, since they are mainly corn-based. One may also question the EFA content of breast milk, since the EFA status of the children proved low, despite the fact that more than half of them was breastfed at the moment of blood collection and that all of them had received breast milk in early life. If this implies that also the mothers had low EFA status, the children might not have been able to accrue sufficient EFAs in the intrauterine period. Low levels of RBC 18:2ω6 might also be explained by rapid RBC turnover, as is also encountered in malnutrition [26]. Rapid RBC turnover results in a high number of young RBC (e.g. reticulocytes), which are characterized by relatively low 18:2ω6 content [27]. Moreover, recurrent gastro-intestinal infections may contribute to malabsorption [10]. Finally, increased oxidative decomposition of PUFA due to deficiency of vitamin E, selenium, or both, may be contributing factors [4,26]. This might be consistent with the lower plasma total vitamin E concentrations in grade 2 malnourished children (Table 2).

Comparison with other studies is difficult. Only Wolff et al. [5] and Vajreswari et al. [8] investigated the RBC PUFA composition. However, they presented data for only some fatty acids [5], or rather small groups [8]. Others [6,12] showed the results in different RBC lipid fractions. Most studies [4-7,9,12] were performed in plasma. Collection of plasma is more easy, but its fatty acid composition may not reflect the overall PUFA status. Measurements in cells may be more meaningful from a functional point of view. A reason for not finding larger differences in EFA status between malnourished children and controls may be the already low EFA levels in the control group, compared with Western standards [14,28].

In contrast to other studies [4-6,12], the RBC 18:2ω6/20:4ω6 ratio of the malnourished children was normal, which suggests a normal activity of the Δ5- and Δ6-desaturase enzymes. Other ratio's (not shown) did not suggest a change in the activities of Δ6-desaturase, Δ5-desaturase or elongase either. Zinc [4,12], iron [29] and protein deficiency [4-8,30] are known to decrease Δ6-desaturase activity. Low levels of zinc are common in protein energy malnutrition [4]. Insufficient protein intake by the presently studied group seems, however, unlikely. Only one of the malnourished children showed clinical evidence of kwashiorkor, which is believed to be partly due to protein deficiency. On the other hand, we found the 22:5ω6/22:4ω6 ratio to be significantly decreased (Table 3). The 22:6ω3/22:5ω3 ratio was also low, but did not reach significant difference (Table 1). Together with low 22:6ω3, these findings suggests low Δ4-desaturation activity. Recent investigations revealed that Δ4-desaturation activity involves three distinct enzymatic steps, including an initial chain elongation, a second Δ6-desaturation and a final chain shortening by peroxisomal β-oxidation [31]. Consequently, low 22:5ω6/22:4ω6 suggests low peroxisomal β-oxidation, since Δ6-desaturase activity and chain elongation showed no evidence to be affected. In contrast to breast-milk, few infant formula contain detectable amounts of LCPUFAs. Therefore, infants who receive infant formula have low levels of 22:6ω3 compared to those who receive breast milk [14,16], possibly because of immature Δ4-desaturation activity [27]. Low 22:6ω3 levels are of particular interest, because 22:6ω3 is important for normal functioning of the central nervous system [11,15].

In conclusion, malnourished children in the north of Pakistan showed biochemical evidence of EFA deficiency, particular of EFAs of the ω6-series. Dietary intake of fats and EFAs is low. Moreover, it is hypothesized that these children may have low Δ4-desaturation activity because of diminished peroxisomal β-oxidation. Gastrointestinal infections and increased oxidative decomposition of PUFAs due to vitamin E deficiency may be contributing factors.

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References

1. Unicef. The State of the Worlds Children. Oxford: Oxford University Press, 1984
2. Pakistan Demographic and Health Survey 1990-91. National Institute of Population Studies, July 1992
3. Seerat E. Hospital experience of malnutrition in "under 5" children. 1988
4. Koletzko B, Abiodun PO, Larya MD, Bremer HJ. Fatty acid composition of plasma lipids in Nigerian children with protein-energy malnutrition. Eur J Ped 1986;145:109-15
5. Wolff JA, Margolis JA, Bujdoso-Wolff K, Matusick E, MacLean WC Jr. Plasma and red blood cell fatty acid composition in children with protein energy malnutrition. Pediatr Res 1984;18:162-7
6. Marin MC, De Tomas ME, Mercuri O, Fernandez A, de Serres CT. Interrelationship between protein-energy malnutrition and essential fatty acid deficiency in nursing infants. Am J Clin Nutr 1991;53:466-8
7. Holman RT, Johnson SB, Mercuri O, Itarte HJ, Rodrigo MA, De Tomas ME. Essential fatty acid deficiency in malnourished children. Am J Clin Nutr 1981;1534-9
8. Vajreswari A, Narayanareddy K, Srinivasa Rao P. Fatty acid composition of erythrocyte membrane lipid obtained from children suffering from kwashiorkor and marasmus. Metabolism 1990;39:779-82
9. Decsi T, Zaknun D, Zaknun J, Sperl W, Koletzko B. Long-chain polyunsaturated fatty acids in children with severe protein-energy malnutrition with and without human immunodeficiency virus-1 infection. Am J Clin Nutr 1995;62:1283-8
10. Torun B, Chew F. Protein-energy malnutrition. In: Shils ME, Olson JA, Shike M. eds. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea and Febiger. 1994:950-76
11. Innis SM. Essential fatty acids in growth and development. Prog Lipid Res 1991;30:39-103
12. Leichsenring M, Sütterlin N, Less S, Bäumann K, Anninos A, Becker K. Polyunsaturated fatty acids in erythrocyte and plasma lipids of children with severe protein-energy malnutrition. Acta Paediatr 1995;84:516-20
13. Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 1991;54:438-63
14. Woltil HA, Van Beusekom CM, Siemensma AD, Polman HA, Muskiet FAJ, Okken A. Erythrocyte and plasma cholesterol ester long chain polyunsaturated fatty acids of low-birth-weight babies fed preterm formula with and without ribonucleotides: comparison with human milk. Am J Clin Nutr 1995;62:943-9
15. Linscheer WG, Vergroesen AJ. Lipids. In: Shils ME, Olson JA, Shike M. eds. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea and Febiger. 1994:47-88
16. Farquharson J, Cockburn F, Patrick WA, Jamieson EC, Logan RW. Infant cerebral cortex phospholipid fatty-acid composition and diet. Lancet 1992;340:810-3
17. Giovannini M, Agostoni C, Salari PC. The role of lipids in nutrition during the first months of life. J Int Med Res 1991;19:351-62
18. Lanting CI, Fidler V, Huisman M, Touwen BCL, Boersma ER. Neurological differences between 9-year-old children fed breast-milk or formula-milk as babies. Lancet 1994;344:1319-22
19. Muskiet FAJ, van Doormaal JJ, Martini IA, Wolthers BG, van der Slik W. Capillary gas chromatographic profiling of total long-chain fatty acids and cholesterol in biological materials. J Chromatogr 1983;278:231-44
20. De Leenheer AP, De Bevere VORC, Claeys AE. Measurement of α-, β-, and γ-tocopherol in serum by lipid chromatography. Clin Chem 1979;25:425-8
21. Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clin Nutr 1979;32:2143-9

22. Connover WJ. Practical nonparametric statistics. 2nd ed. New York: John Wiley & Sons. 1980:215-27
23. Hosmer DW, Lemeshow S. Applied logistic regression. New York: John Wiley & Sons. 1989
24. Solberg HE. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. Clin Chim Acta 1987;170(suppl):13-32
25. Gart J. Point and interval estimation of the common odds ratio in the combination of 2 x 2 tables with fixed marginals. Biometrika 1970;57:471-5
26. Waterlow JC. Protein energy malnutrition. London: Edward Arnold. 1992
27. Hoving EB. Lipid profiling in clinical chemistry. Contributions to neonatal nutrition, perinatology, oxidative stress and sickle cell anaemia. Thesis. Groningen, The Netherlands. 1993
28. van Doormaal JJ, Idema IG, Muskiet FAJ, Martini IA, Doorenbos H. Effects of short-term high dose intake of evening primrose oil on plasma and cellular fatty acid compositions, α-tocopherol levels, and erythropoiesis in normal and Type 1 (insulin-dependent) diabetic men. Diabetologia 1988;31:576-84
29. Smuts CM, Tichelaar HY, Van Jaarsveld PJ, Badenhorst CJ, Kruger M, Laubscher R, et al. The effect of iron fortification on the fatty acid composition of plasma and erythrocyte membranes in primary school children with and without iron deficiency. Prostaglandins Leukot Essent Fatty Acids 1995;52:59-67
30. Narce M, Poisson JP, Belleville J, Chanussot B. Time-course effects of protein malnutrition on hepatic fatty acids Δ6 and Δ5 desaturation in the growing rat. Br J Nutr 1988;60:389-402
31. Voss A, Reinhart M, Sankarappa S, Sprecher H. The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. J Biol Chem 1991;266:19995-2000

2.2. Low erythrocyte docosahexaenoic acid in malnourished, often breast-fed, Pakistani infants. A matter of concern?

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Sir: Long-chain polyunsaturated fatty acids (LCPUFA) are important for the development of the central nervous system, including the retina [2]. Breast milk contains a large range of LCPUFAs, including docosahexanoic (22:6ω3, DHA) and arachidonic (20:4ω6, AA) acids, whereas most formula milks for term infants do not. Newborns do not seem to synthesize sufficient amounts from their precursors to cover their high needs. This results in lower DHA and AA status of formula fed children compared to breast-fed counterparts. For establishment of the LCPUFA status, most investigators measure their contents in RBCs, which are regarded as reliable markers for brain LCPUFA contents. Since low postnatal DHA status has been related to impaired visual acuity and cognitive development [2], concern has risen about brain development of formula fed term and particularly preterm infants. As a consequence, most preterm formulas in Europe are at present supplemented with LCPUFA, notably DHA or a combination of AA and DHA. Low RBC LCPUFAs have also been observed in malnourished children, many of them receiving breast milk and weaning food low in fat [4]. In these children, it has been postulated that the synthesis of LCPUFA from their precursors is decreased [1], making them more dependent on an adequate LCPUFA intake.

Over the last few years we have been evaluating the LCPUFA status of various groups of infants and adults with different dietary patterns and nutritional conditions by establishing their RBC LCPUFA levels. These subjects comprised: Low-birth-weight infants (age 1.5 month), who received either breast milk or a formula without LCPUFA [5, part of the data]; healthy 3.5 years old children and adults (age 22-61 years) in The Netherlands (unpublished data); healthy, breast-fed infants in Jerusalem (age 1-6 month) (unpublished data); and well nourished and malnourished children in North Pakistan (age 2-60 months), either breast fed or not [4]. In these studies we made use of the same sampling techniques, prepurification methods and analyses by capillary gas chromatography with flame ionization detection. Comparison of the outcomes of RBC DHA levels (Table) revealed large differences between the groups. Well nourished breast-fed Jerusalem infants exhibited the highest RBC DHA levels. Malnourished, not breast-fed, Pakistani children had the lowest levels, with 75% of them having RBC DHA levels below the 95% confidence interval of the formula fed Dutch infants! These differences do not seem to be due to imperfect age matching, since apparently healthy Dutch adults do not exhibit much difference in RBC DHA content compared with 42 days old Dutch low-birth-weight infants. Although malnourished breast-fed Pakistani children had a better DHA status than those receiving no human milk, their

Table Erythrocyte docosahexaenoic acid contents in The Netherlands, Jerusalem and Pakistan

	n	Age		RBC DHA (mol%)		
		Median	Range	Median	p2.5	p97.5
The Netherlands						
Well nourished						
Breast-fed	18	42 days	33-46	4.33	3.52	5.13
Formula-fed	81	41 days	37-50	3.07	2.29	3.84
Children	33	3.5 years	3.5-3.5	2.87	2.11	3.88
Adults	69	37 years	22-61	3.75	2.29	5.45
Jerusalem						
Well-nourished						
Breast-fed	31	3 months	1-6	4.51	2.95	5.30
Pakistan						
Well-nourished						
Breast-fed	19	8 months	2-23	3.03	1.96	3.92
Not breast-fed	6	48 months	25-60	2.40	1.76	3.83
Malnourished						
Breast-fed	48	15 months	4-42	2.70	0.77	3.94
Not breast-fed	40	21 months	4-56	1.92	1.10	2.97

Data from the breastfed and formulafed infants from The Netherlands include data from Woltil et al. [5], those from Pakistan derive from Smit et al. [4]. The other data have not been published before. Formula-fed Dutch infants received formula without LCPUFA.
Abbreviations: n, number; RBC, erythrocytes; DHA, docosahexaenoic acid (22:6ω3).

levels were much lower compared to breast-fed infants from The Netherlands and Jerusalem and, surprisingly, 27% of them had RBC DHA levels below the 95% confidence interval of the formula fed Dutch infants. Since the fatty acid composition of human milk is strongly dependent on the maternal dietary intake [3], it may be hypothesized that the poor DHA-status of these malnourished breast-fed children is caused by a marginal DHA-status of these mothers. This finding supports the need for further studies on the adequacy of maternal nutrition during lactation, and - possibly more relevant in terms of prevention - during pregnancy. This applies in particular to high risk, often less privileged, populations. The possible adverse effect of the marginal maternal DHA status in these circumstances on early growth and neuro-development of the offspring remains to be established.

References

1. Koletzko B. Abiodun PO, Larya MD, Bremer HJ (1986). Fatty acid composition of plasma in Nigerian children with protein-energy malnutrition. Eur J Pediatr 145:109-15
2. Makrides M, Neumann M, Simmer K, Pater J, Gibson R (1995) Are long-chain polyunsaturated fatty acids essential nutrients in infancy? Lancet 345:1463-8
3. Makrides M, Neumann MA, Gibson RA (1996). Effect of maternal docosahexaenoic (DHA) supplementation on breast milk composition. Eur J Clin Nutr 50:352-7
4. Smit EN, Dijkstra JM, Schnater TA, Seerat E, Muskiet FAJ, Boersma ER (1997). Effects of malnutrition on the fatty acid composition and plasma vitamin E levels of Pakistani children. Acta Paediatr 86:690-5
5. Woltil HA, Van Beusekom CM, Siemensma AD, Muskiet FAJ, Okken A (1995). Erythrocyte and plasma cholesterol ester long chain polyunsaturated fatty acids of low-birth-weight babies fed preterm formula with and without ribonucleotides: comparison with human milk. Am J Clin Nutr 62:943-9

2.3. Fish oil supplementation improves docosahexaenoic acid status of malnourished infants

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Abstract

Objective: To investigate whether the low docosahexaenoic acid (DHA) status of malnourished, mostly breastfed, Pakistani children can be improved by fish oil (FO) supplementation.
Methods: Ten malnourished children (ages 8-30 months) received 500 mg FO daily during 9 weeks. The supplement contained 62.8 mol% (314 mg) long-chain polyunsaturated fatty acids of the ω3 series (LCPUFAω3) and 22.5 mol% (112 mg) DHA. Seven FO unsupplemented children served as controls. Red blood cell (RBC) fatty acids were analyzed at baseline and at the study end.
Results: FO supplementation augmented RBC DHA from 2.27 ± 0.81 to 3.35 ± 0.76 mol%, without significantly affecting the levels of the LCPUFAω6. Unsupplemented children showed no RBC fatty acid changes. One FO supplemented child with very low initial RBC AA showed a remarkable increase from 4.04 to 13.84 mol%, whereas another with high RBC AA showed a decrease from 15.64 to 10.46 mol%.
Conclusion: FO supplementation improves the DHA status of malnourished children. The supplement is apparently well absorbed and not exclusively used as a source of energy.

2.3.1. Introduction

Essential fatty acids (EFAs) constitute a group of fatty acids that cannot be synthesized *de novo*. Long-chain polyunsaturated fatty acids (LCPUFAs; containing ≥20 carbon atoms and ≥3 double bonds in the methylene-interrupted configuration) derive either from the diet or from desaturation-chain elongation of the two parent EFAs linoleic (18:2ω6; LA) and α-linolenic (18:3ω3; ALA) acids. LCPUFAs are structural components of cell membrane phospholipids and precursors of eicosanoids. They play important roles in the development of the central nervous system, including the retina [1,2]. Postnatal docosahexaenoic acid (22:6ω3; DHA) status is e.g. related to visual acuity [3-6] and neurodevelopment [7,8], whereas arachidonic acid (20:4ω6; AA) has been associated with pre- and postnatal growth [9,10].

In a previous study we found very low erythrocyte (RBC) DHA levels in malnourished mostly breastfed 4-56 months old Pakistani children [11]. A low breastmilk LCPUFAω3 content was identified as the major cause of their poor DHA status (*chapter 3.1*). Apart

from low intake, poor PUFA status of malnourished children may also derive from malabsorption [12,13], impaired desaturation and elongation [11,14-17], peroxidation of PUFA [16] and the use of PUFA as an energy source via β-oxidation [18,19]. Many studies have shown that fish oil (FO) supplementation increases DHA contents of many blood compartments, including RBC and plasma lipid fractions [5,20-25]. It is, however, unknown whether this also applies to malnourished children, since they may have poor fat absorption, or use the supplement as an energy source. In the present study we supplemented 10 malnourished Pakistani children with 500 mg FO daily during 9 weeks and investigated whether it improved their DHA status, as derived from their RBC fatty acid composition. Seven unsupplemented counterparts served as controls.

2.3.2. Subjects and methods

2.3.2.1 *Subjects, supplement and study design*

Seventeen infants were recruited from the Nutrition Rehabilitation Center of the Pediatric Department, Federal Government Services Hospital, Islamabad (Pakistan). Anthropometric, demographic, socio-economic and clinical data were documented. They were classified according to local growth-charts, provided by the WHO. Grades 2 and 3 malnutrition were defined as weight-for-age below the mean minus two SDs and three SDs, respectively, using the data from the United States National Center for Health Statistics (NCHS) as a reference. The study conformed to local ethical standards and the Helsinki declaration of 1975 as revised in 1989.

The children were randomly assigned to receive one 500 mg capsule of FO (Pikasol, Hadsund, Norway) daily for approximately 9 weeks (n=10), or no oil (controls; n=7). Since the capsules proved too big to swallow, they were pierced and the oil was given by spoon. As stated by the manufacturer, each capsule contained 1.5 IU vitamin E. The fatty acid composition, as established by us, is given in Table 1. The daily intakes corresponded with 190 mg eicosapentaenoic acid (EPA), 112 mg DHA and 10 mg AA. EDTA anticoagulated blood (2.5 ml at most) was taken in an undefined metabolic state at baseline and at the study end. All infants received a multi-vitamin syrup and occasionally medical treatment, including antibiotics (mostly erythromycin). The multi-vitamin syrup contained vitamin B complex, vitamin C and lysine (Actoplex-C, Atco Lab. Private Ltd, Karachi, Pakistan). All mothers received nutritional education at the Nutrition Rehabilitation Center. The education included cooking lessons, information about what to feed the child and how to prepare it. Informed consent was obtained from all mothers.

2.3.2.2 *Sample processing and analysis*

The blood samples were immediately cooled in melting ice. Hematological indices were measured within 24 h by means of a Sysmex counter. The remaining volume was centrifuged at 800 g for 10 min in a cooled centrifuge. The plasma and buffy coat were removed and the RBCs were washed three times with isotonic saline. The RBCs were finally resuspended to a hematocrit of about 50% and counted again. For the analysis of RBC fatty acids, 200 µl of this suspension was transferred to a 15 ml teflon-stoppered tube, containing 1 mg butylated hydroxytoluene (antioxidant) and 50 µg margaric acid (17:0; internal quantification standard). The preserved RBC samples were stored at -20ºC and

Table 1. Fatty acid composition of the fish oil capsules

Fatty acids	mol%	Fatty acids	mol%
18:3ω3	1.0[1]	18:1ω9	6.7
18:4ω3	6.1	20:1ω9	1.6
20:4ω3	2.0	22:1ω9	1.0
20:5ω3	37.9	24:1ω9	0.1
22:5ω3	3.8	SAFA	5.9
22:6ω3	22.5	MCSAFA	3.2
18:2ω6	1.4	MUFA	15.8
18:3ω6	0.4	PUFA	78.3
20:2ω6	0.3	sum ω3	73.1
20:3ω6	0.3	sum ω6	5.1
20:4ω6	1.9	sum ω7	6.5
22:4ω6	0.2	sum ω9	9.3
22:5ω6	0.5	LCPUFAω3	66.1
		LCPUFAω6	3.2

[1]Data represent a selection of fatty acids, expressed in mol% (mol/100 mol).
Abbreviations: SAFA, saturated fatty acids; MCSAFA, medium chain SAFA (C6-C14); MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; LCPUFA, long chain PUFA (C≥20, double bonds ≥3).

transported to The Netherlands in dry ice. Fatty acid measurements were done with our previously reported methods by capillary gas chromatography with flame ionization detection [26,27].

2.3.2.3 *Statistics*

Differences between RBC fatty acid contents of FO supplemented infants and controls were analyzed with the Mann Whitney-U test. Differences in RBC fatty acid contents at baseline and at study end were analyzed with the Wilcoxon signed-rank test. $P<0.05$ was considered statistically significant. All statistics were evaluated with SPSS (SPSS 6.0 for Windows, SPSS Inc, Chicago, IL, USA).

2.3.3. Results

The characteristics of the study groups are given in Table 2. The mean FO supplementation period was 9.1 weeks (range: 7-12). For the control group the mean time interval between enrollment and collection of the second blood sample was 9.7 weeks (range: 8-16). Eight infants in the FO supplementation group and all controls had gained weight at the study end. When expressed as percentage of the reference average weight for age (NCHS) their median weights improved from 53.5% (range: 47-68%) to 60% (46-69%) in the FO group and from 63% (57-68) to 68% (60-70%) in the control group.

Selected RBC fatty acids of the children are shown in Table 3. FO supplemented children and controls did not have significantly different RBC fatty acid compositions at enrollment. The controls did not exhibit RBC fatty acid differences between enrollment and study end.

Compared with controls, FO supplemented children had at study end higher RBC 20:5ω3 (p=0.010), 22:6ω3 (p=0.007), sum of ω3 fatty acids (p=0.007), ω3/ω6 (p=0.010), LCPUFAω3/LCPUFAω6 (p=0.002) and 24:1ω9 (p=0.043). FO supplemented children showed increases of RBC 20:5ω3 (p=0.005), 22:5ω3 (p=0.007), 22:6ω3 (p=0.009), sum of ω3 fatty acids (p=0.005), LCPUFAω3 (p=0.005), ω3/ω6 (p=0.005), LCPUFAω3/LCPUFAω6 (p=0.005) and 26:0 ((p=0.022), not shown) from baseline to the study end. There were no changes in RBC ω6 fatty acids.

Table 2. Characteristics of the malnourished children.

	Controls (n=7)	Fish Oil (n=10)
Age (months)	24 (4-56)	17 (8-30)
Weight (kg)	8.0 (4.0-10.3)	5.9 (4.3-8.3)
Grade 2/grade 3	4/3	3/7
Hb (g/dL)	10.4 (7.3-12.2)	8.3 (6.0-10.9)
RBC (10^{12}/L)	4.37 (3.91-5.05)	4.20 (3.02-5.01)
Infections *	5/7	8/10
Breastfed	4/7	4/9

Data expressed as median (range), or fraction.
* Upper respiratory and/or gastrointestinal infections.

2.3.4. Discussion

We investigated whether the DHA status of malnourished Pakistani infants can be improved by FO supplementation. It was found that daily administration of 500 mg FO, containing 62.8 mol% LCPUFAω3, for 9 weeks resulted in about 50% increases of RBC DHA and LCPUFAω3, without affecting the RBC LCPUFAω6 content. The unsupplemented control group did not exhibit RBC fatty acid changes. The results of this study show that the FO supplement is apparently well absorbed in malnourished children and that the LCPUFAω3 are not exclusively used as a source of energy under these conditions.

This is to our knowledge the first report to show the effects of LCPUFA supplementation on the RBC fatty acid composition of malnourished children. Koletzko et al. [17] monitored the plasma fatty acid composition of 8 recovering malnourished children during 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 the essential fatty acid status after 14 days treatment without additional LCPUFA supplements.

The most remarkable changes in the RBC fatty acid composition were recorded in a severely malnourished 21 months old almost exclusively breastfed marasmic girl. Her weight was only 5.5 kg (47% of the reference average) at enrollment and a she had a “dry” skin. After 9 weeks FO supplementation she had gained 1.8 kg (to reach 60% of the reference average). There was no clinical evidence that this weight gain was caused by water retention. Concomitantly she displayed an increase of both RBC ω3 and ω6 fatty acids, mainly at the expense of the sum of the saturated fatty acids (from 65.39 to 48.24

Table 3. Erythrocyte fatty acids of fish oil supplemented children and controls

	Controls (n=7)		Fish Oil (n=10)	
Fatty acids	baseline	9 weeks	baseline	9 weeks
18:3ω3	0.16 ± 0.05	0.15 ± 0.05	0.18 ± 0.12	0.22 ± 0.14
20:5ω3	0.26 ± 0.08	0.29 ± 0.17	0.31 ± 0.33	0.72 ± 0.42[a,d]
22:5ω3	1.62 ± 0.35	1.52 ± 0.47	1.51 ± 0.75	2.08 ± 0.92[b]
22:6ω3	2.11 ± 0.49	2.07 ± 0.71	2.27 ± 0.81	3.35 ± 0.76[b,d]
18:2ω6	9.94 ± 1.99	9.80 ± 2.24	7.43 ± 1.52	7.83 ± 1.99
20:2ω6	0.23 ± 0.07	0.21 ± 0.10	0.18 ± 0.06	0.21 ± 0.07
20:3ω6	1.53 ± 0.17	1.48 ± 0.17	1.32 ± 0.39	1.39 ± 0.29
20:4ω6	14.03 ± 1.11	13.62 ± 1.73	13.01 ± 3.40	13.04 ± 1.49
22:4ω6	3.27 ± 0.36	2.91 ± 0.46	2.64 ± 0.71	2.43 ± 0.48
22:5ω6	0.95 ± 0.17	0.89 ± 0.18	0.92 ± 0.27	0.82 ± 0.17
18:1ω9	10.95 ± 1.07	11.10 ± 1.39	12.90 ± 1.44	12.33 ± 1.62
20:1ω9	0.22 ± 0.04	0.23 ± 0.04	0.25 ± 0.05	0.22 ± 0.06
20:3ω9	0.35 ± 0.09	0.43 ± 0.28	0.60 ± 0.44	0.60 ± 0.32
22:3ω9	0.18 ± 0.12	0.16 ± 0.13	0.29 ± 0.22	0.23 ± 0.14
24:1ω9	3.66 ± 0.37	3.38 ± 0.42	4.08 ± 0.49	3.94 ± 0.49[e]
SAFA	48.59 ± 0.66	50.04 ± 3.93	50.24 ± 5.13	48.88 ± 1.38
MUFA	16.78 ± 1.44	16.44 ± 1.57	19.14 ± 1.53	18.20 ± 1.53
PUFA	34.64 ± 1.95	33.53 ± 3.75	30.64 ± 5.93	32.91 ± 2.17
sumω3	4.16 ± 0.79	4.03 ± 1.24	4.26 ± 1.71	6.37 ± 1.66[a,d]
sumω6	29.95 ± 1.73	28.91 ± 3.34	25.51 ± 5.00	25.70 ± 3.13
sumω7	1.91 ± 0.43	1.70 ± 0.51	1.83 ± 0.50	1.67 ± 0.32
sumω9	15.40 ± 1.41	15.33 ± 1.76	18.15 ± 1.96	17.37 ± 2.11
LCPUFAω3	3.99 ± 0.79	3.88 ± 1.22	4.08 ± 1.60	6.15 ± 1.54[a,c]
LCPUFAω6	19.78 ± 1.43	18.90 ± 2.06	17.90 ± 4.53	17.68 ± 2.01
ω3/ω6	0.14 ± 0.03	0.14 ± 0.05	0.17 ± 0.08	0.26 ± 0.10[a,d]
LCω3/LCω6	0.20 ± 0.03	0.20 ± 0.06	0.23 ± 0.09	0.35 ± 0.11[a,]

Data represent mean ± SD, and are expressed as mol% (mol/100 mol). For abbreviations see Table 1. Controls received nutritional rehabilitation for 9.7 weeks (range: 8-16); the fish oil group was additionally supplemented with 500 mg fish oil (see Table 1) daily for 9.1 weeks (range: 7-12).
[a] significant difference between baseline and 9 weeks at $p \leq 0.005$; [b] significant difference between baseline and 9 weeks at $p \leq 0.01$; [c] significant difference between fish oil and control group at $p \leq 0.005$; [d] significant difference between fish oil and control group at $p \leq 0.01$; [e] significant difference between fish oil and control group at $p \leq 0.05$.

mol%). RBC DHA increased from 0.41 to 2.50 mol%. Surprisingly, a large increase was on account of RBC AA (from 4.04 to 13.84 mol%). It seems unlikely that this increase can be explained by the low AA intake from the supplement (10 mg per day). The RBC 18:2ω6/20:4ω6 ratio (a parameter of combined activities of Δ6-desaturase, chain elongation and Δ5-desaturase) decreased from 1.60 to 0.71 mol/mol, while the RBC 20:3ω6/20:4ω6 ratio (a parameter of Δ5-desaturase activity) did not change. The data of this child suggest low activity of Δ6-desaturase at enrollment, which subsequently improved during the intervention period. A positive effect of ω3 fatty acids on LCPUFAω6 fatty acids has

previously been reported by Bjerve et al. [25]. They observed an increase of plasma and RBC LCPUFAω6 in patients with ω3 fatty acid deficiency following FO supplementation. It was suggested that ω6 fatty acids cannot accumulate normally in cell membranes at the condition of low ω3 fatty acid supply. In contrast to the above case, a 30% decrease of RBC AA was observed in a 16 months old girl with a relatively high RBC AA level at baseline (from 15.64 to 10.46 mol%). Reduction of plasma and RBC AA levels after FO supplementation has previously been noticed in preterm [21,22], and term [5] infants and adults [20]. The contradictory findings in the two cases may indicate that the effect of FO supplementation on RBC AA levels is dependent on baseline RBC AA contents, causing increases at low initial RBC AA status, and decreases at high initial RBC AA status.

We conclude that FO supplementation of malnourished Pakistani children improves their DHA status. Replacement of the presently employed purified FO by the much cheaper cod liver oil (approximately 10% DHA) may give similar results. Another factor in favor of cod liver oil as a supplement would be its high vitamin A and D contents. Malnourished children often have low vitamin A and D status [12,28]. Further investigations are needed to clarify if AA supplementation should be recommended in addition to DHA supplementation to prevent any adverse affects on the LCPUFAω6 status that are caused by augmentation of the LCPUFAω3 status.

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References

1. Innis SM. Essential fatty acids in growth and development. Prog Lipid Res 1991;30:39-101
2. Linscheer WG, Vergroesen J. Lipids. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease, 8th ed. Philadelphia: Lea and Febiger, 1994:47-88
3. Birch EE, Birch DG, Hoffman DR, Uauy R. Dietary essential fatty acid supply and visual acuity development. Invest Ophthalmol Vis Sci 1992;33:3242-53
4. Carlson SE, Werkman SH, Rhodes PG, Tolley EA. Visual acuity development in healthy preterm infants: effect of marine-oil supplementation. Am J Clin Nutr 1993;58:35-42
5. Makrides M, Neumann M, Simmer K, Pater J, Gibson R. Are long-chain polyunsaturated fatty acids essential nutrients in infancy? Lancet 1995;345:1463-8
6. Carlson SE, Ford AJ, Werkman SH, Peeples JM, Koo WWK. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. Pediatr Res 1996;39:882-8
7. Agostoni C, Riva E, Trojan S, Bellù R, Giovanni M. Docosahexaenoic acid status and development quotient of healthy term infants. Lancet 1995;346:638
8. Gibson RA, Neumann MA, Makrides M. Effect of increasing breastmilk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. Eur J Clin Nutr 1997;51:578-84
9. Koletzko B, Braun M. Arachidonic acid and early human growth: Is there a relation? Ann Nutr Metab 1991;35:128-31
10. Carlson SE, Werkman SH, Peeples JM, Cooke RJ, Tolley EA. Arachidonic acid status correlates with first year growth in preterm infants. Proc Natl Acad Sci USA 1993;90:1073-7
11. Smit EN, Dijkstra JM, Schnater TA, Seerat E, Muskiet FAJ, Boersma ER. Effects of malnutrition on the fatty acid composition and plasma vitamin E levels of Pakistani children. Acta Paediatr 1997;86:690-5
12. Torun B, Chew F. Protein-energy malnutrition. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease, 8th ed. Philadelphia: Lea and Febiger.1994:950-76
13. Waterlow JC. Effects of PEM on structure and functions of organs. In: Waterlow JC, ed. Protein-energy malnutrition. London: Edward Arnold, 1992:55-82

14. Marin MC, De Tomas ME, Mercuri O, Fernandez A, De Serres CT. Interrelationship between protein-energy malnutrition and essential fatty acid deficiency in nursing infants. Am J Clin Nutr 1991;53:466-8
15. Holman RT, Johnson SB, Mercuri O, Itarte HJ, Rodrigo MA, De Tomas ME. Essential fatty acid deficiency in malnourished children. Am J Clin Nutr 1981;34:1534-9
16. Leichsenring M, Sütterlin N, Less S, Bäuman K, Anninos A, Becker K. Polyunsaturated fatty acids in erythrocyte and plasma lipids of children with severe protein-energy malnutrition. Acta Paediatr 1995;84:516-20
17. Koletzko B. Abiodun PO, Larya MD, Bremer HJ. Fatty acid composition of plasma in Nigerian children with protein-energy malnutrition. Eur J Pediatr 1986;145:109-15
18. Chen SCH, Dickerman MD. Iron, thyroid hormone and essential fatty acid status of Honduran preschoolers. Nutr Res 1985;5:21-30
19. Parsons HG, O'Loughlin EV, Forbes D, Cooper D, Gall DG. Supplemental calories improve essential fatty acid deficiency in cystic fibrosis patients. Pediatr Res 1988;24:353-6
20. Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease:possible mechanisms. Am J Clin Nutr 1990;52:1-28
21. Carlson SE, Cooke RJ, Rhodes PG, Peeples JM, Werkman SH, Tolley EA. Long-term feeding of formulas high on linolenic acid and marine oil to very low birth weight infants: phospholipid fatty acids. Pediatr Res 1991;30:404-12
22. Hoffman DR, Uauy R. Essentiality of dietary ω3 fatty acids for premature infants: plasma and red blood cell fatty acid composition. Lipids 1992;27:886-95
23. Connor WE, Lowensohn R, Hatcher L. Increased docosahexaenoic acid levels in human newborn infants by administration of sardines and fishoil during pregnancy. Lipids 31;1996:S183-7
24. Henderson RA, Jensen RG, Lammi-Keefe CJ, Ferris AM, Dardick KR. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 1992;27:901-7
25. Bjerve KS, Fischer S, Wammer F, Egeland T. α-Linolenic acid and long-chain ω-3 fatty acid supplementation in three patients with ω-3 fatty acid deficiency: effect on lymphocyte function, plasma and red cell lipids, and prostanoid formation. Am J Clin Nutr 1989;49:290-300
26. Muskiet FAJ, Doormaal JJ van, Martini IA, Wolthers BG, Slik W van der. Capillary gas chromatographic profiling of total long-chain fatty acids and cholesterol in biological materials. J Chromatogr Biomed Appl 1983;278:231-44
27. Steege G van der, Muskiet FAJ, Martini IA, Hutter NH, Boersma ER. Simultaneous quantification of total medium- and long-chain fatty acids in human milk by capillary gas chromatography with split injection. J Chromatogr Biomed Appl 1987;415:1-11
28. Waterlow JC. Protein-energy malnutrition: general introduction. In: Waterlow JC, ed. Protein-energy malnutrition. London: Edward Arnold, 1992:1-13

Chapter 3 Fatty acid composition of breastmilk

3.1. Breastmilk docosahexaenoic acid (DHA) correlates with DHA status of malnourished infants

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Abstract

Objective: To investigate whether low docosahexaenoic acid (22:6ω3; DHA) status of malnourished, mostly breastfed infants is due to low ω3-fatty acid intake via breastmilk.
Methods: The fatty acid composition of the breastmilk of 8 Pakistani mothers and that of the erythrocytes (RBC) of their malnourished children was analysed.
Results: The milk of the Pakistani mothers contained low percentages of all ω3 and most ω6-fatty acids, compared with milk of Dutch mothers. Breastmilk DHA was positively correlated with infant RBC DHA and arachidonic acid (20:4ω6).
Conclusion: DHA status of these malnourished children is strongly dependent on the ω3-fatty acid intake from breastmilk. Augmentation of the infants' ω3-LCPUFA status, or the ω3 and ω6-FA status in general, by supplementation is indicated in deprived circumstances with a difficult access to fresh fish. However, in terms of prevention, maternal supplementation of these LCPUFAs, preferably from early pregnancy onwards, may be a better option in these circumstances.

3.1.1. Introduction

Postnatal docosahexaenoic acid (22:6ω3; DHA) status has been found to be related to e.g. visual acuity, neurodevelopment and behaviour. DHA and other long chain polyunsaturated fatty acids (LCPUFA) are nowadays widely considered to be essential in the pre- and early postnatal periods, because the synthesis of LCPUFA from their precursors, α-linolenic acid (18:3ω3; ALA) and linoleic acid (18:2ω6; LA), does not seem to cover the infants' high needs at this stage of development. Breastmilk contains in general a sufficient quantity of LCPUFA, including DHA and arachidonic acid (20:4ω6; AA) to serve these needs. However, the LCPUFA composition of human milk is to a great extent dependent on the maternal diet [1]. Therefore in North Pakistan, low breastmilk ω3-(LC)PUFA levels might be expected, because of the predominant use of ALA-poor corn oil and ghee, the low intake of green leafy vegetables (a source of ALA) and almost no consumption of, ω3-LCPUFA rich, fish [2]. In a previous study we found low erythrocyte (RBC) DHA levels in malnourished, mostly breastfed, 4-56 months old children, living in the area of Islamabad, North Pakistan [3].

We hypothesised that the low RBC DHA contents of these infants was due to a low ω3-LCPUFA intake via breastmilk. In an attempt to test this hypothesis we studied the FA composition of both the breastmilk of 8 Pakistani mothers and that of the RBC of their malnourished children.

3.1.2. Subjects and methods

The study population consisted of 8 mother-child pairs from a low socio-economic class. They were recruited from the Nutrition Rehabilitation Center of the Pediatric Department, Federal Government Services Hospital, Islamabad. The children were classified as malnourished, defined as weight-for-age below the mean minus 2SD, according to WHO growth charts. EDTA anticoagulated blood (2.5 ml at most) of the children was taken in an undefined metabolic state. Breastmilk samples were collected by manual expression. Fatty acids (FA) were determined as their methyl esters by capillary gas chromatography with flame ionization detection.

Milk FA composition of Pakistani mothers was compared with previously collected mature milk FA data of 25 Dutch mothers. Between-group differences were analyzed with Mann Whitney-U test at $p<0.05$. Correlations of the different PUFA between breastmilk and infant RBC, and between the various PUFA in infant RBC were tested with the Spearman test at $p<0.05$.

3.1.3. Results

The median (range) duration of lactation of the Pakistani mothers was 12.5 (4.5-21) months. The Table shows the breastmilk FA composition, together with the data of the Dutch mothers on postnatal day 89 (77-103). Milk of Pakistani mothers contained significantly lower amounts of all ω3-FA. Among the ω6-FA LA, 18:3ω6, 20:2ω6, 20:3ω6 and AA contents were significantly lower.

Breastmilk DHA and infant RBC DHA were strongly correlated ($r=0.8571$, $p=0.007$). Breastmilk DHA correlated also positively with infant RBC AA ($r=0.7857$, $p=0.021$), but negatively with 18:1ω9 ($r=-0.8571$, $p=0.007$). Milk eicosapentaenoic acid (20:5ω3; EPA) correlated with infant RBC DHA ($r=0.7186$, $p=0.045$). There were no other significant correlations between milk PUFA and infant RBC PUFA. There was a positive correlation between infant RBC DHA and infant RBC AA ($r=0.8571$, $p=0.007$).

Summarizing, milk of Pakistani mothers contained low percentages of all ω3 and most ω6-fatty acids, compared with milk of Dutch mothers. Breastmilk DHA and EPA were positively correlated with infant RBC DHA, while milk DHA also was associated with infant RBC AA.

3.1.4. Discussion

Milk of Pakistani mothers feeding malnourished children was found to contain low percentages of all ω3-PUFA and most of the ω6-PUFA (i.e. LA, 20:2ω6, 20:3ω6 and AA) compared to milk of Dutch mothers. Since the milk FA composition is largely dependent on the maternal diet [1] it is conceivable that the above differences reflect nutritional differences.

Table Fatty acids in mature human milk of Pakistani mothers of malnourished children as compared with Dutch controls

	Pakistan n=8	Dutch n=25
18:3ω3	0.34 (0.28-0.51)[a]	1.12 (0.64-2.19)
20:5ω3	0.02 (0.01-0.09)[d]	0.05 (0.00-0.29)
22:5ω3	0.05 (0.04-0.14)[b]	0.13 (0.09-0.22)
22:6ω3	0.06 (0.04-0.14)[b]	0.14 (0.10-0.65)
sumω3	0.53 (0.37-0.64)[a]	1.45 (0.90-2.54)
LCPUFAω3	0.13 (0.09-0.29)[b]	0.32 (0.23-1.17)
18:2ω6	8.73 (7.45-10.61)[b]	13.84 (6.44-27.69)
18:3ω6	0.05 (0.03-0.12)[c]	0.10 (0.06-0.19)
20:2ω6	0.15 (0.14-0.17)[a]	0.25 (0.18-0.37)
20:3ω6	0.21 (0.15-0.36)[b]	0.29 (0.18-0.43)
20:4ω6	0.26 (0.20-0.44)[d]	0.33 (0.24-0.43)
22:4ω6	0.06 (0.04-0.08)	0.06 (0.04-0.08)
22:5ω6	0.02 (0.00-0.04)	0.02 (0.00-0.04)
sumω6	9.35 (8.22-11.69)[b]	14.80 (7.40-28.97)
LCPUFAω6	0.69 (0.57-0.96)[b]	0.96 (0.77-1.22)

Data represent median (range), and are expressed in mol% (mol/100 mol). Duration of lactation: Pakistan (median 12.5; range 4.5-21 months) and The Netherlands (mean 89; range: 77-103 days); [a] $p<0.0001$; [b] $p<0.005$; [c] $p<0.001$; [d] $p<0.05$.
Abbreviation: LCPUFA, long chain polyunsaturated fatty acid (C ≥ 20, double bonds ≥ 3).

The low Pakistani milk ω3-LCPUFA content is presumably caused by low fish consumption in North Pakistan [2]. In this respect it is noteworthy that fish intake in The Netherlands is also considered to be low, which is reflected in relatively low plasma phospholipid DHA content compared to other countries [4]. The milk ω3-LCPUFA contents were nevertheless two times lower in the Pakistani than that in the Dutch samples.

A strong positive correlation between breastmilk DHA levels and infant plasma and RBC phospholipid DHA in 12 weeks old exclusively breastfed children has been reported by Gibson *et al*. [5]. Similarly, we observed a strong correlation between breastmilk DHA and EPA contents on the one hand and infant RBC DHA content on the other hand. This is of particular interest, since the children in the present study were much older (4.5-21 months) and weaned. It suggests that the DHA status of these malnourished children is still strongly dependent on the DHA and EPA intake from breastmilk and probably much less on the ω3-PUFA intake from weaning food. Prolonged breastfeeding of these malnourished Pakistani children seems therefore not only important for its anti-infective properties and other favourable effects, but also as the major source of dietary ω3-LCPUFA.

Apart from the adequate supply of LCPUFA, of course many more factors are important for cognitive function. Overall nutritional status, iron, iodine and zinc deficiency, seem all to be related to mental development. Finally, poor social and family circumstances and a less stimulating environment could also affect later achievements. These unfavourable conditions do prevail in circumstances where malnutrition is often encountered [6].

Nevertheless, the very low DHA status of these children is a matter of concern, because of its potentially adverse effects on brain development, including that of the retina [1]. Regarding the poor ω3-LCPUFA status of their mothers, it may be expected that this condition was already present during gestation and immediately after birth, exposing the vulnerable foetus and neonate to low levels of ω3-LCPUFA.

Milk DHA was also positively associated with infant RBC AA. Moreover, infant RBC DHA and AA were positively correlated. Others have suggested that ω6-FA cannot accumulate normally in cell membranes at the condition of a low ω3-FA supply [7]. It is therefore tempting to speculate that the low DHA intake by these malnourished children may not only cause low RBC DHA levels, but may also have adverse effects on AA status.

Augmentation of the infants' ω3-LCPUFA status, or the ω3 and ω6-FA status in general, by supplementation is indicated in deprived areas with a difficult access to fresh fish. Fish oil seems to be the most logical supplement. As purified fish oil is rather expensive, the cheaper cod liver oil could be a useful alternative. Moreover, cod liver oil contains vitamin A and D. Malnourished children often suffer from a deficiency of these essential nutrients as well. However, future efforts could be better directed at the supplementation of these LCPUFA to Pakistani women from early pregnancy onwards in order to prevent any adverse affects on perinatal brain development of the child. By doing so both the low transplacental supply to the foetus and a low postnatal supply to the newborn via breastmilk could be avoided. Consequently, the LCPUFA status of early brain development could be improved.

References

1. Hamosh M, Salem N Jr Long-chain polyunsaturated fatty acids. Biol Neonate 1998;74:106-20
2. National Institute of Health, Government of Pakistan, Nutrition division. National nutrition survey 1985-87. Islamabad: PanGraphics Ltd., 1988
3. Smit EN, Dijkstra JM, Schnater TA, Seerat E, Muskiet FAJ, Boersma ER. Effects of malnutrition on the fatty acid composition and plasma vitamin E levels of Pakistani children. Acta Paediatr 1997;86:690-5
4. Otto SJ, v Houwelingen AC, Antal M, Manninen A, Godfrey K, Lopez-Jaramillo P, Hornstra G. Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. Eur J Clin Nutr 1997;51:232-42
5. Gibson RA, Neumann MA, Makrides M. Effect of increasing breastmilk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. Eur J Clin Nutr 1997;51:578-84
6. Simeon DT, Grantham-McGregor SM. Nutritional deficiencies and children's behaviour and mental development. Nutr Res Rev 1990;3:1-24
7. Bjerve KS, Fischer S, Wammer F, Egeland T. α-Linolenic acid and long-chain ω-3 fatty acid supplementation in three patients with ω-3 fatty acid deficiency: effect on lymphocyte function, plasma and red cell lipids, and prostanoid formation. Am J Clin Nutr 1989;49:290-300

3.2. Effect of supplementation of arachidonic acid (AA) or a combination of AA plus docosahexaenoic acid on breastmilk fatty acid composition

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Abstract

We investigated whether supplementation with arachidonic acid (20:4ω6; AA), or a combination of AA and docosahexaenoic acid (22:6ω3; DHA) would affect human milk polyunsaturated fatty acid (PUFA) composition. Ten women were daily supplemented with 300 mg AA, eight with 300 mg AA, 110 mg eicosapentaenoic acid (20:5ω3; EPA) and 400 mg DHA, for one week and eight women served as unsupplemented controls. Milk samples were collected on days 0, 1 and 7. The fatty acid composition of the milk was analysed by capillary gas chromatography with flame ionisation detection. Supplementation with AA alone had no effect on breastmilk AA, but tended to reduce EPA and DHA levels. Administration of a combination of AA, EPA and DHA tended to increase both milk AA and long chain PUFA (LCPUFA)ω3 content. A larger simultaneous increase of milk AA, DHA and EPA than observed in the present study can probably be accomplished by the use of a combination of a lower LCPUFAω6/LCPUFAω3 ratio and higher AA, EPA and DHA dosages.

3.2.1. Introduction

Long chain polyunsaturated fatty acids (LCPUFA) are structural components in cellular membrane phospholipids and precursors of eicosanoids [1,2]. Arachidonic (20:4ω6, AA), an LCPUFAω6 and docosahexaenoic (22:6ω3, DHA), an LCPUFAω3 are the quantitatively most important LCPUFA in brain. Their contents increase at least until 2 years after birth [3]. Present knowledge indicates that LCPUFA are conditionally essential in the perinatal period, because the foetus and (preterm) new-born do not seem to synthesise sufficient amounts of AA and DHA from their precursors linoleic (18:2ω6, LA) and linolenic (18:3ω3, ALA) acid, respectively, to cover their high needs [1,4,5]. DHA status is related to e.g. visual acuity [4,6-8], neurodevelopment [9-14] behaviour [15,16] and brain growth [17], while AA, but also DHA, seems to be associated with bodyweight and growth [17-22].

Human milk contains a full range of polyunsaturated fatty acids (PUFA), including small amounts of the whole series of LCPUFAω6 and LCPUFAω3 found in cellular membranes [23,24]. The fatty acid (FA) composition of human milk is, however, to a certain extent dependent on the maternal diet [25,26]. DHA levels are much higher in milk of women

with high intakes of marine foods [27-29] or LCPUFAω3 enriched eggs [30]. On the other hand, milk AA content does not seem to be so much influenced by the diet [12,28,31-33]. Higher levels of AA were, however, reported in milk from Egyptian, Malaysian and Chinese women as compared to milk from women living in Western countries [34-36]. Also within China, milk AA differed between 5 distinct geographic regions with different dietary patterns [29]. In Pakistan, we found lower AA levels in milk of women who nursed malnourished children compared to Dutch controls [37].

Several supplementation studies with fish oil, rich in LCPUFAω3 [26,38,39] or DHA [40] have shown dose-dependent effects on breastmilk eicosapentaenoic acid (20:5ω3; EPA) and DHA levels. No significant effects of fish oil or DHA supplementation on breastmilk AA levels were observed in three of these reports [26,38,39], while a slight AA decrease was shown in the other [40]. To our knowledge no studies have as yet addressed the question whether AA supplementation affects the human milk FA composition. In this study we investigated the effect of a daily supplement of AA (about 300 mg) or a combination of AA, EPA and DHA (300 mg AA, 110 mg EPA and 400 mg DHA) on the human milk PUFA composition. These supplements were given during one week. A third group of unsupplemented women served as controls.

3.2.2. Subjects and methods

3.2.2.1 *Subjects and study design*

The study was carried out in the Spafford Children Clinic in the Old City of Jerusalem. Mothers who were breast-feeding for a period between 3 and 10 months were recruited to participate. They were randomly allocated to receive daily supplements of 0.8 ml AA oil (OPTIMAR, Gist-Brocades, The Netherlands), 2.5 ml of a combination of AA and DHA oil (0.8 ml AA oil and 1.7 ml DHA oil (EPAX 0626 TG, Gist-Brocades, The Netherlands)), or to serve as unsupplemented controls. Supplements (see below) were taken during one week. Milk samples were collected on days 0 (baseline), 1 and 7. All women gave their informed consent. The study protocol was in agreement with local ethical standards and the Helsinki declaration.

Twenty-nine mothers participated in the study. Ten received AA oil, 9 AA+DHA oil and 10 served as controls. The first milk sample was collected immediately prior to the intake of the first supplement on day 0. The next sample was taken on the following day in the morning before the intake of the supplement (day 1), while the last sample was taken in the morning of day 7. Milk samples of controls were collected at the same clock times. All milk samples (about 5 ml) were taken manually, midstream and from the same breast. Two women in the control group did not report for follow-up, and the first sample of a woman in the AA+DHA group got lost during processing. These three women were excluded from the study. Not excluded were 2 other women, one in the control group and one in the AA group, of whom the sample of day 7 was missing. The final study group was composed of 26 women, of which 10 received AA and 8 AA+DHA. Eight women served as unsupplemented controls.

3.2.2.2 *Supplements*

The FA compositions of the AA and AA+DHA oil supplements, as analysed by us, are given in Table 1. The calculated daily intakes of the AA supplemented group corresponded with 284 mg AA, 313 mg LCPUFAω6, no LCPUFAω3, 288 mg SAFA, 112 mg MUFA and 400 mg PUFA (further referred to as '300 mg AA'). The AA+DHA group received daily 321 mg AA, 399 mg DHA, 113 mg EPA, 387 mg LCPUFAω6, 539 mg LCPUFAω3, 873 mg SAFA, 544 mg MUFA and 1083 mg PUFA (further referred to as '300 mg AA, 110 mg EPA and 400 mg DHA'). Both supplementation groups received daily additional vitamin E (250 IU; Ultra vit E forte, Kenpharm-Ultravit, Veghel, The Netherlands) together with the supplements.

Table 1. Fatty acid composition the AA and AA+DHA oils

FA	AA oil	AA+DHA oil	FA	AA oil	AA+DHA oil
18:3ω3	0.00	0.44	18:1ω9	13.24	13.55
20:5ω3	0.00	4.50	20:3ω9	0.04	0.01
22:5ω3	0.00	0.84	sumω9	13.61	14.64
22:6ω3	0.00	15.94			
sumω3	0.00	22.71			
LCPUFAω3	0.00	21.55			
18:2ω6	7.18	3.59	SAFA	35.94	34.92
18:3ω6	3.34	1.21	MUFA	14.03	21.75
20:2ω6	0.35	0.32	PUFA	50.03	43.33
20:3ω6	3.61	1.23			
20:4ω6	35.51	12.84			
22:4ω6	0.00	0.26			
22:5ω6	0.00	1.17			
sumω6	49.99	20.61			
LCPUFAω6	39.12	15.49			

Data represent a selection of fatty acids, expressed in mol% (mol/100 mol).
Abbreviations: SAFA, saturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; LCPUFA, long chain PUFA (C≥20, double bonds ≥3).
Women were supplemented with either 0.8 ml AA oil or 2.5 ml AA+DHA oil.

3.2.2.3 *Sample processing and analysis*

The milk samples were immediately put in a refrigerator and then stored at -20°C. They were transported to The Netherlands in dry ice. FA measurements were done with our previously reported methods by capillary gas chromatography with flame ionisation detection [41,42].

3.2.2.4 *Statistics*

Within-group differences in milk FA compositions on days 0, 1 and 7 were analysed with the Wilcoxon signed-rank test. Between-group differences were analysed with the Mann Whitney-U test. $P<0.017$ (adjusted for Bonferroni inequality rule type-I errors) was considered significant. All statistical analyses were done with SPSS (SPSS 8.0 for Windows, SPSS Inc, Chicago, IL, USA).

3.2.3. Results

The AA, AA+DHA and control groups did not differ in age (median [range]) (23 years [21-27], 24.5 [19-31], 23 [20-28], resp.), number of children (2 [1-5], 3 [1-7], 2 [1-6], resp.) and duration of lactation (4 months [3-10], 6 [3-10], 5 [3-10], resp.). Their milk PUFA contents and some ratios on days 0, 1 and 7 are presented in Table 2. Figure 1 depicts the courses of AA, EPA and DHA for the three subgroups.

Table 2. PUFA contents of controls and AA and AA+DHA supplemented women at baseline, day 1 and day 7.

Fatty acid	Day	Controls	AA	AA & DHA
18:3ω3	0	1.01 ±0.28	0.94 ±0.46	0.94 ±0.30
	1	1.18 ±0.56	0.92 ±0.34	0.91 ±0.26 [&]
	7	1.25 ±0.27	0.86 ±0.40	1.21 ±0.38 [&]
20:5ω3	0	0.05 ±0.05	0.03 ±0.04	0.05 ±0.04 [&]
	1	0.05 ±0.06	0.02 ±0.03 [a]	0.07 ±0.04 [&,a]
	7	0.04 ±0.02 [a,b]	0.01 ±0.01 [a,c]	0.06 ±0.03 [b,c]
22:6ω3	0	0.13 ±0.06	0.16 ±0.08	0.20 ±0.13
	1	0.22 ±0.16	0.12 ±0.02 [a]	0.25 ±0.09 [a]
	7	0.15 ±0.05	0.12 ±0.04 [a]	0.21 ±0.08 [a]
sum ω3	0	1.28 ±0.37	1.22 ±0.58	1.30 ±0.39
	1	1.57 ±0.64	1.15 ±0.37	1.36 ±0.31
	7	1.54 ±0.31	1.07 ±0.41 [a]	1.60 ±0.41 [a]
LCPUFAω3	0	0.28 ±0.13	0.28 ±0.17	0.36 ±0.19 [&]
	1	0.39 ±0.22	0.23 ±0.07 [a]	0.44 ±0.17 [&,a]
	7	0.29 ±0.09	0.21 ±0.04 [a]	0.38 ±0.12 [a]
18:2ω6	0	17.07 ±4.20	18.61 ±3.38	18.55 ±5.82
	1	18.67 ±4.39	17.88 ±4.81	17.13 ±6.29
	7	19.61 ±3.12	16.70 ±4.81	18.16 ±3.90
20:4ω6	0	0.46 ±0.06	0.53 ±0.13	0.55 ±0.15
	1	0.47 ±0.09	0.50 ±0.11	0.61 ±0.12
	7	0.49 ±0.07	0.50 ±0.07	0.69 ±0.23
22:5ω6	0	0.05 ±0.03	0.05 ±0.01 [&]	0.05 ±0.02
	1	0.04 ±0.01	0.04 ±0.01 [&]	0.05 ±0.02
	7	0.05 ±0.01	0.04 ±0.02	0.05 ±0.01

Table 2. PUFA contents of controls and AA and AA+DHA supplemented women at baseline, day 1 and day 7.

Fatty acid	Day	Controls	AA	AA & DHA	
sum ω6	0	18.56 ±4.27	20.26 ±3.68	20.19 ±5.73	
	1	20.22 ±4.64	19.39 ±5.03	18.77 ±6.24	
	7	21.13 ±3.29	18.15 ±4.96	19.98 ±3.82	
LCPUFAω6	0	1.31 ±0.17	1.48 ±0.34	1.44 ±0.34	
	1	1.37 ±0.22	1.37 ±0.29	1.46 ±0.28	
	7	1.37 ±0.19	1.30 ±0.25	1.62 ±0.39	
18:2ω6/	0	17.82 ±5.43	23.57 ±9.92	21.99 ±10.97	
18:3ω3	1	16.89 ±3.49	21.41 ±7.87	19.96 ±9.23	&
	7	16.13 ±2.97	21.96 ±9.18	16.04 ±5.15	&
20:4ω6/	0	3.85 ±1.15	3.61 ±1.09	3.34 ±1.44	
22:6ω3	1	2.92 ±1.49	4.28 ±1.02 [a]	2.63 ±0.72	&,a
	7	3.50 ±0.81	4.53 ±1.15	3.55 ±0.93	&
ω6/ω3	0	15.22 ±4.85	19.32 ±7.67	17.58 ±9.49	
	1	13.75 ±3.30	18.11 ±6.05	14.83 ±7.54	
	7	13.94 ±1.94	18.37 ±6.69	13.19 ±4.06	
LCPUFAω6/	0	5.33 ±1.80	6.01 ±1.87	4.72 ±2.06	&
LCPUFAω3	1	4.32 ±1.73	6.37 ±2.14 [a]	3.61 ±1.17	&,$,a
	7	4.98 ±1.35	6.34 ±1.71	4.51 ±1.32	$

[1] Data represent mean ± SD and are expressed as mol% (mol/100 mol).
Values with the same subscript are significantly different at $p < 0.017$. Letters for between group and symbols for within group differences.
The supplemented women received daily 300 mg AA or 300 mg AA, 110 mg EPA and 400 mg DHA for 1 week. Midstream breastmilk samples were taken in the morning, prior to intake of the supplement.

3.2.3.1 *Between-group differences.*

There were no between-group differences in milk PUFA on day 0. Comparison between the AA and AA+DHA group on day 1 showed that the AA+DHA group had higher 20:5ω3 ($p=0.001$), 22:6ω3 ($p<0.0001$) and LCPUFAω3 ($p=0.001$), and lower 20:4ω6/22:6ω3 ratio ($p=0.006$) and LCPUFAω6/LCPUFAω3 ratio ($p=0.002$). On day 7 the control group had higher 20:5ω3 compared with the AA group ($p=0.008$), but lower 20:5ω3 compared with the AA+DHA group ($p=0.014$). Comparison between the AA and AA+DHA group on day 7 showed that the AA+DHA group had higher 20:5ω3 ($p=0.002$), 22:6ω3 ($p=0.015$), LCPUFAω3 ($p=0.004$) and sumω3 ($p=0.015$).

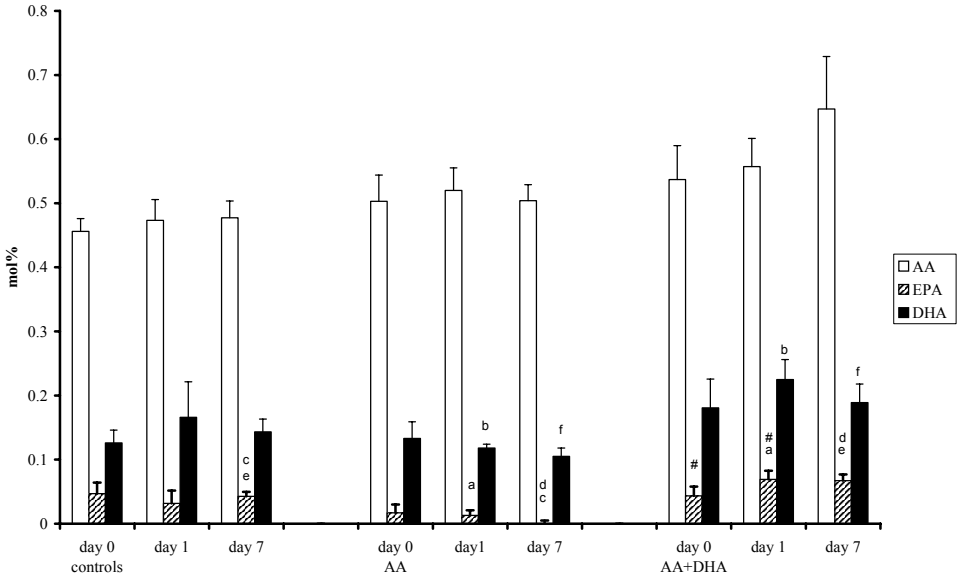

Figure 1. Breastmilk AA, EPA and DHA (median, sem) of controls and AA and AA+DHA supplemented women at baseline, day1 and day 7. Bars with the same superscript are significantly different at p<0.017. Letters stand for between group differences and symbols for within group differences.

3.2.3.2 *Within-group differences.*

The control group did not exhibit changes in milk PUFA during the 1 week study period. The AA group showed a decrease of 22:5ω6 from day 0 to day 1 (p=0.007). In the AA+DHA group 20:5ω3 (p=0.012) and LCPUFAω3 (p=0.012) increased from day 0 to day 1. From day 1 to day 7 18:3ω3 (p=0.012), the 20:4ω6/22:6ω3 ratio (p=0.012) and the LCPUFAω6/LCPUFAω3 ratio (p=0.012) also increased. Decreases were observed for the LCPUFAω6/LCPUFAω3 ratio from day 0 to day 1 (p=0.012) and the 18:2ω6/18:3ω3 ratio from day 1 to day 7 (p=0.012).

3.2.4. Discussion

We investigated the effects of a one week daily supplementation with about 300 mg AA (AA group) and a combination of about 300 mg AA, 110 mg EPA and 400 mg DHA (AA+DHA group) on the breastmilk PUFA composition of healthy mothers in their 3rd-10th month of lactation. Midstream milk samples were collected on days 0, 1 and 7 at the same clock time in the morning, prior to supplement intake. A midstream sample is known to provide a representative reflection of the milk FA composition [43]. We found that the intake of AA alone had no effect on breastmilk AA, but that it tended to reduce

LCPUFAω3 levels (Table 2, Figure 1). In contrast, the milk AA content of the AA+DHA group showed a tendency to increase. The EPA and LCPUFAω3 contents of the AA+DHA group were significantly higher after 1 day, but had returned to baseline levels after 1 week. The lowering effect of the AA supplement on milk LCPUFAω3 became apparent by lower EPA and DHA levels on days 1 and 7 of the AA group compared with the AA+DHA group. The control group held an intermediate position (Figure 1).

This is to our knowledge the first report on the effects of AA supplementation on human breastmilk FA composition. Such investigations might have been hampered by the uniformity of the breastmilk AA content, despite the differences in dietary AA intakes [12, 28,31-33], and the possible adverse effects of an extremely high AA intake (6 g daily) on prostaglandin synthesis [44]. We estimated the daily AA intake of the women in our study at 200 mg or less, taking into account a daily AA intake of 200-1000 mg by omnivorous US adults [45], and the relatively low intake of AA rich food products like meat and eggs of our study population. Therefore we considered the present 300 mg AA supplement to be safe, since the resulting total daily intake of around 500 mg AA would be well within the range for US adults. Moreover, a recent study in healthy adults using an AA rich oil, resulting in an AA intake of 1700 mg per day, showed no adverse effects on health [46]. Even higher amounts of AA (3.6 g) were reported to be harmless, but in that study the AA supplement was accompanied by DHA [47]. The women in this study reported no adverse effects of the supplementation on health.

Breast milk AA levels are remarkably similar in omnivores, vegetarians and vegans [32,33]. For Chongqing Chinese women the general belief is that they have to replenish their body nutrient stores after a pregnancy. Therefore a daily consumption of more than 8 eggs, 200 g chicken meat and 50 g pork (approximately 1100 mg AA, based on food AA content given by reference 48) is not uncommon during the first months after delivery. Yet, they showed no significantly higher milk AA content than their urbanised counterparts from Hong Kong (0.76 vs 0.61 wt%) with an estimated six times lower AA intake [36]. The higher milk AA levels reported from Asian and African countries compared to Western countries [29,34-36] are probably caused by other factors than AA intake. Chen *et al*. [36] have suggested that they are due to higher intakes of *trans* FA in Western countries, since these are known to inhibit essential FA desaturation and elongation. The low milk AA levels encountered in Northern Pakistan [37] probably reflect the poor essential FA status of these women. In view of the above, it may be expected that a moderate AA supplementation would not change milk AA levels of adequately nourished healthy women, and this is indeed what we observed in the present study.

The effects of fish oil (i.e. EPA+DHA) or DHA supplements on the breastmilk FA composition are well documented. They demonstrate a positive relation between their dietary intakes and breastmilk contents [26,38-40]. AA levels were slightly reduced in one of these studies. Makrides *et al*. [40] showed a small decline of breastmilk AA after 12 weeks supplementation with various DHA dosages (0.2, 0.4, 0.9 and 1.3 g DHA daily). The women consuming 0.9 g DHA daily had significant lower breastmilk AA levels compared to the control group (0.33±0.06 g% vs 0.41±0.06 g%). A decrease of milk AA levels after fish oil supplementation might be a reason for concern, notably in those women with marginal baseline LCPUFAω6 status, like Pakistani women [37]. That an adverse effect of LCPUFAω3 supplementation on the breastmilk AA content can be counteracted by co-administration of AA is suggested by the results of the present study, in which we used a

combination of 300 mg AA, 110 mg EPA and 400 mg DHA. This combination even caused a tendency of AA to increase, which was not found after supplementation with AA alone. Somewhat to our surprise we found that in this group milk EPA and LCPUFAω3 were only moderately increased after 1 day of supplementation and that these changes had disappeared after 1 week. An adverse effect of the AA supplement on milk LCPUFAω3 is probably the underlying cause of the much smaller changes in milk LCPUFAω3 levels compared to other studies in which similar LCPUFAω3 dosages, but no AA, have been used [39,40]. This observation may be on account of the well known competition between AA on the one hand and EPA and DHA on the other [1], resulting in a slight preference for AA incorporation into milk lipids at the presently employed LCPUFAω6/LCPUFAω3 ratio and total LCPUFA dosage.

We conclude that one week supplementation with a moderate dose of 300 mg AA per day has no significant effect on breastmilk AA, but tended to decrease the milk LCPUFAω3 content. However, administration of a combination of 300 mg AA plus 110 mg EPA and 400 mg DHA, tended to increase both milk AA and LCPUFAω3 content. A larger simultaneous increase of milk AA, DHA and EPA can probably be accomplished by the use of a combination of a lower LCPUFAω6/LCPUFAω3 ratio and higher AA, EPA and DHA dosages than employed in the present study.

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References

1. Innis S. M. Essential fatty acids in growth and development. Prog Lipid Res 1991;30:39-101
2. Linscheer W. G., Vergroesen J. Lipids. In: Shils M. E., ed. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea and Febiger, 1994:47-88
3. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129-38
4. Makrides M., Neumann M., Simmer K., Pater J., Gibson R. Are long-chain polyunsaturated fatty acids essential nutrients in infancy? Lancet 1995;345:1463-8
5. Decsi T., Koletzko B. Polyunsaturated fatty acids in infant nutrition. Acta Paediatr Suppl 1994;395:31-7
6. Birch E. E., Birch D. G., Hoffman D. R., Uauy R. Dietary essential fatty acid supply and visual acuity development. Invest Ophthalmol Vis Sci 1992;33:3242-53
7. Carlson S. E., Werkman S. H., Rhodes P. G., Tolley E. A. Visual acuity development in healthy preterm infants: effect of marine-oil supplementation. Am J Clin Nutr 1993;58:35-42
8. Carlson S. E., Ford A. J., Werkman S. H., Peeples J. M., Koo W. W. K. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. Pediatr Res 1996;39:882-8
9. Agostoni C., Riva E., Trojan S., Bellù R., Giovanni M. Docosahexaenoic acid status and development quotient of healthy term infants. Lancet 1995;346:638
10. Gibson R. A., Neumann M. A., Makrides M. Effect of increasing breastmilk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. Eur J Clin Nutr 1997;51:578-4
11. Gibson R. A., Makrides M. The role of long chain polyunsaturated fatty acids (LCPUFA) in neonatal nutrition. Acta Paediatr 1998;87:1017-22
12. Hamosh M., Salem N. Jr. Long-chain polyunsaturated fatty acids. Biol Neonate 1998;74:106-20
13. Willats P., Forsyth J. S., DiModugno M. K., Varma S., Colvin M. Effects of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. Lancet 1998;352:688-91
14. Woltil H. A., van Beusekom C. M., Okken-Beukens M., Schaafsma A., Muskiet F. A. J., Okken A. Development of low-birthweight infants at 19 months of age correlates with early intake and status of long-chain polyunsaturated fatty acids. Prostaglandins Leukot Essent Fatty Acids 1999;61:235-41

15. Hamazaki T., Sawazaki S., Itomura M., et al. The effect of docosahexaenoic acid on aggression in young adults. J Clin Invest 1996;97:1129-33
16. Stevens L. J., Zentall S. S., Deck J. L., Abate M. L., Watkins B. A., Lipp S. R. Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. Am J Clin Nutr 1995;62:761-8
17. Woltil H. A., van Beusekom C. M., Schaafsma A., Muskiet F. A., Okken A. Long-chain polyunsaturated fatty acid status and early growth of low birth weight infants. Eur J Pediatr 1998;157:146-52
18. Hoffman D. R., Uauy R. Essentiality of dietary ω3 fatty acids for premature infants: plasma and red blood cell fatty acid composition. Lipids 1992;27:886-95
19. Koletzko B., Braun M. Arachidonic acid and early human growth: Is there a relation? Ann Nutr Metab 1991;35:128-31
20. Decsi T., Zaknun D., Zaknun J., Sperl W., Koletzko B. Long-chain polyunsaturated fatty acids in children with severe protein-energy malnutrition with and without human immunodeficiency virus-1 infection. Am J Clin Nutr 1995;62:1283-8
21. Xiang M., Zetterstrom R. Relation between polyunsaturated fatty acids and growth. Acta Paediatr Suppl 1999;88:78-82
22. Xiang M., Lei S., Li T., Zetterstrom R. Composition of long chain polyunsaturated fatty acids in human milk and growth of young infants in rural areas of northern China. Acta Paediatr 1999;88:126-31
23. Jensen R. G. Lipids in human milk- Composition and fatsoluble vitamins. In: Lebenthal E, ed. Textbook of gastroenterology and nutrition in infancy. New York: Raven Press, 1989:157-208
24. Jackson K. A., Gibson R. A. Weaning foods cannot replace breastmilk as sources of long-chain polyunsaturated fatty acids. Am J Clin Nutr 1989;50:980-2
25. Read W. W. C., Lutz P. G., Tashjian A. Human milk lipids. II. The influence of dietary carbohydrates and fat on the fatty acids of mature milk. Am J Clin Nutr 1965;17:180-3
26. Harris W. S., Connor W. E., Lindsey S. Will dietary ω-3 fatty acids change the composition of human milk? Am J Clin Nutr 1984;40:780-5
27. Innis S. M., Kuhnlein H. V. Long-chain n-3 fatty acids in breast milk of Inuit women consuming traditional foods. Early Human Dev 1988;18:185-9
28. Koletzko B., Thiel I., Abiodun P. O. The fatty acid composition of human milk in Europe and Africa. J Pediatr 1992;120:S62-70
29. Chulei R., Xiaofang L., Hongseng M., et al. Milk composition in women from five different regions of China: The great diversity of milk fatty acids. J Nutr 1995;125:2993-8
30. Cherian G., Sim J. S. Changes in the breast milk fatty acids and plasma lipids of nursing mothers following consumption of n-3 polyunsaturated fatty acid enriched eggs. Nutrition 1996;12:8-12
31. Villalpando S., Butte N. F., Flores-Huerta S. F., Thotathuchery M. Qualitative analysis of human milk produced by women consuming a maize-predominant diet typical of rural Mexico. Ann Nutr Metab 1998;42:23-32
32. Sanders T. A. B., Reddy S. The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant. J Pediatr 1992;120:S71-7
33. Finley D. A., Lonnerdal B., Dewey K. G., Grivetti L. E. Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians. Am J Clin Nutr 1985;41:787-800
34. Borschel M. W., Elkin R. G., Kirksey A., Story J. A., Galal O., Harrison G. G., Jerome N. W. Fatty acid composition of mature human milk in Egyptian and American women. Am J Clin Nutr 1986;44:330-5
35. Kneebone G. M., Kneebone R., Gibson R. A. Fatty acid composition of breast milk from three racial groups from Penang, Malaysia. Am J Clin Nutr 1985;41:765-9
36. Chen Z. Y., Kwan K. Y., Tong K. K., Ratnayake W. M. N., Li H. Q., Leung S. S. F. Breast milk fatty acid composition: A comparative study between Hong Kong and Chongqing Chinese. Lipids 1997;32:1061-7
37. Smit E. N., Oelen E. A., Seerat E., Muskiet F. A. J., Boersma E. R. Breastmilk docosahexaenoic acid (DHA) correlates with DHA status of malnourished infants. Arch Dis Child 2000;6:493-4
38. Henderson R. A., Jensen R. G., Lammi-Keefe C. J., Ferris A. M., Dardick K. R. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 1992;27:901-7
39. Helland I. B., Saarem K., Saugstad O. D., Drevon C. A. Fatty acid composition in maternal milk and plasma during supplementation with cod liver oil. Eur J Clin Nutr 1998;52:839-45
40. Makrides M., Neumann M. A., Gibson R. A. Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. Eur J Clin Nutr 1996;50:352-7
41. Muskiet F. A. J., Doormaal J. J. van, Martini I. A., Wolthers B. G., Slik W. van der. Capillary gas chromatographic profiling of total long-chain fatty acids and cholesterol in biological materials. J Chromatogr Biomed Appl 1983; 278:231-44

42. Steege G. van der, Muskiet F. A. J., Martini I. A., Hutter N. H., Boersma E. R. Simultaneous quantification of total medium- and long-chain fatty acids in human milk by capillary gas chromatography with split injection. J Chromatogr Biomed Appl 1987;415:1-11
43. Hall B. Uniformity of human milk. Am J Clin Nutr 1979;32:304-12
44. Seyberth H. W., Oelz O., Kennedy T., et al. Increased arachidonate in lipids after administration to man: effects on prostaglandin biosynthesis. Clin Pharmacol Ther 1975;18:521-9
45. Phinney S. D., Odin R. S., Johnson S. B., Holman R. T. Reduced arachidonate in serum phospholipids and cholesteryl esters associated with vegetarian diets in humans. Am J Clin Nutr 1990;51:385-92
46. Nelson G. J., Schmidt P. C., Bartolini G., et al. The effect of dietary arachidonic acid on plasma lipoprotein distributions, apoproteins, blood lipid levels, and tissue fatty acid composition in humans. Lipids 1997;32:427-33
47. Innis S. M., Hansen J. W. Plasma fatty acid responses, metabolic effects, and safety of microalgal and fungal oils rich in arachidonic and docosahexaenoic acids in healthy adults. Am J Clin Nutr 1996;64:159-67
48. Taber L., Chiu C., Whelan J. Assessment of the arachidonic acid content in foods commonly consumed in the American diet. Lipids 1998;33:1151-7

3.3. Estimated biological variation of the mature human milk fatty acid composition

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Summary

We estimated the biological variation (CV_{biol}) of 28 fatty acids (FA) in 465 mature human milk samples from The Netherlands, Caribbean, Jerusalem, Tanzania and Pakistan, by using data from the observed variation (CV_{obs}) and analytical variation (CV_{anal}). CV_{biol} of the various regions was remarkably similar. The average CV_{biol} of 455 samples, Pakistan excluded, ranged from 12.7% for 16:0 and 18.9% for 18:1ω9 to 68% for 22:6ω3 and about 100% for 20:5ω3. Those of 20:4ω6, 18:2ω6 and 18:3ω3 were 28.0, 33.0 and 37.3%, respectively. Because of the large CV_{biol} and the many dietary changes in recent history it seems impossible to consider the present human milk FA composition as the 'gold standard' for infant formula. Optimal human milk FA composition should rather derive from populations that consume traditional diets or from scientific data that show the function of the individual FAs in neonatal development.

3.3.1. Introduction

Human milk is the single best food for babies and young infants [1,2]. Its superiority relates to many factors, including its specific fatty acid (FA) composition. Some studies have stressed the uniformity of the human milk FA composition [3,4], but Jensen [5] recently pointed out that the range of milk FA contents is wide and that there is lack of sufficient reliable data showing the ranges of the biologically important species. This information is e.g. important for the manufacturing of infant formulas, since the human milk composition is still considered to be the 'gold standard' for most nutrients.

One class of biologically important FAs in breast milk are long chain polyunsaturated FAs (LCPs), including arachidonic acid (20:4ω6) and docosahexaenoic acid (22:3ω6). LCPs (≥20 carbon atoms and ≥3 double bonds in the methylene-interrupted *cis*-configuration) derive either from the diet (mainly meat and fish, respectively) or from desaturation, chain elongation and chain shortening of the two parent essential FAs, linoleic acid (18:2ω6; ω6 series) and α-linolenic acid (18:3ω3; ω3-series). They are structural components of virtually all cell membrane phospholipids and precursors of biologically active eicosanoids and hydroxy FAs [6]. The two quantitatively most important LCPs in brain are 20:4ω6 and 22:6ω3, and their contents increase until at least 2 years postpartum [7]. Formula fed babies have for long been known to have lower LCP status than breast-fed infants, since LCPs are not present in the vegetable oils that constitute the basis of infant formulas [8,9]. Breast fed

children score higher on mental development and visual acuity tests than formula fed children [10,11], which could possibly relate to their higher LCP status, notably that of 22:6ω3 [12,13,14]. Docosahexaenoic acid, or 22:6ω3 plus 20:4ω6, are nowadays added by many manufacturers of formulas for preterm babies and to a lesser extent for term infants [8,9]. Such formulas augment LCP status and have in preterm infants been shown to cause transient improvement of neurodevelopment [15,16]. The results in term infants are, however, equivocal [17,18].

Use of the human milk FA composition as the gold standard is hampered by its dependence on the maternal diet [19,20] and uncertainty regarding the appropriate maternal diet. For instance, the milk medium chain FA content can be drastically increased by a high carbohydrate intake [20,21,22]. Milk 18:2ω6 and *trans*-FA contents are related to maternal 18:2ω6 [22,23] and *trans*-FA intakes [5], respectively, and 22:6ω3 levels are much higher in milk of women with high intakes of marine foods, fish oil or LCPω3 enriched eggs [24,25,26,27,28]. On the other hand, the milk 20:4ω6 content does not seem to be influenced much by the diet [29,30,31] or 20:4ω6 supplementation [32]. Dietary habits in the Western world have changed drastically over the last 200 years. Among these are a higher fat intake at the expense of carbohydrates, intake of 18:2ω6-rich, 18:3ω3-poor, vegetable oils and margarines for cholesterol lowering, use of *trans*-FA rich hydrogenated fats, and declining fish consumption. It is e.g. estimated that these changes altered the dietary ω6/ω3 ratio from around 1:1 to 10:1 in Europe and the USA [33,34], and to 4:1 in Japan [35]. All of these changes have affected the human milk composition [5,33].

There have been several reviews on the mature milk FA composition in different countries [4,5,19]. Fortunately there is no influence of the clock time or method of sampling on the FA composition [19]. Comparisons to arrive at a reasonable estimate of biological variation (CV_{biol}) are, however, difficult if not impossible, since the underlying data represent some combination of genuine CV_{biol} and (usually unknown) analytical precision and accuracy. Precision is very much dependent on the (relative) amount of analyte [36], and standardization to minimize the bias is difficult because of the poor availability of certified standard FAs. Most investigators rely on the relation between the relative gas chromatographic peak areas of the analyzed FA methyl esters and their relative weight amounts (g%), but this relation might be seriously affected by evaporation steps in the preanalytical phase and injector-related discrimination in the analytical phase, which both jeopardize any analysis of homologous series with highly different boiling points [37]. Another analytical imperfection may derive from the use of different capillary gas chromatographic columns, since it is well known that the FA separation characteristics are dependent on the polarity of the employed stationary phase [38].

We have over the last 25 years collected mature breast milk samples from 11 different regions, including The Netherlands, Caribbean Region, Jerusalem, Tanzania and Pakistan. All samples were analyzed in the same laboratory with a single analytical technique, i.e. that of high-resolution capillary gas chromatography-flame ionization detection with an apolar stationary phase and use of odd carbon numbered medium chain FAs and margarinic acid (17:0) as internal quantification standards [36]. Using this data set and the previously reported between-series precision of our method, we estimated the genuine CV_{biol} of the analyzed milk FAs.

3.3.2. Materials and methods

3.3.2.1 *Milk samples*

A total of 465 mature breast milk samples were collected over the last 25 years. Analysis took place soon after collection. The regions from which these samples derived were: The Netherlands [n=222: 99 collected at mean (SD) postnatal days 14.4 (3.5), 98 at day 42.1 (2.7) and 25 at day 89.2 (5.6)], Antigua (n=23), Belize (n=10), Curaçao (n=47), Dominica (n=17), St. Lucia (n=12), St. Vincent (n=30), Surinam (n=20), Jerusalem (n=63), Tanzania (n=11) and Pakistan (n=10). The 159 samples from Antigua, Belize, Curaçao, Dominica, St. Lucia, St. Vincent and Surinam will be collectively referred to as deriving from the 'Caribbean Region'. Data on the FA compositions of The Netherlands [9], Tanzania and the Caribbean Region [21], Jerusalem [32] and Pakistan [39] have been published before. Milk from Dutch mothers derived from 24-hour samples that were collected by vacuum pump. Ten percent was kept for analyses and the remainder was administered to the baby by bottle or cup. The milk from all other women were midstream samples of about 5 ml that were collected by manual expression.

3.3.2.2 *Sample processing and analysis*

The milk samples were immediately put in a refrigerator and then stored at -20^0C. Samples from abroad were transported to The Netherlands in dry ice. The FA composition of the milk was analyzed by capillary gas chromatography with split injection and flame ionization detection [36]. Medium chain FAs (MCFA; C_6-C_{14}) were calculated with the bracketing method [37], whereas long chain FAs ($\geq C_{16}$) were quantified on the basis of 17:0 as an internal calibration standard assuming that equal weight amounts of FAs give rise to equal peak areas [40]. All data are expressed in mol/100 mol.

3.3.2.3 *Data processing and statistics*

The analytical variation (CV_{anal}) was taken from the series-to-series precision of 26 of the 28 FAs as previously reported by us [36]. Based on the inverse relationship between FA contents (mol%) and their CV_{anal} (in %) we estimated the CV_{anal} of the two unknowns, i.e. 18:3ω3 and 22:5ω6, at 5 and 15%, respectively. The inter-individual CV_{biol} was calculated from the observed variation (CV_{obs}) by the following equation: $CV_{biol} = \sqrt{(CV^2_{obs} - CV^2_{anal})}$.

3.3.3. Results and discussion

Different views on the variation of the human milk FA composition are illustrated by the titles of two articles: '*Uniformity* of human milk' published in 1979 [3] and 'Milk composition in women from five different regions of China: The great *diversity* of milk fatty acids' published in 1995 [41]. Koletzko *et al*. in their review on the FA composition of milk from Europe and Africa [4], concluded that saturated FA (SAFA), monounsaturated FA (MUFA), 18:2ω6 and LCPω3 are to a certain extent depended on the maternal diet,

Table 1. Fatty acid composition of mature human milk from The Netherlands, Caribbean Region, Jerusalem, Tanzania and Pakistan.

	All (n=455)		The Netherlands (n=222)		Caribbean Region (n=159)	
	median	range	median	range	median	range
6:0	0.28	0.03 - 0.69	0.30	0.17 - 0.52	0.17	0.03 - 0.48
8:0	0.66	0.11 - 1.76	0.66	0.45 - 0.94	0.67	0.24 - 1.76
10:0	3.00	0.57 - 6.15	2.73	1.58 - 4.27	3.62	0.57 - 6.15
12:0	9.78	2.14 - 34.90	8.20	2.91 - 15.75	13.82	4.12 - 34.90
14:0	8.84	1.57 - 27.61	7.89	3.68 - 14.21	11.54	4.09 - 26.00
16:0	21.90	12.68 - 29.21	23.21	14.45 - 28.82	20.89	14.29 - 29.21
18:0	6.35	1.08 - 9.68	7.18	4.84 - 9.68	5.45	2.14 - 8.77
20:0	0.20	0.03 - 0.91	0.21	0.03 - 0.37	0.20	0.07 - 0.91
22:0	0.09	0.00 - 0.34	0.10	0.05 - 0.21	0.09	0.00 - 0.34
24:0	0.07	0.00 - 0.31	0.07	0.03 - 0.16	0.07	0.00 - 0.31
MCSAFA	22.41	4.65 - 67.92	19.89	9.19 - 35.20	30.62	9.90 - 67.92
LCSAFA	38.35	25.53 - 47.15	38.89	27.28 - 46.09	39.09	27.07 - 47.15
18:3ω3	0.92	0.27 - 2.71	1.02	0.64 - 2.71	0.67	0.27 - 2.00
20:5ω3	0.05	0.00 - 1.18	0.05	0.00 - 0.29	0.05	0.00 - 0.36
22:5ω3	0.12	0.00 - 0.31	0.12	0.08 - 0.24	0.13	0.00 - 0.31
22:6ω3	0.21	0.08 - 1.63	0.19	0.09 - 0.84	0.33	0.09 - 1.63
LCPω3	0.38	0.10 - 1.68	0.36	0.20 - 1.33	0.52	0.16 - 1.68
ω3	1.30	0.16 - 3.08	1.42	0.90 - 3.08	0.98	0.16 - 2.79
14:1ω5	0.28	0.03 - 0.69	0.37	0.03 - 0.69	0.23	0.05 - 0.52
18:2ω6	12.67	3.51 - 30.03	12.84	6.01 - 28.21	11.26	3.51 - 25.94
18:3ω6	0.09	0.00 - 0.33	0.09	0.03 - 0.20	0.07	0.00 - 0.23
20:2ω6	0.31	0.08 - 0.99	0.31	0.17 - 0.57	0.32	0.08 - 0.99
20:3ω6	0.36	0.18 - 0.78	0.33	0.18 - 0.78	0.38	0.20 - 0.68
20:4ω6	0.42	0.19 - 0.99	0.37	0.21 - 0.62	0.50	0.19 - 0.99
22:4ω6	0.09	0.00 - 0.50	0.07	0.04 - 0.16	0.12	0.00 - 0.50
22:5ω6	0.04	0.00 - 0.18	0.03	0.00 - 0.08	0.05	0.00 - 0.18
LCPω6	1.23	0.59 - 3.25	1.11	0.71 - 1.72	1.40	0.59 - 3.25
ω6	14.08	4.14 - 31.35	14.04	7.22 - 29.53	12.80	4.14 - 27.36
16:1ω7	2.33	0.65 - 5.89	2.33	0.76 - 4.99	2.58	0.89 - 5.89
18:1ω7	2.89	0.50 - 7.63	3.13	1.57 - 5.34	2.98	0.79 - 7.63
ω7	5.36	1.65 - 10.34	5.50	2.66 - 9.40	5.55	1.96 - 10.34
18:1ω9	25.20	7.17 - 40.05	26.49	19.06 - 34.47	21.38	7.17 - 34.59
20:1ω9	0.35	0.06 - 1.10	0.37	0.22 - 0.69	0.38	0.06 - 1.10
20:3ω9	0.05	0.00 - 0.20	0.05	0.00 - 0.09	0.06	0.00 - 0.20
24:1ω9	0.05	0.00 - 0.46	0.04	0.00 - 0.46	0.05	0.00 - 0.27
ω9	25.60	7.28 - 40.45	26.99	19.48 - 35.11	21.84	7.28 - 35.63
MUFA	31.48	9.54 - 46.60	33.04	22.20 - 44.74	28.06	9.54 - 42.91
PUFA	15.49	5.03 - 32.24	15.53	8.41 - 32.15	13.93	5.03 - 27.82
LCω3+LCω6	1.64	1.02 - 3.96	1.50	1.05 - 2.21	1.92	1.09 - 3.96

Table 1. Fatty acid composition of mature human milk from The Netherlands, Caribbean Region, Jerusalem, Tanzania and Pakistan.

	All (n=455)		The Netherlands (n=222)		Caribbean Region (n=159)	
	median	range	median	range	median	range
LCω6/LCω3	3.18	0.40 - 9.85	3.19	0.67 - 5.73	2.87	0.40 - 9.62
ω6/ω3	10.36	3.54 - 101.38	9.56	3.54 - 23.95	11.04	3.71 - 101.38
20:3ω9/20:4ω6	0.12	0.00 - 0.47	0.13	0.00 - 0.23	0.11	0.00 - 0.47
20:4ω6/22:6ω3	1.97	0.15 - 5.71	2.04	0.30 - 3.75	1.60	0.15 - 3.83
18:2ω6/18:3ω3	13.44	4.77 - 42.89	11.81	4.77 - 30.55	14.53	9.13 - 32.91

Data are in mol%. 'All' indicates pooled data from all samples, those of Pakistan excluded. The 159 samples from the 'Caribbean Region' derive from Antigua (n=23), Belize (n=10), Curaçao (n=47), Dominica (n=17), St. Lucia (n=12), St. Vincent (n=30) and Surinam (n=20).

Table 1. continued. Fatty acid composition of mature human milk from The Netherlands, Caribbean Region, Jerusalem, Tanzania and Pakistan.

	Jerusalem (n=63)		Tanzania (n=11)		Pakistan (n=10)	
	median	range	median	range	median	range
6:0	0.32	0.07 - 0.69	0.35	0.21 - 0.54	0.32	0.16 - 0.42
8:0	0.57	0.11 - 1.30	0.86	0.66 - 1.26	0.46	0.28 - 0.77
10:0	2.80	0.75 - 5.58	3.83	2.17 - 4.84	2.28	1.43 - 3.32
12:0	9.67	2.14 - 16.53	19.87	11.28 - 28.43	10.03	5.05 - 11.87
14:0	7.98	1.57 - 15.93	13.22	7.75 - 27.61	10.99	4.94 - 14.04
16:0	18.97	12.68 - 28.19	18.57	14.16 - 28.65	27.94	18.99 - 34.36
18:0	4.93	2.57 - 8.11	3.63	1.08 - 4.44	5.20	3.97 - 7.70
20:0	0.14	0.08 - 0.23	0.10	0.05 - 0.12	0.17	0.12 - 0.19
22:0	0.07	0.02 - 0.13	0.05	0.00 - 0.07	0.07	0.05 - 0.11
24:0	0.06	0.03 - 0.13	0.05	0.00 - 0.07	0.06	0.03 - 0.09
MCSAFA	21.74	4.65 - 35.25	39.72	23.91 - 59.14	25.36	12.77 - 27.79
LCSAFA	32.51	25.53 - 42.31	38.49	32.93 - 43.97	44.48	33.71 - 49.59
18:3ω3	0.97	0.46 - 2.01	0.82	0.44 - 1.84	0.34	0.25 - 1.84
20:5ω3	0.04	0.00 - 0.16	0.06	0.00 - 1.18	0.02	0.00 - 0.09
22:5ω3	0.10	0.05 - 0.23	0.11	0.00 - 0.21	0.05	0.04 - 0.14
22:6ω3	0.16	0.08 - 0.49	0.17	0.10 - 0.40	0.06	0.03 - 0.19
LCPω3	0.32	0.13 - 0.78	0.40	0.10 - 1.54	0.14	0.09 - 0.38
ω3	1.25	0.73 - 2.43	1.17	0.54 - 2.16	0.53	0.37 - 2.21
14:1ω5	0.12	0.04 - 0.36	0.18	0.10 - 0.36	0.13	0.07 - 0.27
18:2ω6	16.57	10.48 - 30.03	12.47	5.15 - 23.56	8.73	7.13 - 22.71
18:3ω6	0.15	0.00 - 0.33	0.10	0.05 - 0.22	0.05	0.00 - 0.12
20:2ω6	0.28	0.16 - 0.63	0.23	0.11 - 0.39	0.16	0.14 - 0.37
20:3ω6	0.42	0.22 - 0.78	0.32	0.18 - 0.50	0.21	0.15 - 0.36
20:4ω6	0.48	0.28 - 0.81	0.49	0.31 - 0.71	0.26	0.20 - 0.44
22:4ω6	0.10	0.05 - 0.20	0.10	0.07 - 0.12	0.06	0.04 - 0.08

Table 1. continued. Fatty acid composition of mature human milk from The Netherlands, Caribbean Region, Jerusalem, Tanzania and Pakistan.

	Jerusalem (n=63)		Tanzania (n=11)		Pakistan (n=10)	
	median	range	median	range	median	range
22:5ω6	0.04	0.00 - 0.12	0.06	0.00 - 0.07	0.02	0.00 - 0.05
LCPω6	1.34	0.91 - 2.11	1.30	0.73 - 1.46	0.69	0.57 - 1.30
ω6	18.18	11.99 - 31.35	13.92	5.92 - 25.06	9.35	7.78 - 24.10
16:1ω7	1.79	0.65 - 4.21	1.94	1.28 - 5.07	2.23	1.20 - 5.02
18:1ω7	1.74	0.73 - 4.00	1.48	0.50 - 4.53	4.05	2.22 - 5.80
ω7	3.73	1.65 - 6.51	3.96	1.83 - 7.75	6.17	4.35 - 9.31
18:1ω9	28.14	18.46 - 40.05	17.31	7.83 - 23.94	24.19	21.22 - 28.94
20:1ω9	0.26	0.15 - 0.54	0.15	0.06 - 0.26	0.25	0.21 - 0.32
20:3ω9	0.05	0.00 - 0.09	0.05	0.03 - 0.08	0.06	0.03 - 0.07
24:1ω9	0.05	0.02 - 0.10	0.00	0.00 - 0.04	0.04	0.02 - 0.07
ω9	28.61	18.78 - 40.45	17.64	7.95 - 24.23	24.53	21.52 - 29.31
MUFA	33.18	21.85 - 46.60	22.37	9.95 - 30.86	30.93	26.31 - 35.77
PUFA	19.90	12.93 - 32.24	15.57	7.12 - 26.11	9.96	8.22 - 26.36
LCω3+LCω6	1.68	1.12 - 2.89	1.64	1.02 - 3.00	0.86	0.68 - 1.68
LCω6/LCω3	4.21	2.39 - 9.85	3.08	0.95 - 9.09	4.94	2.25 - 8.76
ω6/ω3	13.62	7.99 - 36.04	9.24	5.14 - 24.63	19.02	10.89 - 27.24
20:3ω9/20:4ω6	0.09	0.00 - 0.20	0.11	0.04 - 0.19	0.20	0.11 - 0.29
20:4ω6/22:6ω3	2.88	1.60 - 5.71	2.24	1.52 - 4.77	4.23	1.65 - 7.78
18:2ω6/18:3ω3	17.24	9.02 - 40.85	13.55	6.04 - 42.89	26.90	12.37 - 33.94

Data are in mol%. 'All' indicates pooled data from all samples, those of Pakistan excluded. The 159 samples from the 'Caribbean Region' derive from Antigua (n=23), Belize (n=10), Curaçao (n=47), Dominica (n=17), St. Lucia (n=12), St. Vincent (n=30) and Surinam (n=20).

while LCPω6 are hardly affected. Apart from diet, milk FA composition is to a certain extent influenced by other factors like time postpartum, gestational age, parity and certain diseases [5,19]. The CV_{obs} of the breast milk FA is however not a synonym for the genuine CV_{biol}, since it also contains CV_{anal}.

Table 1 shows the medians and ranges for 28 FAs and some of their sums and ratios in milk from The Netherlands, the Caribbean Region, Jerusalem, Tanzania and Pakistan, together with the pooled data of all human milk samples, with the exception of Pakistan. The Pakistani data were not included in the final calculation of the CV_{biol} (see below), since we doubt whether the milk of the investigated mothers can be considered to contain sufficient essential FAs. Their milk had the highest LCSAFA, 16:0, 20:3ω9/20:4ω6, ω6/ω3, 18:2ω6/18:3ω3, LCPω6/LCPω3 and 20:4ω6/22:6ω3, and the lowest polyunsaturated FA (PUFA), ω6, ω3, LCPω6 and LCPω3. This profile is consistent with their marginal essential FA status, notably that of ω3 FAs, due to the low intake of vegetable oil and the very low fish consumption in North Pakistan [39].

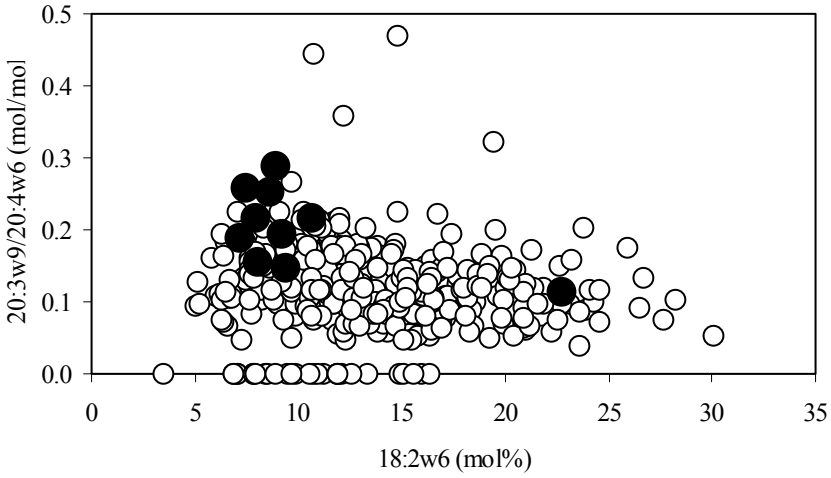

Figure 1. Relationship between mature milk 18:2ω6 content and the 20:3ω9/20:4ω6 ratio. Data are in mol% (18:2ω6) or mol/mol (20:3ω9/20:4ω6). Open circles indicate data from 455 samples (i.e. those of Pakistan excluded, see Table 1). Closed circles indicate data from 10 milk samples from Pakistan. The Mead acid (20:3ω9) content and the 20:3ω9/20:4ω6 ratio in plasma and erythrocytes are markers of essential fatty acid deficiency. The usefulness of these parameters in human milk has as yet not been fully established.

We also noticed that the 20:3ω9/20:4ω6 ratio in milk of Pakistani was amongst the highest and the 18:2ω6 content amongst the lowest of all samples (Figure 1). The 20:3ω9/20:4ω6 ratio is considered to be a general marker of essential FA deficiency in plasma and erythrocytes [6], but its usefulness in milk has not been fully established. The milk of the Jerusalem women had the highest ω6, PUFA, ω9 and MUFA contents, notably on account of highest 18:2ω6 and 18:1ω9, respectively. This profile is consistent with predominant local use of corn, sunflower and olive oils. The milk of the Tanzanian women was especially rich in MCSAFA, notably 12:0 and 14:0, most probably because of the high consumption of carbohydrates, which are known to be converted into MCSAFA in the human breast [42]. The milk of the Caribbean mothers contained the highest LCPω3, 22:6ω3, LCPω6, 20:4ω6 and LCP and fair amounts of MCSAFA, which may be consistent with high consumption of fish and carbohydrates.

Figure 2 shows the previously reported CV_{anal} [36], together with the CV_{biol}, as calculated for all human milk samples, except for those of Pakistan. The Figure indicates that most of the observed variance is indeed caused by CV_{biol}, with relatively little contribution from CV_{anal}. A clinical chemical quality criterion is that CV_{anal} should be equal or less than 50% of the mean CV_{biol} [43]. This criterion was met for all examined FAs except for 18:3ω6 and

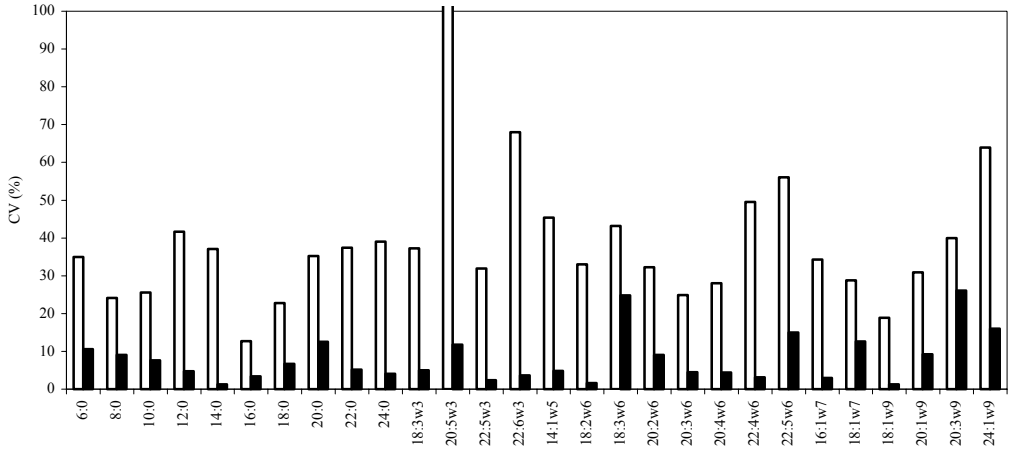

Figure 2. Comparison of the between-series analytical variation (CVanal) and the calculated inter-individual biological variation (CVbiol) for 28 mature human milk fatty acids. Data derive from 455 human milk samples (i.e. those of Pakistan excluded, see Table 1). Open bars indicate CV_{biol}. Closed bars indicate CV_{anal}. The Figure indicates that most of the observed variance is caused by biological variation, with relatively little contribution from analytical variation.between FA that are exclusively synthesized in the mammary gland (12:0, 14:0) on the one hand and those from extra-mammary gland synthesis and adipose tissue stores (16:0, 18:0 and 18:1ω9) on the other hand.

20:3ω9. Correction for CV_{anal} showed that CV_{biol} is genuinely high, ranging from 12.7% for 16:0 and 18.9% for 18:1ω9 to 68% for 22:6ω3 and about 100% for 20:5ω3. The human milk 16:0 and 18:1ω9 contents seem to be the most constant of all FAs, possibly because of their major derivation from adipose tissue [44]. It should be noted that the ranges of these FAs are nevertheless large. When all data are taken together (Table 1) 16:0 ranged from 12.68-29.21%, with an even higher extreme in the milk of Pakistani women (i.e. 34.36%), whereas 18:1ω9 ranged from 7.17-40.05%, with data from Jerusalem showing the highest values. The very high CV_{biol} of 20:5ω3 and 22:6ω3 are somewhat artificial, since the distributions of these FAs are skewed to the right. Correction for non-Gaussian distribution proved however impossible by lack of the original quality control data. It is well known that ingestion of fish oil rapidly augments the 20:5ω3 and 22:6ω3 contents of human milk [27], which conceivably makes them subject to both large inter- and intra-individual variation. The majority of FAs (n=15) showed a CV_{biol} ranging from 25 to 40%. The rather high CV_{biol} is notably remarkable for 20:4ω6 (28.0%). Supplementation of (omnivorous) lactating mothers with 300 mg 20:4ω6 for 1 week did not augment their milk 20:4ω6 in our previous study [32], and omnivores, vegans and vegetarians have comparable milk 20:4ω6 contents [31,33]. Chen *et al*. [29] have suggested that the milk 20:4ω6 content may partially depend on *trans*-FA intakes, but it is unlikely that these FAs are at the basis of the

comparably high 20:4ω6 variation in countries such as Tanzania (Table 1). It seems that the major factors that determine the milk 20:4ω6 content remain as yet unclear.

The various regions showed remarkable similarity in the calculated CV_{biol} for the various FAs (data not shown). The CV_{biol} of The Netherlands was the lowest for 17 of the 28 analyzed FAs, probably indicating low inter-individual variance in dietary habits. The Caribbean Region showed the highest CV_{biol} for 22:6ω3, suggesting large differences in fish intake between the various countries within this region. Milk from Tanzania showed a high CV_{biol} for 12:0, 14:0, 16:0, 18:0 and 18:1ω9. This probably reflects competition between FA that are exclusively synthesized in the mammary gland (12:0, 14:0) on the one hand and those from extra-mammary gland synthesis and adipose tissue stores (16:0, 18:0 and 18:1ω9) on the other hand. It has previously been shown that a high carbohydrate intake leads within 8 hours to an increase of the milk MCSAFA contents, at the expense of 18:0, 18:1ω9 and to a lesser extent 16:0 [45]. The milk from Pakistani women had the highest CV_{biol} for almost all ω6 FAs. This could be due to high inter-individual variation in the low amounts of vegetable oils consumed by these women. Taken together it seems that the highest CV_{biol} is encountered for those milk FAs that are affected by short-term dietary intakes (such as 20:5ω3 and 22:6ω3 from fish and fish oil and MCSAFA from carbohydrates). The resulting high intra-individual variation of these FAs might be an important factor in their encountered high inter-individual variation.

We conclude that the CV_{anal} of the human milk FA composition is low compared with the genuine inter-individual CV_{biol}. The largest CV_{biol} was observed for the short-term dietary dependent 20:5ω3 and 22:6ω3, and the smallest for those that derive mainly from adipose tissue (16:0 and 18:1ω9). In view of the large CV_{biol} and the many dietary changes in recent history it seems impossible to consider the present human milk FA composition as the 'gold standard' for infant formula manufacturing. The optimal human milk FA composition should rather derive from populations that consume traditional diets consistent with our genetic background. A more practical and objective approach is to base it on scientific data that show the functions of the individual FAs in neonatal development.

References

1. Bernt KM, Walker WA. Human milk as a carrier of biochemical messages. Acta Paediatr Suppl 1999;430:27-41
2. Uauy R, De Andraca I. Human milk and breastfeeding for optimal mental development. J Nutr 1995;125:S2278-80
3. Hall B. Uniformity of human milk. Am J Clin Nutr 1979;32:304-12
4. Koletzko B, Thiel I, Abiodun PO. The fatty acid composition of human milk in Europe and Africa. J Pediatr 1992;120:S62-70
5. Jensen RG. Lipids in human milk. Lipids 1999;34:1243-71
6. Innis SM. Essential fatty acids in growth and development. Prog Lipid Res 1991;30:39-101
7. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129-38
8. Decsi T, Koletzko B. Polyunsaturated fatty acids in infant nutrition. Acta Paediatr Suppl 1994;395:31-7
9. Huisman M, van Beusekom CM, Lanting CI, Nijeboer HJ, Muskiet FAJ, Boersma ER. Triglycerides, fatty acids, sterols, mono- and disaccharides and sugar alcohols in human milk and current types of infant formula milk. Eur J Clin Nutr 1996;50:255-60
10. Birch E, Birch D, Hoffman D, Hale L, Everett M, Uauy R. Breast-feeding and optimal visual development. J Pediatr Ophtalmol Strabismus 1993;30:33-8
11. Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992;339:261-4

12. Agostoni C, Riva E, Trojan S, Bellù R, Giovanni M. Docosahexaenoic acid status and development quotient of healthy term infants. Lancet 1995;346:638
13. Makrides M, Neumann M, Simmer K, Pater J, Gibson R. Are long-chain polyunsaturated fatty acids essential nutrients in infancy? Lancet 1995;345:1463-8
14. Woltil HA, van Beusekom CM, Okken-Beukens M, Schaafsma A, Muskiet FA, Okken A. Development of low-birthweight infants at 19 months of age correlates with early intake and status of long-chain polyunsaturated fatty acids. Prostaglandins Leukot Essent Fatty Acids 1999;61:235-41
15. Simmer K. Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev 2000;2:CD000375
16. SanGiovanni JP, Parra-Cabrera S, Colditz GA, Berkey CS, Dwyer JT. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. Pediatrics 2000;105:1292-8
17. Simmer K. Longchain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database Syst Rev 2000;2:CD000376
18. SanGiovanni JP, Berkey CS, Dwyer JT, Colditz GA. Dietary essential fatty acids, long-chain polyunsaturated fatty acids, and visual resolution acuity in healthy fullterm infants: a systematic review. Early Hum Dev 2000;57:165-88
19. Jensen RG. Lipids in human milk. Composition and fat soluble vitamins. In: Lebenthal E, ed. Textbook of gastroenterology and nutrition in infancy. 2nd ed. New York: Raven Press. 1989:157-208
20. Read WWC, Lutz PG, Tashjian A. Human milk lipids. II. The influence of dietary carbohydrates and fat on the fatty acids of mature milk. Am J Clin Nutr 1965;17:180-3
21. Muskiet FAJ, Offringa PJ, Boersma ER. Lipid content and fatty acid composition of human milk in relation to developing countries. In Boersma ER et al, eds. A holistic approach to perinatal care and the prevention of handicap, pp 294-305. Erven B. van der Kamp Publishers, Groningen. 1988.
22. Harzer G, Dieterich I, Haug M. Effects of the diet on the composition of human milk. Ann Nutr Metab 1984;28:231-9
23. Insull W, Hirsch J, James T, Ahrens EH. The fatty acids of human milk.II. Alterations produced by manipulation of caloric balance and exchange of dietary fats. J Clin Invest 1959;39:443-50
24. Innis SM, Kuhnlein HV. Long-chain n-3 fatty acids in breast milk of Inuit women consuming traditional foods. Early Human Dev 1988;18:185-9
25. Makrides M, Neumann MA, Gibson RA. Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. Eur J Clin Nutr 1996;50:352-7
26. Harris WS, Connor WE, Lindsey S. Will dietary ω-3 fatty acids change the composition of human milk? Am J Clin Nutr 1984;40:780-5
27. Henderson RA, Jensen RG, Lammi-Keefe CJ, Ferris AM, Dardick KR. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 1992;27:901-7
28. Jensen CL, Maude M, Anderson RE, Heird W. Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. Am J Clin Nutr 2000;71(suppl):292S-9S
29. Chen ZY, Kwan KY, Tong KK, Ratnayake WMN, Li HQ, Leung SSF. Breast milk fatty acid composition: A comparative study between Hong Kong and Chongqing Chinese. Lipids 1997;1061-7
30. Sanders TAB, Reddy S. The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant. J Pediatr 1992;120:S71-7
31. Finley DA, Lonnerdal B, Dewey KG, Grivetti LE. Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians. Am J Clin Nutr 1985;41:787-800
32. Smit EN, Koopmann M, Boersma ER, Muskiet FAJ. Effect of supplementation of arachidonic acid (AA) or a combination of AA plus docosahexaenoic acid on breastmilk fatty acid composition. Prostaglandins Leukot Essent Fatty Acids 2000;62:335-40
33. Sanders TAB. Polyunsaturated fatty acids in the food chain in Europe. Am J Clin Nutr 2000;71(suppl):176S-8S
34. Kris-Etherton PM, Shaffer Taylor D, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 2000;71(suppl):179S-88S
35. Sugano M, Hirahara F. Polyunsaturated fatty acids in the food chain in Japan. Am J Clin Nutr 2000;71(suppl):189S-96S
36. Steege G van der, Muskiet FAJ, Martini IA, Hutter NH, Boersma ER. Simultaneous quantification of total medium- and long-chain fatty acids in human milk by capillary gas chromatography with split injection. J Chromatogr Biomed Appl 1987;415:1-11

37. Volmer M, Neiborg G, Muskiet FAJ. Simultaneous capillary gas chromatographic profiling of medium- and long-chain fatty acid methyl esters with split injection. Correction for injection-related discrimination by the 'bracketing' method. J Chromatogr 1988;434:385-94
38. Muskiet FAJ, Doormaal JJ van. Fatty acids, fatty acids containing lipids, and their gas chromatographic analysis. In: Clement RE, ed. Gas chromatograp-hy: Biochemical, biomedical and clinical applications. New York: John Wiley & Sons Inc, 1990:217-43
39. Smit EN, Oelen EA, Seerat E, Muskiet FAJ, Boersma ER. Breastmilk docosahexaenoic acid (DHA) correlates with DHA status of malnourished infants. Arch Dis Child 2000;6:493-4
40. Muskiet FAJ, Doormaal JJ van, Martini IA, Wolthers BG, Slik W van der. Capillary gas chromatographic profiling of total long-chain fatty acids and cholesterol in biological materials. J Chromatogr Biomed Appl 1983;278:231-44
41. Chulei R, Xiaofang L, Hongseng M, Xiulan M, Guizheng L, Gianhong D, et al. Milk composition in women from five different regions of China: The great diversity of milk fatty acids. J Nutr 1995;125:2993-8
42. Thompson BJ, Smith S. Biosynthesis of fatty acids by lactating human breast epithelial cells: An evaluation of the contribution to the overall composition of human milk fat. Pediatr Res 1985;19:139-43
43. Shultz EK. Analytical goals and clinical interpretation of laboratory procedures. In: Burtis CA, Ashwood ER eds. Tietz textbook of clinical chemistry, WB Saunders Co, Philadelphia, second edition 1994, Chapter 14, pp 485-507
44. Hachey DL, Thomas MR, Emken EA, Garza C, Brown-Booth L, Adlof RO, Klein PD. Human lactation: maternal transfer of dietary triglycerides labeled with stable isotopes. J Lipid Res 1987;28:1185-92
45. Read WWC, Lutz PG, Tashjian A. Human milk lipids. III. Short-term effects of dietary carbohydrate and fat. Am J Clin Nutr 1965;17:184-7

3.4. Fatty acids in formulas for term infants. Compliance of present recommendations with the actual human milk fatty acid composition of geographically different populations

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Submitted

Abstract

Background: Recommendations for formula fatty acids (FA) are largely based on the mature human milk FA composition.
Objective: We investigated whether current recommendations for formula FA for term infants comply with the actual breastmilk FA composition of geographically distinct populations. To provide more realistic grounds for future recommendations we also investigated human milk FA interrelationships.
Design: We collected 455 mature breastmilk samples in different countries over the past 25 years. Recommendations of different organizations were projected on their FA data. FA interrelationships were calculated with Spearman rank tests.
Results: Many samples from non-western communities did not meet the recommendations for formula 12:0, 14:0 and 18:2ω6, since these are mainly based on breastmilk of mothers living in western countries. Recommendations for 18:3ω3 and 18:2ω6/18:3ω3 were not met by many milk samples from distinct populations either, probably because addition of 18:3ω3 aims partially at compensation for the lack of 22:6ω3. Striking discrepancies were observed for 20:4ω6 and 22:6ω3, which may relate to the as yet poorly developed recommendations for long chain polyunsaturated FA. A selection of investigated breastmilk FA (12:0, 14:0, 16:0, 18:0, 18:3ω3, 22:6ω3, 18:2ω6, 20:4ω6, 18:1ω9) were either positively or negatively interrelated.
Conclusion: Future recommendations, if based on human milk, may preferably derive from its FA balance, as indicated by the FA interrelationships. A 'humanized' formula FA composition would in this sense be any composition that cannot be distinguished from that of breastmilk by techniques such as principal component analysis.

3.4.1. Introduction

Human milk is the single best food for babies and young infants [1]. Its superiority relates to many factors, including its specific fatty acid (FA) composition. The FA composition of human milk is, however, strongly dependent on maternal diet and to a small extent affected

by other factors like time postpartum, gestational age, parity and certain diseases [2]. Together these, and probably other, factors give rise to high inter-individual biological variation [3]. Most national and international authorities have based their recommendations for the manufacturing of infant formulas on mature human milk FA composition as the gold standard [4]. Possibly because of lack of additional information, these 'standards' are mostly based on milk obtained from mothers with Caucasian ethnicity, consuming typically western diets. For instance, based on human milk data derived from Sweden, Germany and the United Kingdom, the ESPGHAN recommends a formula linoleic acid/α-linolenic acid (18:2ω6/18:3ω3) ratio around 10 g/g, with a range of 5-15 [5]. Yet there are several reasons why not only milk from western women should be taken into consideration. First, at present there is no scientific evidence that the composition of breastmilk obtained from Caucasian women is superior to that of other ethnic populations. On the contrary, motor development of breastfed black children from African descent is often precocious as compared to Caucasian breastfed children raised in Western countries [6]. Second, there is growing concern regarding the tremendous changes in the intakes of total fat, ω6 and ω3FA and *trans*-FA over the past 100-150 years in western countries [7], which may each have influenced the present western human milk FA composition [2]. On the other hand, due to increased migration, many non-Caucasian ethnic populations (with their typical dietary patterns) have settled in the USA and Europe, and many Europeans and North-Americans have included food from Asian, African and South-American countries into their diets.

Next to human milk, recommendations may also be based on estimated requirements or proposed potential beneficial effects of supplementation of certain FA. The minimal requirement of 18:2ω6 is estimated at 1% of energy, which together with a safety margin has resulted in a recommendation of a minimum of 11 g% 18:2ω6. A maximum intake to prevent untoward effects was set by ESPGHAN at 20 g% [5]. Another expert panel provided a wider margin for 18:2ω6 with a minimum of 8 g% and a maximum of 35 g% of total FA [8]. The latter recommendations were based on the large range in human milk samples and the observation that no adverse effects have been reported of formulas with LA beyond 35% as used in the past. There is good evidence from randomized controlled trials that enrichment of formula with long-chain polyunsaturated FA (LCP), notably 22:6ω3, improves early cognitive and visual development, especially in preterm and possibly also in term newborns (reviewed in references 9-11). Although, these effects seem to be transient, they have nevertheless been the basis for recommendations by several authorities to add 22:6ω3 and 20:4ω6 to infant formula for preterm and also term infants [12-15]. Others were more hesitant to include LCP in formula for term children and advised addition of LCP, but not beyond 1 and 2 g% for 22:6ω3 and 20:4ω6, respectively [5,16,17]. The American Life Sciences Research Office (LSRO) did not recommend addition of 22:6ω3 and 20:4ω6 to infant formulas for term newborns in 1998, but wished to reassess their point of view within 5 years when the results of more randomized controlled trials will be available [8].

We were interested to investigate whether current recommendations for term infant formula FA composition comply with that of human milk obtained from different populations living in a wide range of industrialized and non-industrialized countries. For this purpose we projected these recommendations on a human milk FA data set that we have compiled over the past 25 years. To provide more realistic grounds for future recommendations based on FA balance we also investigated the interrelationships between various milk FA.

3.4.2. Materials and methods

3.4.2.1 *Milk samples*

A total of 455 mature breast milk samples were collected over the past 25 years. The countries and places from which these samples derived were: The Netherlands (n=222), Antigua (n=23), Belize (n=10), Curaçao (n=47), Dominica (n=17), St. Lucia (n=12), St. Vincent (n=30), Surinam (n=20), Jerusalem (n=63) and Tanzania (n=11). The samples from Antigua, Belize, Curaçao, Dominica, St. Lucia, St. Vincent and Surinam (n=159) will collectively be referred to as deriving from the 'Caribbean Region'. Data on the milk FA compositions in The Netherlands [18], Tanzania and the Caribbean Region [19] and Jerusalem [20] have been published before. The Dutch women were of Caucasian origin and those from the Caribbean region were of African origin with eating habits varying between western and traditional diets. The Jerusalem women were of Middle-Eastern origin (Palestinians) and those from Tanzania of African origin. The latter two consumed local traditional diets.

The original data were calculated in mol%, but were recalculated to g% for the present study. Milk from Dutch mothers derived from 24-hour samples that were collected by vacuum pump. Ten percent was kept for analyses and the remainder was administered to the baby by bottle or preferably by cup. The milk samples from all other women were midstream samples of about 5 ml that were collected by manual expression. The milk samples were immediately put in a refrigerator and then stored at -20^{0}C. Samples from outside The Netherlands were transported to The Netherlands in dry ice. All FA compositions of the milk samples were analyzed by capillary gas chromatography with split injection and flame-ionization detection in the same laboratory [21].

3.4.2.2 *Recommendations*

To standardize current recommendations and to enable their comparison with human milk FA data we recalculated the recommendations, if necessary, into g% of total FA. The following recommendations for the formula FA composition were investigated (see Table 1):

EU Commission directive, 1991 [22]: 12:0 ≤15 g% and 14:0 ≤15 g%, 18:2ω6 ≥9 g% but ≤19 g%, as calculated from a minimum fat content of 3.3 and a maximum of 6.5 g/100 kcal and a 300-1200 mg 18:2ω6 intake from 100 kcal.

EU Commission directive (amendment), 1996 [17]: LCPω3 ≤1 g% and LCPω6 ≤2 g%, 20:5ω3 ≤22:6ω3.

ESPGHAN, 1991 [5]: 18:2ω6 ≥11.4 g% but ≤20 g%, as calculated from a minimum fat content of 4.4 and maximum of 6.0 g/100 kcal and a 500-1200 mg 18:2ω6 intake from 100 kcal. LCPω3 ≤1 g% and LCPω6 ≤2 g%, 18:2ω6/18:3ω3 ≥5 g/g but ≤15 g/g.

FAO/WHO, 1994 [13]: 18:2ω6 ≥11.4 g%, 18:3ω3 ≥1.0 g%, 20:4ω6 ≥0.8 g%, 22:6ω3 ≥0.4 g%, as calculated from 35 g fat/L, an intake of 150 ml/kg bodyweight and 600 mg 18:2ω6, 50 mg 18:3ω3, 40 mg 20:4ω6 and 20 mg 22:6ω3 per kg bodyweight.

UK Statutory Instruments (amendment), 1997 [16]: 18:3ω3 ≥1.5 g%, as calculated from a minimum fat content of 3.3 g/100 kcal (taken from ref. 22) and the recommendation of 50 mg/100 kcal. 20:4ω6 ≤1 g%, LCPω3 ≤1 g% and LCPω6 ≤2 g%, 20:5ω3 ≤22:6ω3,

Table 1. Prevalence (%) of human milk samples with FA compositions beyond recommendations

Commission	Recommendation	Netherlands n=222	Caribbean n=159	Jerusalem n=63	Tanzania n=11	All n=455
CD91	12:0<=15g %	0.0	20.8	0.0	54.5	8.6
CD91	14:0<=15g%	0.0	17.0	1.6	36.4	7.0
LSRO	18:2ω6=>8g%	2.3	10.7	0.0	18.2	5.3
CD91	18:2ω6=>9g%	8.1	16.4	0.0	18.2	10.1
GR, WS	18:2ω6=>10g%	15.3	24.5	0.0	27.3	16.7
FAO, ESP	18:2ω6=>11g%	24.3	35.8	0.0	27.3	25.1
CD91	18:2ω6<=19g%	12.2	8.2	44.4	9.1	15.2
ESP	18:2ω6<=20g%	8.6	4.4	41.3	9.1	11.6
LSRO	18:2ω6<=35g%	0.0	0.0	0.0	0.0	0.0
FAO	18:3ω3=>1.0g%	36.9	80.4	49.2	72.7	51.7
GR	18:3ω3=>1.4g%	80.2	96.4	76.2	81.8	84.1
SI, WS	18:3ω3=>1.5g%	85.6	96.4	87.3	90.9	89.0
LSRO	18:3ω3=>1.75g%	93.2	98.2	95.2	90.9	94.9
LSRO	18:3ω3<=4g%	0.0	0.0	0.0	0.0	0.0
SI, ESP	18:2ω6/18:3ω3=>5g/g	0.5	0.0	0.0	0.0	0.2
LSRO	18:2ω6/18:3ω3=>6g/g	0.9	0.0	0.0	0.0	0.5
SI, ESP	18:2ω6/18:3ω3<=15g/g	25.2	45.5	73.0	36.4	38.5
LSRO	18:2ω6/18:3ω3<=16g/g	19.4	33.9	58.7	27.3	29.9
CHF	20:4ω6=>0.35g%	14.0	2.5	1.6	0.0	7.9
WS	20:4ω6=>0.5g%	80.6	21.4	23.8	27.3	50.8
GR	20:4ω6=>0.6g%	96.8	49.1	63.5	54.5	74.5
FAO	20:4ω6=>0.8g%	100.0	89.9	95.2	90.9	95.6
SI	20:4ω6<=1g%	0.0	1.9	0.0	0.0	0.7
CHF	22:6ω3=>0.2g%	29.7	5.0	44.4	27.3	23.1
WS	22:6ω3=>0.35g%	86.9	32.1	95.2	72.7	68.6
FAO, GR	22:6ω3=>0.4g%	92.3	43.4	96.8	81.8	75.6
WS	20:5ω3<0.10g%	8.6	11.3	6.3	18.2	11.4
CD96, SI	20:5ω3<22:6ω3	0.0	0.0	0.0	0.0	0.0
SI, ESP, CD96	LCPω6<=2g%	0.0	4.4	1.6	0.0	1.8
SI, ESP, CD96	LCPω3<=1g%	1.8	13.8	0.0	0.0	5.7

Note: 18:3ω3 was not investigated in 47 of the 159 Caribbean samples.
Abbreviations: CD91, Commission Directive 1991 [22]; LSRO, Life Science Research Office [8]; GR, Health council of the Netherlands (Gezondheidsraad) [14]; WS, Workshop Statement [12]; FAO, Food and Agriculture Organization of the United Nations [13]; ESP, ESPGHAN [5]; SI, Statutory Instruments [16]; CHF, Child Health Foundation [15]; CD96, Commission Directive 1996 [17].

18:2ω6/18:3ω3 ≥5 g/g but ≤15 g/g.
LSRO, 1998 [8]: 18:2ω6 ≥8 g% but ≤35 g%, 18:3ω3 ≥1.75 g% but ≤4 g%. With a fat content between 4.4-6.4 g/100 kcal this corresponds with 350-2240 mg/100 kcal 18:2ω6, 77-256 mg/100 kcal 18:3ω3. 18:2ω6/18:3ω3 ≥6 g/g but ≤16 g/g.

Workshop Statement, 2000 [12]: 18:2ω6 ≥10.0 g%, 18:3ω3 ≥1.50 g%, 20:4ω6 ≥0.50 g%, 22:6ω3 ≥0.35, 20:5ω3 <0.10 g%.
Health Council of the Netherlands (Gezondheidsraad), 2001 [14]: 18:2ω6 ≥10 g%, 18:3ω3 ≥1.4 g%, 20:4ω6 ≥0.6 g%, 22:6ω3 ≥0.4 g%, as calculated from an estimated FA intake of 600 mg 18:2ω6, 80 mg 18:3ω3, 40 mg 20:4ω6 and 20 mg 22:6ω3 per kg bodyweight.
Child Health Foundation, 2001 [15]: 20:4ω6 ≥0.35 g%, 22:6ω3 ≥0.2 g%.

3.4.2.3 *Fatty acid composition of commercially available formulas*

The FA compositions, recalculated to g%, of ten previously investigated infant formulas for term infants [18] were used for comparison with current recommendations and the FA compositions of human milk.

3.4.2.4 *Data evaluation*

Recommendations regarding infant formula contents of 12:0, 14:0, 18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3, 22:6ω3, LCPω6 and LCPω3, and the 18:2ω6/18:3ω3 and 20:5ω3/22:6ω3 ratios were investigated. The number of human milk samples with contents beyond recommended amounts were calculated. The outcome was expressed as a percentage of all samples within a region, and as percentages of all samples. FA interrelationships were calculated with Spearman rank tests, using SPSS (SPSS 8.0 for Windows, SPSS Inc, Chicago, IL, USA).

3.4.3. Results

3.4.3.1 *Prevalence of human milk samples with FA contents beyond formula recommendations*

Table 1 shows the percentages of human milk samples with FA contents beyond those recommended for formula. The magnitudes of the deviations for 12:0, 14:0, 18:2ω6, 18:3ω3 and 18:2ω6/18:3ω3 may be derived from Figure 1. It was found that 12:0 and 14:0 exceeded the ≤15 g% recommendation in about 21 and 17% of the milk samples from the Caribbean and in about 55 and 36% of those from Tanzania, respectively. The lowest prevalence of 12:0 >15 g% within the Caribbean amounted to 9% in Curaçao and the highest to 41% for Dominica (data not shown). The milk 18:2ω6 content was below 8 g% in only 2% of the Dutch human milk samples, but in about 11 and 18% of the samples from the Caribbean and Tanzania, respectively. The prevalence of 18:2ω6 <8 g% in the Caribbean region ranged from 0% in Curaçao to 50% in St. Lucia (not shown). Virtually none of the human milk samples met the 18:3ω3 ≥1.75 g% criterion of the LSRO and 30% of all investigated human milk samples had 18:2ω6/18:3ω3 >16 g/g. Only 3 samples (0.7%) contained more than 1 g% 20:4ω6, while on the other hand only 4% met the 20:4ω6 ≥0.8 g% criterion of the FAO. Around 24% reached the highest recommendation for 22:6ω3 (i.e. ≥0.4 g%), while 77% met the 22:6ω3 ≥0.2 g% criterion. All human milk samples exhibited 20:5ω3/22:6ω3 ratios below 1, but 11% had 20:5ω3 ≥0.10 g%. Around 2 and 6% of the milk samples exceeded the criteria of LCPω6 ≤2 g% and LCPω3 ≤1 g%, respectively.

Table 2. Correlations between selected fatty acids in breastmilk

Fatty acid		12:0	14:0	16:0	18:0	18:3ω3	22:6ω3	18:2ω6	20:4ω6
14:0	r	0.896							
	P	0.0001							
16:0	r	-0.410	-0.159						
	P	0.0001	0.001						
18:0	r	-0.544	-0.364	0.558					
	P	0.0001	0.0001	0.0001					
18:3ω3	r	-0.403	-0.394	-0.097	0.244				
	P	0.0001	0.0001	0.050	0.0001				
22:6ω3	r	0.364	0.356	0.018	-0.190	-0.205			
	P	0.0001	0.0001	0.698	0.0001	0.0001			
18:2ω6	r	-0.329	-0.507	-0.494	-0.149	0.433	-0.288		
	P	0.0001	0.0001	0.0001	0.001	0.0001	0.0001		
20:4ω6	r	0.227	0.107	-0.242	-0.412	-0.229	0.486	0.155	
	P	0.0001	0.023	0.0001	0.0001	0.0001	0.0001	0.001	
18:1ω9	r	-0.779	-0.756	0.219	0.348	0.212	-0.407	0.143	-0.216
	P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.002	0.0001

Spearman's correlation coefficient for 455 mature human milk samples from The Netherlands (n=222), The Caribbean Region (n=159), Jerusalem (n=63), and Tanzania (n=11).

3.4.3.2 *Milk fatty acid interrelationships*

Correlation coefficients for the association between selected milk FA of the whole data set are shown in Table 2. These FA were chosen because of either their quantitative or presumed qualitative importance in breastmilk. Correlations between all 28 analyzed FA are available on request. The strongest positive correlation was observed between 12:0 and 14:0 (r=0.896, P=0.0001), whereas the strongest negative correlation was observed between 12:0 and 18:1ω9 (r=-0.779, P=0.0001). The two FA that did not correlate with each were 16:0 and 22:6ω3 (r=0.018, P=0.698).

Figure 1 shows the relationship between 18:2ω6 and 18:3ω3, 12:0 and 14:0, 18:2ω6 and 16:0, and 18:1ω9 and 12:0. The data derive from all investigated human milk samples. The FA contents of ten infant formulas are also indicated. Although six out of ten investigated infant formulas had 12:0 and 14:0 within the recommendations, only one proved in agreement with the genuine physiological relationship between human milk 12:0 and 14:0. Also for the relationships between 18:2ω6/18:3ω3, 18:2ω6/16:0 and 18:1ω9/12:0, it was observed that several formulas had compositions that were not in line with human milk when considered from a two-dimensional point of view.

3.4.4. Discussion

We investigated whether current recommendations for the formula FA composition comply with the FA composition of a large data set of human milk samples derived from geographically distinct populations. The aim of our study is not to disqualify human milk

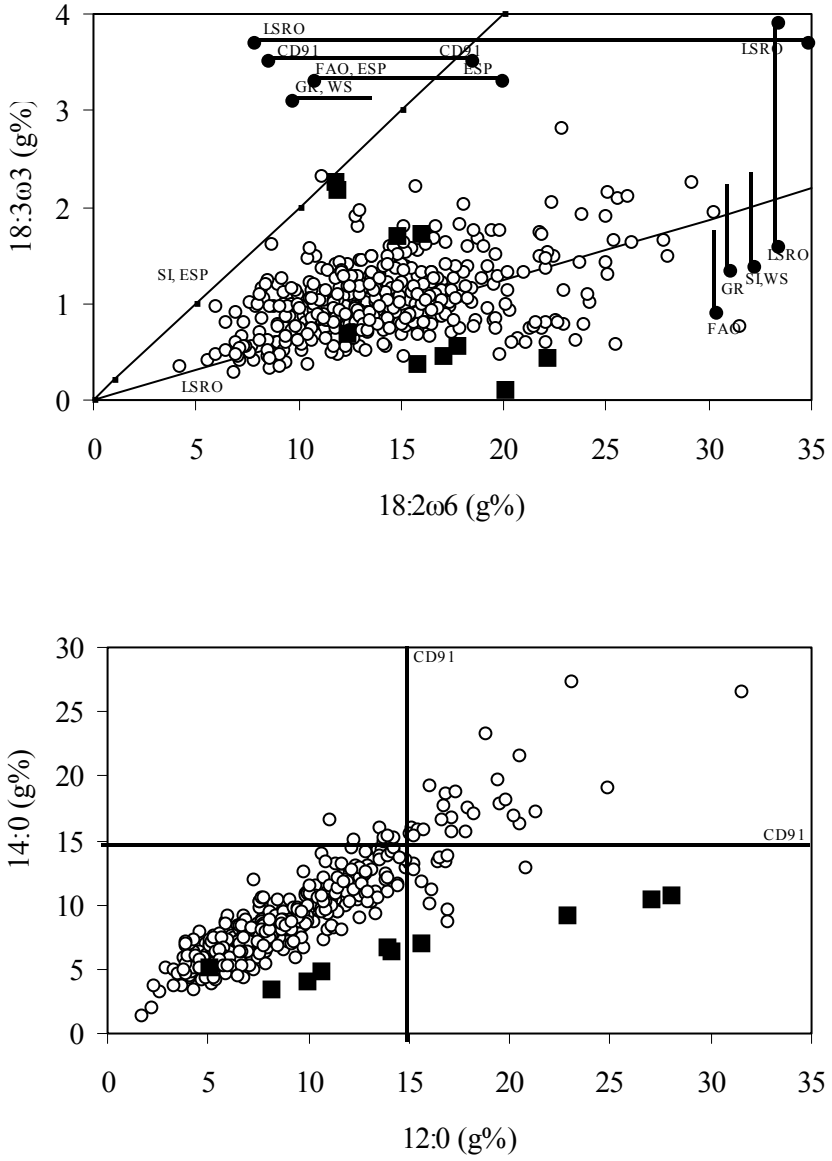
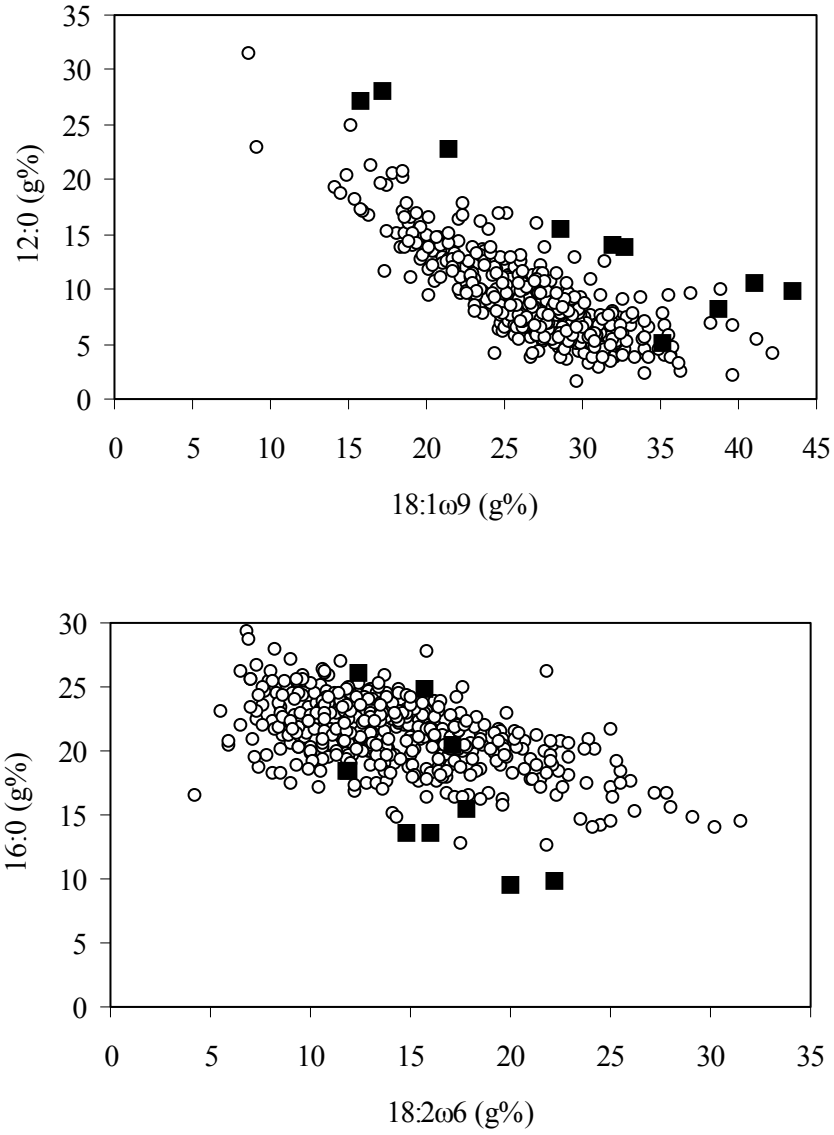


Figure 1: Relations between selected fatty acids in human milk and formula. ○, human milk (n=455); ■ formula (n=10). Recommendations for the 18:2ω6 lower limit vary between 8 and 11 g%, and for the upper limit between 19 and 35 g%. The 18:3ω3 lower limit recommendation varies between 1.0 and 1.75 g% and the upper limit is 4 g%. The lowest and the highest limits for the 182ω6/18:3ω3 ratio are 5 g/g and 16 g/g respectively. Recommendations for 12:0 and 14:0 are ≤15 g%. For abbreviations and detailed recommendations see Table 1.



<u>Figure 1 (continued)</u>: Relations between selected fatty acids in human milk and formula. ○, human milk (n=455); ■ formula (n=10). Recommendations for the 18:2ω6 lower limit vary between 8 and 11 g%, and for the upper limit between 19 and 35 g%. The 18:3ω3 lower limit recommendation varies between 1.0 and 1.75 g% and the upper limit is 4 g%. The lowest and the highest limits for the 182ω6/18:3ω3 ratio are 5 g/g and 16 g/g respectively. Recommendations for 12:0 and 14:0 are ≤15 g%. For abbreviations and detailed recommendations see Table 1.

on the basis of its FA composition, nor to disqualify current recommendations, but rather to point at discrepancies and their possible causes. By emphasizing the interrelationships between human milk FA, we propose a more integrated approach that might be of use for future recommendations of the formula FA composition.

Our data showed that especially breastmilk from women living in non-western communities did not meet the recommendations for 12:0, 14:0 and 18:2ω6 (Table 1). This is not surprising in view of the fact that current recommendations for formula FA composition are mainly based on milk from Caucasian mothers consuming predominantly typically western diets. The discrepancies are probably caused by the higher intakes of carbohydrates by women eating non-western diets, which are known to increase mammary gland *de novo* synthesis of 12:0 and 14:0 [23]. These dietary habits are apparently associated with lower milk 18:2ω6 contents, as illustrated by the inverse relations between 12:0, 14:0 on the one hand and 18:2ω6 on the other (Table 2).

Another discrepancy between recommendations and actual milk FA composition regards the 18:3ω3 content. A high percentage of human milk samples did not reach the 18:3ω3 ≥1.75 g% and 18:2ω6/18:3ω3 ≤15 g/g criteria, and even the less stringent recommendations of 18:3ω3 ≥1.0 g% and 18:2ω6/18:3ω3 ≤16 g/g were met by no more than 48 and 70% of all investigated samples, respectively. A high 18:3ω3 content and a low 18:2ω6/18:3ω3 ratio stimulates, up to a certain extent, the synthesis of LCPω3 in newborns [24]. In contrast to many formulas, human milk contains LCPω3 and breastfed babies have consequently better LCPω3 status, compared to babies receiving formula without LCPω3 [25,26]. It implies that, from the point of view of 'balance', there might be a need in the future to define some relationships between 18:2ω6, 18:3ω3 and their long chain metabolites, notably for those formulas containing each of these FA.

The most striking discrepancies were observed for 20:4ω6 and 22:6ω3. These are caused by the wide differences in present recommendations. The LSRO does not advice addition of 20:4ω6 and 22:6ω3 [8], whereas the FAO, the International Workshop on the essentiality of and recommended dietary intakes for ω6 and ω3 FA, and the Health Council of the Netherlands recommend rather high amounts [12-14]. For 20:4ω6, the upper limit of 1 g% as set by the UK Statutory Instrument [16] is close to the lower limit of 0.8 g% issued by the FAO [13]. The FAO criteria for 20:4ω6 (i.e. ≥0.8 g%) and 22:6ω3 (≥0.4 g%) were met by only 4 and 24% of all milk samples, respectively. The observation that fish intake increases milk 22:6ω3 [27,28], is e.g. reflected in the samples of Caribbean women. Especially women from the island of Dominica had high milk 22:6ω3 (up to 2.1 g%), which by far exceed the UK Statutory Instrument recommendation of LCPω3 ≤1 g%. On the other hand, about 10% of the Dutch and Palestinian women had milk 22:6ω3 as low as 0.1 g%, probably reflecting low fish intake by these women. In contrast to 22:6ω3, milk 20:4ω6 seems almost unaffected by either short-term or long-term dietary changes [28-30]. We found both the lowest and the highest milk 20:4ω6 (0.3 and 1.1 g%, respectively) in the Caribbean women (data not shown). Recently the Child Health Foundation [15] advised amounts of ≥0.35 g% for 20:4ω6 and ≥0.2 g% for 22:6ω3, which are around half of the amounts as recommended by the FAO and the International Workshop [12,13]. It seems that there is at present little agreement on the recommended 20:4ω6 and 22:6ω3 contents. As suggested previously, future recommendations might be based on the human milk

balance of parent essential FA and their metabolites or, on randomized controlled trials that show benefits of the investigated compositions on e.g. growth and neurodevelopment of formula fed infants [4,11].

The breastmilk FA composition differs between various populations and these differences are probably mainly on account of different diets. We therefore suggest using interrelationships between human milk FA as the basis for future recommendations, since they reflect the naturally occurring physiological balance. Table 2 shows that almost all of the qualitatively and quantitatively most important FA were either positively or negatively related. This is partially caused by a, so-called, closure effect (i.e. all FA add up to 100%). It may however be proposed that a humanized formula FA composition would be any composition that cannot be distinguished from that of human milk on the basis of this FA balance. Examples of non-balanced FA compositions in this sense are depicted in Figure 1, which shows that many formulas complied with recommendations in a univariate sense, but not so when studied in a two-dimensional model. Proof of balance would become more complicated if all current 28 milk FA are to be taken into account simultaneously. The solution might be the construction of a multivariate model in which the relations between all FA are studied simultaneously by means of e.g. principal component analysis. But even if the FA composition of formula would resemble that of human milk many other factors will yet remain unaccounted for. Examples are the FA (stereo) chemical *sn*-locations and combinations on the glycerol moieties of either phospholipids or triglycerides, the complex interaction with other components in human milk, and the immunological and psychological aspects of breastfeeding [1,4,31,32].

In conclusion, we have shown that the human milk FA composition of different populations is often beyond FA recommendations for infant formula. These discrepancies are mainly caused by basing FA recommendations on breastmilk of mothers living in western countries (i.e. 12:0, 14:0 and 18:2ω6). Furthermore, current recommendations compensate for the lack of 22:6ω3 by increasing the recommended amounts of 18:3ω3. Finally, recommendations for LCP are poorly developed because of lack of solid long-term evidence. In view of the encountered strong human milk FA interrelationships, it is proposed that future recommendations may also derive from this physiological balance, that reflects the outcome of mammary gland *de novo* synthesis, transport, metabolism, competition and many other complex, mostly genetically determined, biochemical processes. A humanized formula FA composition would in that sense be any composition that cannot be distinguished from that of human milk by techniques such as principal component analysis.

References

1. Bernt KM, Walker WA. Human milk as a carrier of biochemical messages. Acta Paediatr Suppl 1999;430:27-41.
2. Jensen RG. Lipids in human milk. Lipids 1999;34:1243-71.
3. Smit EN, Martini IA, Mulder H, Boersma ER, Muskiet FAJ. Estimated biological variation of the mature human milk fatty acid composition. Prostaglandins Leukot Essent Fatty Acids 2002;"in press".
4. Koletzko B, Sinclair A. Long-Chain polyunsaturated fatty acids in diets for infants: choices for recommendation and regulating bodies and for manufacturing of dietary products. Lipids 1999;43:215-20.
5. ESPGAN committee on nutrition. Committee report. Comment on the content and composition of lipids in infant formulas. Acta Pædiatr Scand 1991;80:887-96.
6. van der Veere CN, Luteyn AJ, Sorhaindo BA, et al. Obstetrical condition and neonatal neurological outcome in Dominica, the Caribbean. A comparative study. Trop Geogr Med. 1992;44:338-45.

7. Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr 1999;70(S):560S-9.
8. Life Sciences Research Office Report, American Society for Nutritional Sciences. Executive summary for the report: Assessment of nutrient requirements for infant formulas. J Nutr 1998;128S:S2059-2293.
9. Simmer K. Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev 2000;(2):CD000375.
10. Simmer K. Longchain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database Syst Rev 2000;(2):CD000376.
11. Uauy R, Mena P. Lipids and neurodevelopment. Nutr Rev 2001;59S:S34-48.
12. Simopoulos AP, Leaf A, Salem Jr N. Workshop statement on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids 2000;63:119-21.
13. FAO/WHO report of a joint expert consultation. Fats and oils in human nutrition. Paper No 57. FAO, Rome, Italy, 1994.
14. Health Council of The Netherlands. Dietary reference intake: energy, proteins, fats and digestible carbohydrates. Publication nr. 2001/19. The Hague, Health Council of The Netherlands, 2001.
15. Koletzko B, Agostoni C, Carlson SE, Clandinin T, et al. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. Report of workshop. Acta Paediatr 2001;90:460-4.
16. UK Statutory Instrument 1997 No. 451. The infant formula and follow-on formula (amendment) regulations 1997. World Wide Web: http://www.hmso.gov.uk/si/si1997/97045101.htm (accessed 10 December 2001).
17. Commission on the European Communities (1996). Commission Directive 94/6/EEC of 16 February 1996 amending directive 91/321/EEC on infant formulae and follow-on formulae. Off J Eur Comm 39(L49):12-6.
18. Huisman M, van Beusekom CM, Lanting CI, Nijeboer HJ, Muskiet FAJ, Boersma ER. Triglycerides, fatty acids, sterols, mono- and disaccharides and sugar alcohols in human milk and current types of infant formula milk. Eur J Clin Nutr 1996;50:255-60.
19. Muskiet FAJ, Offringa PJ, Boersma ER. Lipid content and fatty acid composition of human milk in relation to developing countries. In Boersma ER et al, eds. A holistic approach to perinatal care and the prevention of handicap. Groningen: Erven B. van der Kamp Publishers, 1988:294-305.
20. Smit EN, Koopmann M, Boersma ER, Muskiet FAJ. Effect of supplementation of arachidonic acid (AA) or a combination of AA plus docosahexaenoic acid on breastmilk fatty acid composition. Prostaglandins Leukot Essent Fatty Acids 2000;62:335-40.
21. Steege G van der, Muskiet FAJ, Martini IA, Hutter NH, Boersma ER. Simultaneous quantification of total medium- and long-chain fatty acids in human milk by capillary gas chromatography with split injection. J Chromatogr Biomed Appl 1987;415:1-11.
22. Commission on the European Communities (1991). Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae. Off J Eur Comm L 175, 04/07/1991:0035-49.
23. Read WWC, Lutz PG, Tashjian A. Human milk lipids. II. The influence of dietary carbohydrates and fat on the fatty acids of mature milk. Am J Clin Nutr 1965;17:180-3.
24. Clark KJ, Makrides M, Neumann MA, Gibson RA. Determination of the optimal ratio of linoleic acid to α-linoleic acid in infant formulas. J Pediatr 1992;120:S151-8.
25. Decsi T, Thiel I, Koletzko B. Essential fatty acids in full term infants fed breast milk or formula. Arch Dis Child 1995;72:F23-8.
26. Sanders TAB, Naismith DJ. A Comparison of the influence of breast-feeding and bottle-feeding on the fatty acid composition of the erythrocytes. Br J Nutr 1979;41:619-23.
27. Innis SM, Kuhnlein HV. Long-chain n-3 fatty acids in breast milk of Inuit women consuming traditional foods. Early Human Dev 1988;18:185-9.
28. Koletzko B, Thiel I, Abiodun PO. The fatty acid composition of human milk in Europe and Africa. J Pediatr 1992;120:S62-70.
29. Chen ZY, Kwan KY, Tong KK, Ratnayake WMN, Li HQ, Leung SSF. Breast milk fatty acid composition: A comparative study between Hong Kong and Chongqing Chinese. Lipids 1997;32:1061-7.
30. Smit EN, Koopmann M, Boersma ER, Muskiet FAJ. Effect of supplementation of arachidonic acid (AA) or a combination of AA plus docosahexaenoic acid on breastmilk fatty acid composition. Prostaglandins Leukot Essent Fatty Acids 2000;62:335-40.
31. Winter CH, Hoving EB, Muskiet FAJ. Fatty acid composition of human milk triglyceride species. Possible consequences for optimal structures of infant formula triglycerides. J Chromatogr 1993;616:9-24.
32. Uauy R, De Andraca I. Human milk and breastfeeding for optimal mental development. J Nutr 1995;125:S2278-80.

Chapter 4 Biochemical parameters of essential fatty acid deficiency

4.1. Assessment of essential fatty acid and ω3-fatty acid status by measurement of erythrocyte 20:3ω9 (Mead acid), 22:5ω6/22:6ω3 and 22:5ω6/22:4ω6

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Abstract

Background. Early suspicion of essential fatty acid deficiency (EFAD) or ω3-deficiency may rather focus on polyunsaturated fatty acid (PUFA) or long-chain PUFA (LCP) analyses than clinical symptoms. We determined cut-off values for biochemical EFAD and ω3-deficiency by measurement of erythrocyte 20:3ω9 (Mead acid), 22:5ω6/22:6ω3 and 22:5ω6/22:4ω6.
Methods. Cut-off values, based on 97.5 percentiles, derived from an apparently healthy omnivorous group (6 Dominica breast-fed newborns, 32 breast-fed and 27 formula+LCP-fed Dutch low-birth weight infants, 31 Jerusalem infants, 33 Dutch 3.5-years old infants, 69 omnivorous Dutch adults and 7 Dominica mothers) and an apparently healthy group with low dietary LCP intake (81 formula-fed Dutch low-birth weight infants, 12 Dutch vegans). They were validated by their application in an EFAD suspected group of 108, mostly malnourished, Pakistani children, three pediatric patients with chronic fat-malabsorption (abetalipoproteinemia, congenital jejunal and biliary atresia) and one patient with a peroxisomal disorder.
Results. Erythrocyte 20:3ω9, 22:5ω6/22:6ω3 and 22:5ω6/22:4ω6 proved age-dependent up to 0.2 years. Cut-off values for ages above 0.2 years were: 0.46 mol% 20:3ω9 for EFAD, 0.22 mol/mol 22:5ω6/22:6ω3 for ω3-marginality, 0.48 mol/mol 22:5ω6/22:6ω3 for ω3-deficiency and 0.33 mol/mol 22:5ω6/22:4ω6 for low 22:6ω3 precursor status. Increases beyond the 20:3ω9 and 22:5ω6/22:6ω3 cut-off values identified EFAD in 33.3% Pakistani children and 3 pediatric patients, ω3-deficiency in 35.2% Pakistani children and all 4 pediatric patients, and ω3-marginality in 60.2% Pakistani children. Increased 22:5ω6/22:4ω6 might be useful to detect a low status of 22:6ω3-precursors.
Conclusion. Present cut-off values may serve for PUFA supplement intervention until better concepts have emerged.

4.1.1. Introduction

Essential fatty acid (EFA) deficiency (EFAD) is a clinical condition that derives from inadequate status of fatty acids (FA) of both the ω6 and ω3 families. Isolated ω3-deficiency

is recognized as a separate condition next to EFAD, but isolated ω6-deficiency in humans is probably rare. Among the clinical features of EFAD and isolated ω3-deficiency are impaired growth, skin lesions, infertility, kidney abnormalities, fatty liver, polydipsia, increased susceptibility to infections, reduced learning and impaired vision [1-4]. These symptoms are nonspecific and may develop after long standing marginal EFA status [5]. Therefore, present clinical chemical cut-off values for EFAD and isolated ω3-deficiency are mostly based on the detection of disbalances between the ω3, ω6, ω7, ω9 and saturated FA families [6]. Such cut-off values do not necessarily relate to the presence of clinically detectable symptoms or disease development as gold standards, and a biochemical deficiency is therefore not to be confused with a clinically detectable deficiency.

The parent EFA linoleic (18:2ω6) and α-linolenic (18:3ω3) acids, and their long-chain polyunsaturated FA homologues of the ω3 and ω6 series (LCP; ≥C20 with at least 3 double bonds in methylene interrupted cis-configuration) are structural elements of membrane phospholipids and precursors of hydroxy FA and eicosanoids via the lipoxygenase and cyclooxygenase pathways [1,6]. Parent EFA derive exclusively from the diet, whereas LCP derive either from the diet or synthesis from parent EFA. Important food sources are vegetable oils like sunflower (18:2ω6) or soybean (18:2ω6 and 18:3ω3) oils, meat (arachidonic acid, 20:4ω6) and fish (eicosapentaenoic acid, 20:5ω3 and docosahexaenoic acid, 22:6ω3). LCP synthesis from 18:2ω6 and 18:3ω3 occurs by desaturation, chain-elongation and chain-shortening, with Δ6-desaturation as the first and rate-limiting step. Both 18:2ω6 (to 20:4ω6) and 18:3ω3 (to 22:6ω3 via 20:5ω3), but also non-essential oleic acid (18:1ω9) compete for conversion by Δ6-desaturase. This enzyme has preference for its substrates in the order 18:3ω3>18:2ω6>18:1ω9, implying that some combination of low 18:3ω3 and 18:2ω6 status is needed to allow 18:1ω9 to serve as a Δ6-desaturase substrate for the formation of 20:3ω9 (also known as ‘Mead acid’) and 22:3ω9. Mead acid incorporation into tissue lipids is associated with platelet hyperactivity [7], vasoconstriction [8] and altered cell-cell adhesion [9] but its feeding to rats has no adverse effects on health or growth [10].

Both 20:3ω9 and the so-called triene-tetraene (20:3ω9/20:4ω6) ratio are widely used as markers for biochemical EFAD [6,11,12]. The total plasma 20:3ω9/20:4ω6 ratio has for many years been the EFAD 'gold standard'. With time, the upper-limit has been reduced from 0.4 to 0.2 [13]. Mead acid increases prior to a 20:4ω6 decrease, since tissues and notably brain [6] tend to conserve 20:4ω6 at developing EFA deficiency [10,14]. Total plasma may also not be the preferred compartment, since its FA profile derives from at least four different lipid classes, which are located in a variety of lipoproteins with different functions, origins, targets, turnover rates and interindividual compositions. Erythrocyte (RBC) FA contents might provide a more reliable parameter of cellular EFA-status, which reflects bone marrow FA availability and plasma-RBC phospholipid exchange processes of the preceding 2-3 months. RBC FA derive solely from RBC plasma membrane phospholipids, contain the full range of LCP, are well defined with respect to their dietary dependence [6] and relate to the FA composition of brain [15,16]. RBC FA might on the other hand be somewhat dependent on RBC age-distribution [17].

Isolated ω3-deficiency has attracted attention since its recognition in a 6 years old girl who received long-term ω3 FA poor total parental nutrition [18]. Isolated ω3- (or ω6-) deficiency does not necessarily cause augmented 20:3ω9, because of the 18:1ω9

desaturation suppressing effect of the remaining sufficient 18:2ω6 (or 18:3ω3). Establishment of decreased 22:6ω3, or increased 20:4ω6/22:6ω3 [6], 22:5ω6/22:6ω3 [6] or 22:5ω6/22:4ω6 [20] have been advocated for the assessment of isolated ω3-deficiency. Increased 20:4ω6/22:6ω3 and 22:5ω6/22:6ω3 point at disbalances between members of the LCPω6 and LCPω3 families in favor of LCPω6. Increased 22:5ω6/22:4ω6 finds its origin in predominant Δ4-desaturation of 22:4ω6, secondary to an insufficient amount of the competing member of the ω3-family, 22:5ω3.

We established cut-off values, based on 97.5 percentiles, of RBC 20:3ω9 and 22:5ω6/22:6ω3 for assessment of biochemical EFAD and ω3-deficiency and investigated the added value of RBC 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 as parameters of ω3 status. The study population was composed of apparently healthy subgroups of different ages consuming either an LCP-containing omnivorous diet or a low-LCP diet (i.e. formula-fed infants and vegans). The cut-off values were validated by investigating their ability to detect EFAD and ω3-deficiency in a group of mostly malnourished Pakistani infants with known low EFA and/or ω3 status, in 3 patients with chronic fat-malabsorption of various causes, and in one patient with a peroxisomal disorder. The Pakistani data were also used for the comparison of the diagnostic sensitivities of RBC 22:5ω6/22:6ω3, 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 as indices of ω3-status.

4.1.2. **Subjects and Methods**

4.1.2.1 *Study groups*

The RBC FA data of this study are a collection of results from studies conducted by our research group during the past 10 years. The data derived from employment of the same pre-analytical and analytical methods for RBC FA analyses [29,30] in a single laboratory. The selected study population was composed of two apparently healthy groups and two EFAD-suspected groups (Table 1). Details regarding their dietary backgrounds can be obtained from the respective papers.

The apparently healthy subjects were either assigned to an omnivorous group or a group with low dietary LCP intake. The omnivorous group comprised 6 Dominica newborns (studied at two occasions), 32 human milk fed low-birth weight (LBW) Dutch infants (studied at three occasions) and 27 LBW Dutch babies who received formula with LCP (of which 1 was studied at one, 1 at two and 25 at three occasions), 31 Jerusalem infants, 33 Dutch 3.5-years old infants, 69 omnivorous Dutch adults and 7 Dominica mothers, totaling 326 data points. The low-dietary-LCP group comprised 81 LBW Dutch infants who received formula without LCP (studied at three occasions) and 12 Dutch adults consuming a vegan diet, totaling 255 data points. All LBW (≤ 2.50 kg) Dutch babies were born in the Groningen Martini Hospital, The Netherlands. They comprised a group of healthy, predominantly preterm, babies (gestational ages at birth 31-40 weeks; 66% ≤37 weeks) who participated in various studies on the effect of diet (i.e. breastmilk and formula with and without LCP) on LCP status. Their RBC FA were determined around postnatal days 11, 21 and 42 [21-23]. The 7 healthy Dominica mothers and 6 of their healthy babies (cord blood) were studied at delivery. These exclusively breast-fed infants were also investigated in the 20-22 days postnatal period [24]. The group of healthy breast-fed Jerusalem infants (ages 1

Table 1. Erythrocyte fatty acids and ratios.

	n	Age *(years)*	18:2ω6 *(mol%)*	20:4ω6 *(mol%)*	22:4ω6 *(mol%)*	22:5ω6 *(mol%)*	22:6ω3 *(mol%)*	20:3ω9 *(mol%)*	22:5ω6/ 22:6ω3 *(mol/mol)*	20:4ω6/ 22:6ω3 *(mol/mol)*	22:5ω6/ 22:4ω6 *(mol/mol)*
Omnivorous group											
Age <0.2 years	119										
Dominica newborns	6	0.003 (0.003-0.003)	3.38 (3.08-4.05)	14.61 (12.94-16.16)	2.52 (2.33-3.00)	1.01 (0.96-1.26)	5.58 (4.74-5.90)	0.83 (0.59-1.10)	0.19 (0.17-0.26)	2.68 (2.42-3.25)	0.41 (0.36-0.48)
Dutch LBW infants [1]	68	0.03 (0.01-0.04)	6.87 (4.99-8.95)	13.10 (9.61-16.54)	2.90 (2.04-3.81)	1.12 (0.73-1.80)	4.06 (2.32-5.09)	0.60 (0.35-1.27)	0.28 (0.20-0.46)	3.38 (2.53-4.67)	0.38 (0.32-0.53)
Dutch LBW infants [1] + Dominica infants	56 + 6	0.06 (0.05-0.07)	7.84 (5.44-9.97)	12.62 (9.11-16.06)	2.80 (2.08-3.80)	1.05 (0.76-1.70)	4.48 (2.53-5.73)	0.56 (0.27-1.12)	0.25 (0.17-0.44)	2.98 (2.22-4.33)	0.38 (0.30-0.51)
Dutch LBW infants [1] + Jerusalem infants	50 + 12	0.09 (0.08-0.17)	8.62 (6.78-9.99)	13.06 (9.18-14.87)	2.88 (2.15-3.74)	0.99 (0.67-1.55)	4.37 (2.51-5.56)	0.42 (0.25-1.06)	0.23 (0.15-0.43)	2.99 (1.90-3.93)	0.35 (0.27-0.50)
Age >=0.2 years	128										
Jerusalem	19	0.33 (0.21-0.50)	8.37 (6.85-11.50)	14.64 (11.15-16.04)	2.76 (2.25-3.30)	0.78 (0.69-0.97)	4.61 (3.63-5.34)	0.27 (0.15-0.46)	0.17 (0.13-0.22)	3.02 (2.72-3.60)	0.29 (0.24-0.33)
Dutch 3.5 years old infants	33	3.51 (3.48-3.53)	10.10 (8.39-12.95)	14.37 (12.97-15.26)	2.95 (2.26-3.63)	0.66 (0.50-0.84)	2.87 (2.11-3.88)	0.27 (0.17-0.46)	0.23 (0.16-0.37)	4.91 (3.76-7.00)	0.23 (0.17-0.29)
Dutch omnivorous adults + Dominica mothers	69 + 7	34.5 (19-61)	10.23 (7.91-12.99)	13.75 (10.13-15.88)	2.75 (1.77-3.53)	0.51 (0.35-0.72)	3.89 (2.34-6.73)	0.25 (0.13-0.42)	0.13 (0.07-0.22)	3.67 (1.56-6.44)	0.18 (0.14-0.33)
Low-dietary-LCP group											
Age <0.2 years	243										
Dutch LBW infants [2]	81	0.03 (0.02-0.04)	7.56 (6.15-10.27)	13.74 (12.31-15.94)	3.02 (2.35-3.95)	1.19 (0.80-1.58)	3.91 (2.97-5.06)	0.69 (0.34-1.29)	0.31 (0.18-0.47)	3.59 (2.60-4.76)	0.39 (0.30-0.51)
Dutch LBW infants [2]	81	0.05 (0.05-0.07)	9.10 (7.90-11.38)	12.58 (11.11-14.53)	2.91 (2.26-3.88)	1.13 (0.77-1.46)	3.65 (2.73-4.63)	0.61 (0.34-1.11)	0.32 (0.18-0.47)	3.55 (2.63-4.80)	0.39 (0.27-0.50)
Dutch LBW infants [2]	81	0.11 (0.10-0.14)	10.21 (9.18-12.85)	11.77 (10.29-13.97)	2.88 (2.39-3.67)	1.04 (0.73-1.30)	3.07 (2.29-3.84)	0.51 (0.29-0.81)	0.34 (0.21-0.48)	4.00 (2.95-5.12)	0.35 (0.27-0.45)
Age >=0.2 years	12										
Dutch vegan adults	12	34.5 (25-57)	11.20 (10.19-15.36)	14.22 (12.55-16.16)	1.59 (0.65-2.80)	0.54 (0.32-0.72)	1.59 (1.06-3.46)	0.24 (0.15-0.32)	0.30 (0.10-0.50)[3]	8.82 (3.83-11.25)[3]	0.31 (0.17-0.92)[3]
EFAD suspected group											
Age >=0.2 years											
Pakistani infants	108	1.25	8.66	14.38	2.89	1.03	2.47	0.39	0.43	5.66	0.36

Table 1. Erythrocyte fatty acids and ratios.

	n	Age *(years)*	18:2ω6 *(mol%)*	20:4ω6 *(mol%)*	22:4ω6 *(mol%)*	22:5ω6 *(mol%)*	22:6ω3 *(mol%)*	20:3ω9 *(mol%)*	22:5ω6/ 22:6ω3 *(mol/mol)*	20:4ω6/ 22:6ω3 *(mol/mol)*	22:5ω6/ 22:4ω6 *(mol/mol)*
		(0.21-5.00)	(4.91-12.20)	(11.94-16.38)	(1.91-3.90)	(0.69-1.46)	(1.26-4.00)	(0.19-1.54)	(0.21-0.75)	(3.66-11.69)	(0.25-0.54)
Pediatric patients											
1 Jejunal atresia with apple peel syndrome	1	0.67	4.58	13.12	2.02	1.78	1.35	3.14	1.32	9.72	0.88
2 Abetalipoproteinemia	1	5	2.22	12.34	4.38	1.87	1.43	2.23	1.31	8.63	0.43
3 Biliary atresia	1	0.67	8.70	11.85	3.24	1.23	0.96	0.56	1.28	12.34	0.31
4 Peroxisomal disorder	1	1.5	11.53	12.79	2.65	0.65	0.69	0.35	0.94	18.54	0.25

Ages are medians (range), Erythrocyte fatty acids are medians (2.5-97.5 percentiles). LBW, low-birth-weight; LCP, long chain polyunsaturated fatty acids; EFAD, essential fatty acid deficiency. 1, Human milk or formula+LCP fed infants; 2, formula (without LCP) fed infants; 3, data for n=11 (one outlier excluded). One Pakistani infant aged <0.2 years is not included in this Table. The cut-off value for EFAD was established by taking the mean of the 97.5 percentiles of the Jerusalem infants >=0.2 years and Dutch 3.5 years old infants.Two cut-off values were established for RBC 22:5ω6/22:6ω3, 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6. The first was defined as the border between ω3-sufficiency and ω3-marginality and derived from the mean 97.5 percentiles of the Jerusalem infants and the Dutch omnivorous adults plus Dominica mothers. The second was defined as the border between ω3-marginality and ω3-deficiency and derived from the 97.5 percentile(s) of the entire low-dietary-LCP group (RBC 22:5ω6/22:6ω3) or of the several low-dietary-LCP groups (RBC 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6).

6 months) were recruited from the 'Spafford mother and child health clinic' in East-Jerusalem (unpublished data). The 3.5 years old healthy Dutch children participated in a study on the effect of perinatal exposure to PCB's and dioxins on neurological development [25]. The adult Dutch vegans and 15 of the adult omnivores participated in a study on the LCP status of the vegan diet [26]. The remaining 54 adult Dutch omnivores donated blood for the establishment of RBC FA reference values (unpublished results).

The EFAD and/or ω3-deficient groups were composed of 109 Pakistani infants and 4 pediatric patients. The, mostly malnourished, Pakistani infants (ages 2-60 months) were recruited from the Federal Government Service Hospital in Islamabad [27,28]. They were classified as malnourished according to the Gomez classification, i.e. a weight below 75% of average for age, using the reference data from the United States National Center for Health Statistics. Of these children, 58.7% were breast-fed (70.3% malnourished, 29.7% not malnourished) and 38.5% were formula-fed (85.7% malnourished, 14.3% not malnourished). Nutritional status of 2.8% was unknown. Three pediatric patients suspected of EFAD and/or ω3-deficiency were diagnosed with abetalipoproteinemia (genetically confirmed), jenunal atresia (with apple peel syndrome) and biliary atresia. The other patient was diagnosed with a peroxisomal disorder, characterized by increased plasma 26:0/22:0 ratio, pipecolic acid concentration and phytanic acid.

Approval for the intervention studies with LBW babies was obtained from the medical ethical committee of the Groningen Martini Hospital. The study protocols of the 3.5 years old Dutch children and omnivorous and vegan Dutch adults were approved by the medical ethical committee of the Groningen University Hospital. All study protocols were in agreement with local ethical standards and the Helsinki declaration of 1975, as revised in 2000.

4.1.2.2 *Blood sampling, processing and analyses*

EDTA-anticoagulated blood samples were collected by venepuncture and immediately cooled in melting ice. The samples were centrifuged at 800 g for 10 min in a cooled centrifuge. Plasma and buffy coat were removed and the RBC washed three times with isotonic saline. The RBC were finally suspended to a hematocrit of about 50%. For the analysis of RBC FA, 200 μl of this suspension was transferred to a 15-ml Teflon-stoppered tube, containing 1 mg butylated hydroxytoluene (antioxidant) and 50.0 μg margaric acid (17:0; internal quantification standard). Preserved RBC samples were stored at –20 ^{o}C. The samples from Dominica, Jerusalem and Pakistan were transported to The Netherlands in dry ice. FA methyl esters were prepared by acid-catalyzed transmethylation. They were separated by gas chromatography on an apolar capillary column and detected with a flame ionization detector [29]. The RBC FA composition was calculated by assuming that equal peak areas give rise to equal weight amounts [30]. Data were expressed as mol% (FA composition) or mol/mol (FA ratios). The within-run and day-to-day precisions for 15 RBC FA have been described in detail [29]. They vary characteristically between 1.9-12.0% and 1.1-17.6%, dependent on FA abundance. The between-series precisions (in %) of some of the presently evaluated RBC FA amount to: 3.7 (18:2ω6), 5.3 (20:4ω6), 8.0 (22:5ω3) and 14.9 (22:6ω3).

4.1.2.3 *Mathematical procedures*

RBC FA data of the apparently healthy omnivorous and low-dietary-LCP groups (Table 1) were used for establishment of cut-off values. Age-dependency was investigated by Spearman rank test at $p<0.05$. Cut-off values were defined as the 97.5 percentiles (P97.5), according to recommendations of the International Federation of Clinical Chemistry [31]. The cut-off value for RBC 20:3ω9 served for classification into EFA sufficient (≤P97.5) and EFAD (>P97.5). The RBC 22:5ω6/22:6ω3 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 were used for classification into ω3-sufficient (≤P97.5 of omnivorous group), ω3-marginal (>P97.5 of omnivorous group but ≤P97.5 of low-dietary LCP group) and ω3-deficient (>P97.5 of low-dietary LCP group). These cut-off values were validated in the Pakistani children and 4 pediatric patients by their classification as EFAD+ω3-deficient, EFAD+ω3-marginal, EFAD+ω3-sufficient, EFA-sufficient+ω3-deficient, EFA-sufficient+ω3-marginal or EFA-sufficient+ω3-sufficient.

4.1.3. Results

4.1.3.1 *RBC 20:3ω9 and 22:5ω6/22:6ω3 cut-off values for EFA and ω3-status*

RBC 20:3ω9 of all subjects, Dominica newborns excluded, proved age-dependent ($r=-0.709$, $p<0.0001$, Figure 1 top), but became age-independent from 0.2 years. Since the P97.5 of the four subgroups aged ≥0.2 years seemed age-dependent (Table 1), we decided to take the average of the Jerusalem infants and Dutch 3.5 years old infants to find a value of 0.46 mol% as the upper limit of EFA sufficiency for all subjects aged ≥0.2 years. The RBC 22:5ω6/22:6ω3 of the omnivorous group, Dominica newborns excluded, was age-dependent ($r= -0.668$, $p<0.0001$, Figure 1 bottom). The RBC 22:5ω6/22:6ω3 of the omnivorous group was still age-dependent beyond the age of 0.2 years ($r= -0.546$, $p<0.0001$), but the P97.5 values of the Jerusalem infants and the Dutch omnivorous adults plus Dominica mothers proved remarkably similar (Table 1). Despite the higher P97.5 of the Dutch 3.5 years old infants (see Discussion), we decided to take the average of the Jerusalem infants ≥0.2 years and the Dutch omnivorous adults plus Dominica mothers to find a value of 0.22 mol/mol as the upper limit of ω3-sufficiency for subjects aged ≥0.2 years. The RBC 22:5ω6/22:6ω3 of the low-dietary-LCP group was age-independent (Figure 1 bottom) and its 4 subgroups had similar P97.5 values (Table 1). We therefore decided to average their P97.5 to find a value of 0.48 mol/mol as the upper limit of ω3-marginality for all ages.

4.1.3.2 *Validation of RBC 20:3ω9 and 22:5ω6/22:6ω3 cut-off values*

The 0.46 mol% RBC 20:3ω9 cut-off value for EFAD and the 0.22 and 0.48 mol/mol 22:5ω6/22:6ω3 cut-off values for ω3-marginality and ω3-deficiency were evaluated by investigating their influence on the classification of all subjects aged ≥0.2 years, i.e. 140 apparently healthy controls, 108 of the 109 malnourished Pakistani children and the four pediatric patients (Figure 2). Because of the definition of the cut-off values at a P97.5, it is obvious that 2.5 percent of several apparently healthy subgroups were classified as EFAD,

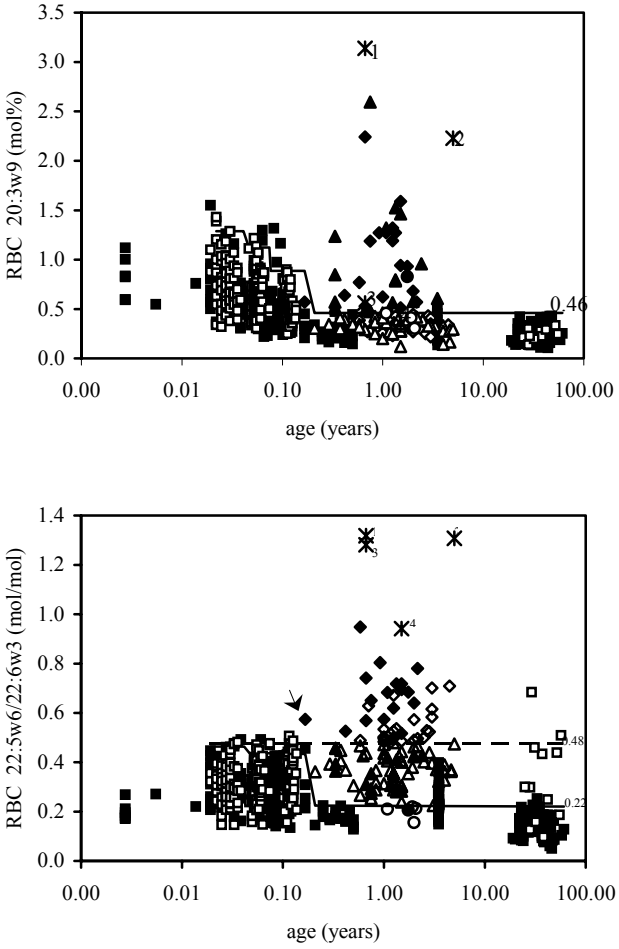


Figure 1. ■, *Omnivores;* □, *Low-dietary-LCP group;* ♦, *Pakistan EFAD+ω3-deficient;* ▲, *idem +ω3-marginal;* ●, *idem +ω3-sufficient;* ◊, *Pakistan EFA-sufficient +ω3-deficient;* Δ, *idem +ω3-marginal;* ○, *idem +ω3-sufficient;* *, *Patients.*
Erythrocyte 20:3ω9 (top) and 22:5ω6/22:6ω3 (bottom) as a function of age. RBC, erythrocyte; EFA, essential fatty acid; EFAD, EFA deficiency. RBC 20:3ω9 is a marker for EFA status and RBC 22:5ω6/22:6ω3 is a marker of ω3 status. Notice the log scale of the x-axis. For subject numbers in subgroups see Table 1. RBC 20:3ω9 and 22:5ω6/22:6ω3 of the omnivorous group became age-independent from 0.2 years. RBC 22:5ω6/22:6ω3 of the low-dietary-LCP group was age-independent. For subjects aged ≥0.2 years, the RBC 20:3ω9 cut-off value for EFAD was 0.46 mol%; their RBC 22:5ω6/22:6ω3 cut-off values for ω3-marginality and deficiency were 0.22 and 0.48 mol/mol, respectively. For cut-off values of subjects <0.2 years, see Table 1. The arrow indicates a Pakistani child aged <0.2 years, whose data were not used for the evaluation of the cut-off values. For diagnosis of patients 1-4, see Table 1.

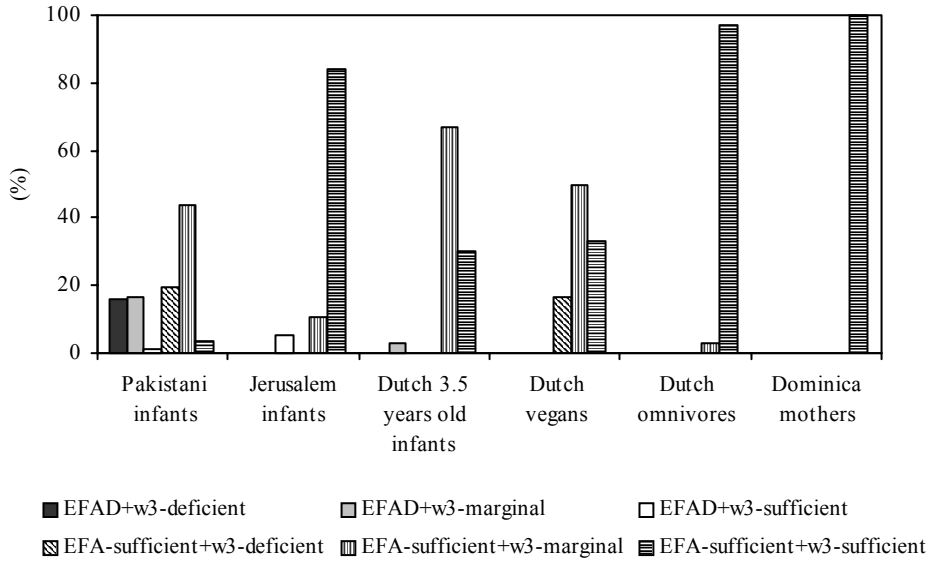


Figure 2. Classification of controls and malnourished Pakistani infants according to EFA and ω3 status.
EFA, essential fatty acid; EFAD, EFA deficiency. All subjects were above 0.2 years of age. For subject numbers in subgroups see Table 1. The applied cut-off values were 0.46 mol% RBC 20:3ω9 for EFAD, 0.22 mol/mol RBC 22:5ω6/22:6ω3 for ω3-marginality and 0.48 mol/mol RBC 22:5ω6/22:6ω3 for ω3-deficiency (see Figure 1). The distribution of the Pakistani infants was: 15.7% EFAD+ω3-deficient, 16.7% EFAD+ω3-marginal, 0.9% EFAD+ω3-sufficient, 19.5% EFA-sufficient+ω3-deficient, 43.5% EFA-sufficient+ω3-marginal, and 3.7% EFA-sufficient+ω3-sufficient.

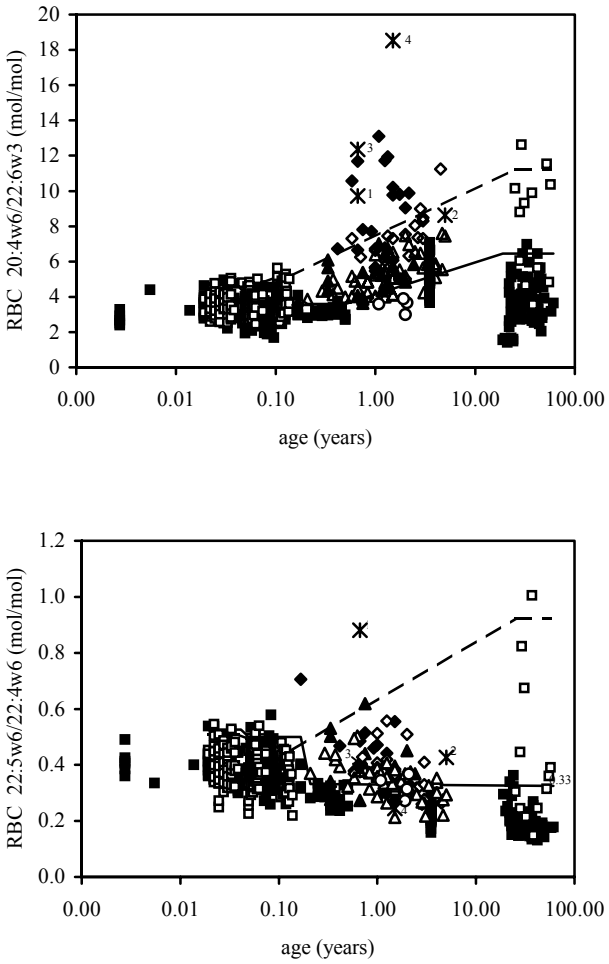
ω3-marginal or ω3-deficient. Of the 108 Pakistani infants 33.3% were found to be EFAD and 66.7% EFA-sufficient. Thirtyfive (35.2) percent were ω3-deficient, 60.2% ω3-marginal and 4.6% ω3-sufficient. When combined they were classified as 15.7% EFAD+ω3-deficient, 16.7% EFAD+ω3-marginal, 0.9% EFAD+ω3-sufficient, 19.5% EFA-sufficient+ω3-deficient, 43.5% EFA-sufficient+ω3-marginal and 3.7% EFA-sufficient+ω3-sufficient. The RBC 20:3ω9 and 22:5ω6/22:6ω3 data of the four pediatric patients suspected of EFAD/ω3-deficiency are shown in Table 1 and Figure 1. The three patients with chronic fat-malabsorption had both increased 20:3ω9 and 22:5ω6/22:6ω3, indicating EFAD+ω3-deficiency. The patient with the peroxisomal disorder was classified as EFA sufficient+ω3-deficient, with a 20:3ω9 below the cut-off value in combination with increased 22:5ω6/22:6ω3, due to very low 22:6ω3.

4.1.3.3 *RBC 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 for ω3-status*

Figure 3 illustrates the values of 20:4ω6/22:6ω3 (top) and 22:5ω6/22:4ω6 (bottom) for establishment of ω3-status. The P97.5 of RBC 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 for the omnivorous and low-dietary-LCP groups proved age-dependent beyond the age of 0.2 years, except for the 22:5ω6/22:4ω6 of omnivores. The cut-off values of RBC 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 were, analogous to 22:5ω6/22:6ω3, based on the P97.5 of the Jerusalem infants ≥0.2 years and the Dutch omnivorous adults plus Dominica mothers (upper limit of ω3-sufficiency) and the P97.5 of the four low-dietary-LCP subgroups (upper limit of ω3-marginality). Interconnection of these cut-off values allowed classification of the Pakistani children and the four patients by visual inspection. It showed that the use of RBC 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 caused a shift towards lower prevalence of ω3-deficiency and higher prevalence of ω3-marginality and ω3-sufficiency in the Pakistani group. With 20:4ω6/22:6ω3 cut-off values the distribution became 14.7% ω3-deficient, 75.2% ω3-marginal and 10.1% ω3-sufficient and with 22:5ω6/22:4ω6 cut-off values it became 0.9% ω3-deficient, 68.8% ω3-marginal and 30.3% ω3-sufficient. Three of the 4 pediatric patients were classified as ω3-deficient and one as ω3-marginal (abetalipoproteinemia) with use of RBC 20:4ω6/22:6ω3, whereas use of RBC 22:5ω6/22:4ω6 cut-off values classified one patient (jejunal atresia) as ω3-deficient, two patients as ω3-marginal and one patient (peroxisomal disorder) as ω3-sufficient.

4.1.4. Discussion

Suspicion of low EFA or ω3 FA status may rather focus on clinical chemical tests than the presence of clinical symptoms. Early diagnosis is important, since it is increasingly recognized that subclinical micronutrient deficiencies may cause disease in the long run. We established cut-off values for RBC 20:3ω9 and 22:5ω6/22:6ω3 for establishment of biochemical EFAD, ω3-marginality and ω3-deficiency and investigated the added value of 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 as parameters of ω3 status. The cut-off values are based on the 97.5 percentiles of two major groups of apparently healthy subjects, who either consumed diets with LCP (breast-fed babies, babies receiving LCP-enriched formula, omnivorous infants and adults) or diets with very little LCP (babies receiving formula without LCP and vegans). Recruitment of healthy LBW, predominantly preterm, babies for establishment of cut-off values may be questioned. However, preterm and term babies have similar RBC LCP contents at birth and do not exhibit major differences in postnatal RBC LCP courses upon the same feeding regimen [6]. Newborns are known to have high 20:3ω9 and 22:5ω6/22:6ω3 [32], and it was found that both the postnatal decline of 20:3ω9 and 22:5ω6/22:6ω3 in omnivorous subjects reached stable levels from the age of about 0.2 years (2.4 months). No 22:5ω6/22:6ω3 decrease seems to take place when the diet is virtually devoid of LCP, since infants who received formula without LCP and adult vegans had remarkably similar 22:5ω6/22:6ω3 ratios (Table 1). We therefore defined two levels for ω3-status, i.e. one based on the RBC 22:5ω6/22:6ω3 P97.5 of omnivores (upper limit of ω3-sufficiency) and a second based on the P97.5 of formula-fed infants and vegans (upper limit ω3-marginality). The term ‘marginality’ was introduced to indicate that there is no evidence that the well-known low ω3-status of vegans [26] should be regarded as a state of



*<u>Figure 3</u>. ■, Omnivores; □, Low-dietary-LCP group; ♦, Pakistan EFAD+ω3-deficient; ▲, idem +ω3-marginal; ●, idem +ω3-sufficient; ◊, Pakistan EFA-sufficient +ω3-deficient; Δ, idem +ω3-marginal; ○, idem +ω3-sufficient; *, Patients.*

Erytrocyte 20:4ω6/22:6ω3 (top) and 22:5ω6/22:4ω6 (bottom) as a function of age. RBC, erythrocyte; EFA, essential fatty acid; EFAD, EFA deficiency. RBC 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 are markers of ω3 status. Notice the log scale of the x-axis. for subject numbers in subgroups see Table 1. Cut-oof values for both 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 were age-dependent beyond the age of 0.2 years, except for the 22:5ω6/22:4ω6 cutt-off value for omnivores. Classification of the ω3-status of the Pakistani infants by symbol is based on the 22:5ω6/22:6ω3 cutt-off value (see Figure 1). Application of the 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 cutt-off values in the Pakistani group gave rise to a shift towards lower prevalence of ω3-deficiency and higher prevalence of ω3-marginality and ω3-sufficiency compared with the use of the 22:5ω6/22:6ω3 cutt-off value. The arrow indicates a Pakistani child aged <0.2 years, whose data were not used for the evaluation of the cut-off values. For diagnosis of patients 1-4, see Table 1.

ω3-deficiency. Data of the 3.5 years old Dutch infants were not used for the calculation of the upper limit of ω3-sufficiency. These children exhibited expected higher RBC 22:5ω6/22:6ω3 P97.5, compared with the Jerusalem infants ≥0.2 years and the Dutch omnivorous adults plus Dominica mothers (Table 1). Fish intake of 1-4 years old Dutch children is known to be 70% lower than that of Dutch adults on a g/kcal/day basis [33] and many of them should probably be regarded to consume a vegan diet with regard to LCPω3 intake.

The finally selected cut-off values for 20:3ω9 (i.e. 0.46 mol%) and 22:5ω6/22:6ω3 (i.e. 0.22 and 0.48 mol/mol, Figure 1) cannot be easily validated, since there is no 'gold standard' for the diagnosis of EFAD or ω3-deficiency. We therefore decided to validate these outcomes in, mostly malnourished, North-Pakistani infants who are known to have very low intakes of vegetable oils and fish, causing high incidences of EFAD and ω3-deficiency with occasional symptoms consistent with these conditions [27,34]. It was found that the encountered high percentages EFAD, ω3-marginality and ω3-deficiency are indeed consistent with their diets (Figure 2). Malnutrition is, however, by far more complex than EFAD alone, and we felt that current cut-off values required further confirmation in non-malnutrition cases of EFAD and ω3-deficiency. It was found that the cut-off values enabled detection of EFAD and ω3-deficiency in patients with inherited disfunctional chylomicron assembly (abetalipoproteinemia) and congenital atresia of the jejunum and biliary ducts. The patient with the peroxisomal disorder had high 22:5ω6/22:6ω3, due to very low 22:6ω3. The low 22:6ω3 levels of patients with peroxisomal disorders are considered to derive from their low 24:6ω3 to 22:6ω3 retroconversion capacity, due to insufficient peroxisomal β-oxidation [35]. The value of the present cut-off values should however be investigated more closely in larger groups of patients with miscellaneous causes of chronic fat-malabsorption (e.g. cystic fibrosis, liver disease), increased EFA demand (cancer, trauma) and inborn errors that affect EFA-metabolism (such as the Zellweger's syndrome).

We used the same method for the calculation of cut-off values for RBC 20:4ω6/22:6ω3 and 22:5ω6/22:4ω6 as employed for 22:5ω6/22:6ω3. We subsequently compared the added value of the former two ratios to detect low ω3 status in the Pakistani children and the 4 pediatric patients. It was found that RBC 20:4ω6/22:6ω3 is age-dependent beyond the age of 0.2 years and that this ratio is also less sensitive for the detection of ω3-deficiency in the Pakistani children and pediatric patients, compared with 22:5ω6/22:6ω3. The RBC 22:5ω6/22:4ω6 ratio on its turn was age-independent from 0.2 years in omnivores, but proved less sensitive for the detection of low ω3-status compared with both the 22:5ω6/22:6ω3 and 20:4ω6/22:6ω3 ratios in the Pakistani children and the pediatric patients. Low sensitivity of the 22:5ω6/22:4ω6 ratio is disappointing, since analogous to 20:3ω9 for EFAD, this ratio may be regarded as a 'functional' marker [36] that detects competition between FA of the ω3 and ω6 series in favor of ω6. Moreover, the intakes of both 22:5ω6 and 22:4ω6 from the diet are probably very low, which leaves this ratio to be a closer reflection of enzymatic activity than ratios that contain potentially diet-derived LCP such as 22:6ω3 and 20:4ω6. The most plausible explanation for the discrepancy is that each of the presently investigated ratios reflects different aspects of ω3 status. Increased 22:5ω6/22:6ω3 and increased 20:4ω6/22:6ω3 may both predominantly indicate low dietary 22:6ω3 intake and/or absorption. Increased 22:5ω6/22:4ω6 may predominantly point at low status of ω3 FA up to 22:5ω3, collectively referred to as 22:6ω3 precursors. We propose

that both RBC 22:5ω6/22:6ω3 and 22:5ω6/22:4ω6 might be useful for ω3 status assessment and to additionally employ the 0.33 mol/mol RBC 22:5ω6/22:4ω6 cut-off value for the detection of low status of 22:6ω3 precursors in subjects of 0.2 years and beyond (Figure 3 bottom). Patients with metabolic disorders of EFA metabolism might, however, cause conflicting results of these parameters. The patient with the peroxisomal disorder had normal 22:5ω6/22:4ω6 caused by disturbed conversion of 22:4ω6 to 22:5ω6. His increased 22:5ω6/22:6ω3 and 20:4ω6/22:6ω3 stems from similarly disturbed conversion of 22:5ω3 to 22:6ω3 in combination with a low 22:6ω3 intake.

The two groups of Dutch infants with ages below 0.2 years exhibited deviating 22:5ω6/22:6ω3 cut off-values with advancing age (Table 1; Figure 1 bottom). Their deviation with time is due to the intake of 22:6ω3 from breastmilk or LCP-enriched formula, and the lack of dietary 22:6ω3 intake by feeding formula without LCP [6,37]. There is evidence that enrichment of formula with 22:6ω3 improves early visual development, notably in prematures [37,38]. This effect is transient, but has nevertheless been the basis to add 22:6ω3 to formula for prematures in many countries. Enrichment of formulas for term infants is also gaining increasing support. Whether 0.01-0.17 years (0.5-2.0 months) old infants with RBC 22:5ω6/22:6ω3 ratios in the about 0.43-0.48 mol/mol range should be classified as ω3-marginal, or even ω3-deficient, seems therefore rather a matter of opinion than a scientifically proven fact. It is in this context also interesting to point at the very low cord blood RBC 22:5ω6/22:6ω3 ratio of the breast-fed Dominica newborns (median 0.19; P97.5 0.26 mol/mol) (Figure 1 bottom; Table 1), with similarly low 22:5ω6/22:6ω3 around postnatal day 21 (0.19; 0.27 mol/mol; data not shown in Table 1) and the even lower 22:5ω6/22:6ω3 of their mothers (0.09; 0.16 mol/mol; data not shown in Table 1). The Dominica mothers were known to have high fish intake and consequently high ω3-status, as also witnessed by their high breastmilk 22:6ω3 contents [24]. In contrast, the much higher RBC 22:5ω6/22:6ω3 of Dutch formula- and breast-fed infants and Dutch adults, is probably related to the typical North-European diet with low intake of fish and high dietary 18:2ω6/18:3ω3 ratio due to the predominant use of 18:2ω6-rich oils [39]. Intake of both fish and 18:3ω3 have been associated with lower risk of coronary artery disease in omnivores [40,41], and it seems therefore attractive to lower the 22:5ω6/22:6ω3 cut-off level for ω3-marginality (for omnivorous subjects) to that of the 97.5 percentile of the Dominica infants and their mothers. Such a cut-off value would classify the majority of the Western subjects as ω3-marginal and thereby illustrates the difficulty to define a border between ω3-sufficiency and marginality. It seems that any cut-off value for ω3 or 22:6ω3 status assessments may eventually have to be based on hard clinical evidence and not on a 97.5 percentile of an apparently healthy omnivorous population with inherently high risk of cardiovascular disease.

In conclusion, we calculated cut-off values for assessment of biochemical EFA status by measurement of RBC 20:3ω9, RBC 22:5ω6/22:6ω3 and RBC 22:5ω6/22:4ω6. The cut-off values are based on 97.5 percentiles of apparently healthy populations and apply for subjects aged ≥0.2 years (2.4 months). They amount to 0.46 mol% RBC 20:3ω9 for EFAD, 0.22 mol/mol RBC 22:5ω6/22:6ω3 for ω3-marginality, 0.48 mol/mol RBC 22:5ω6/22:6ω3 for ω3-deficiency and 0.33 mol/mol RBC 22:5ω6/22:4ω6 for low status of ω3 fatty acids up to 22:5ω3. It is important to realize that values beyond these cut-off values have not been validated on the basis of clinically detectable symptoms, but that they rather point at

states of altered substrate competition (20:3ω9, 22:5ω6/22:4ω6) and altered LCPω6/LCPω3 balance (22:5ω6/22:6ω3), which might be consistent with subclinical deficiencies or imminent clinical deficiencies. Employment of present cut-off values indicated high prevalence of biochemical EFAD and ω3-deficiency in mostly malnourished North-Pakistani infants with very low intakes of vegetable oils and fish, in three patients with chronic fat-malabsorption and in one patient with a peroxisomal disorder. In view of lack of toxicity, increasing concern of the (long-term) consequences of low micronutrient status and relatively low costs, we suggest to use these cut-off values, for the decision of dietary supplement intervention until better concepts have emerged.

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References

1. Bjerve KS. Omega 3 fatty acid deficiency in man: Implications for the requirement of alpha-linolenic acid and long-chain omega 3 fatty acids. World Rev Nutr Diet 1991;66:133-42
2. Holman RT. The slow discovery of the importance of omega 3 essential fatty acids in human health. J Nutr 1998;128:427S-33
3. Linscheer WG, Vergroesen J. Lipids. In: Shils ME. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea and Febiger. 1994:47-88
4. Hansen EA, Wiese HF, Boelsche AN, Haggard ME, Adam DJD, Davis H. Role of linoleic acid in infant nutrition. Pediatrics 1963;31:S171-92
5. O'Neill JA, Caldwell MD, Meng HC. Essential fatty acid deficiency in surgical patients. Ann Surg 1977;185:535-41
6. Innis SM. Essential fatty acids in growth and development. Prog Lipid Res 1991;30:39-101
7. Lagarde M, Burtin M, Sprecher H, Dechavanne M, Renaud S. Potentiating effect of 5,8,11-eicosatrienoic acid on human platelet aggregation. Lipids 1983;18:291-4
8. Orchard MA, Lewis PJ, Ongari MA, Waddell KA, Blair IA. Free 5,8,11-Eicosatrienoic Acid in Human Umbilical Artery. In: Lewis et al., ed. Prostacyclin in Pregnancy. New York, Raven Press, 1983:59-63
9. Eynard AR, Jiang WG, Mansel RE. Eicosatrienoic acid (20:3 n-9) inhibits the expression of E-cadherin and desmoglein in human squamous cell carcinoma in vitro. Prostaglandins Leukot Essent Fatty Acids 1998;59:371-7
10. Cleland LG, Neumann MA, Gibson RA, Hamazaki T, Akimoto K, James MJ. Effect of dietary n-9 eicosatrienoic acid on the fatty acid composition of plasma lipid fractions and tissue phospholipids. Lipids 1996;31:829-37
11. Holman RT. The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. J Nutr 1960;70:405-10
12. Lundberg WO. The significance of cis,cis,cis,-5,8,11-eicosatrienoic acid in essential fatty acid deficiency. Nutr Rev 1980;38:233-5
13. Holman RT, Smythe L, Johnson S. Effect of sex and age on fatty acid composition of human serum lipids. Am J Clin Nutr 1979;32:2390-9
14. Korotkova M, Strandvik B. Essential fatty acid deficiency affects the fatty acid composition of the rat small intestinal and colonic mucosa differently. Biochim Biophys Acta 2000;1487:319-25
15. Carlson SE, Carver LD, House SG. High fat diets varying in ratios of polyunsaturated to saturated fatty acids and linoleic to linoleic acid: a comparison of rat neural and red cell membrane phospholipids. J Nutr 1986;116:718-25
16. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. Am J Clin Nutr 1994;60:189-94
17. Van Gastel C, Van den Berg D, De Gier J, Van Deenen LLM. Some lipid characteristics of normal red blood cells of different age. Br J Haematol 1965;11:193-9
18. Holman RT, Johnson SB, Hatch TF. A case of human linolenic acid deficiency involving neurological abnormalities. Am J Clin Nutr 1982;35:617-23

19. Hoffman DR, Uauy R. Essentiality of dietary ω3 fatty acids for premature infants: plasma and red blood cell fatty acid composition. Lipids 1992;27:886-95
20. Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci U S A 1986;83:4021-5
21. Woltil HA, van Beusekom CM, Siemensma AD, Muskiet FAJ, Okken A. Erythrocyte and plasma cholesterol ester long chain polyunsaturated fatty acids of low-birth-weight babies fed preterm formula with and without ribonucleotides: comparison with human milk. Am J Clin Nutr 1995;62:943-9
22. Woltil HA, van Beusekom CM, Schaafsma A, Muskiet FAJ, Okken A. Long-chain polyunsaturated fatty acid status and early growth of low birth weight infants. Eur J Pediatr 1998;157:146-52
23. Woltil HA, van Beusekom CM, Okken-Beukens M, Schaafsma A, Muskiet FAJ, Okken A. Development of low-birthweight infants at 19 months of age correlates with early intake and status of long-chain polyunsaturated fatty acids. Prostaglandins Leukot Essent Fatty Acids 1999;61:235-41
24. van Beusekom CM, Nijeboer HJ, van der Veere CN, Luteyn AJ, Offringa PJ, Muskiet FAJ, et al. Indicators of long chain polyunsaturated fatty acid status of exclusively breastfed infants at delivery and after 20-22 days. Early Hum Dev 1993;32:207-18
25. Boersma ER, Lanting CI. Environmental exposure to polychlorinated biphenyls (PCBs) and dioxins. Consequences for longterm neurological and cognitive development of the child lactation. Adv Exp Med Biol 2000;478:271-87
26. Fokkema MR, Brouwer DAJ, Hasperhoven MR, Hettema Y, Bemelmans WJE, Muskiet FAJ. Polyunsaturated fatty acid status of Dutch vegans and omnivores. Prostaglandins Leukot Essent Fatty Acids 2000;63:279-85
27. Smit EN, Dijkstra JM, Schnater TA, Seerat E, Muskiet FAJ, Boersma ER. Effects of malnutrition on the fatty acid composition and plasma vitamin E levels of Pakistani children. Acta Paediatr 1997;86:690-5
28. Smit EN, Oelen EA, Seerat E, Boersma ER, Muskiet FAJ. Fish oil supplementation improves docosahexaenoic acid status of malnourished infants. Arch Dis Child 2000;5:366-9
29. Muskiet FAJ, Doormaal JJ van, Martini IA, Wolthers BG, Slik W van der. Capillary gas chromatographic profiling of total long-chain fatty acids and cholesterol in biological materials. J Chromatogr Biomed Appl 1983;278:231-44
30. Volmer M, Neiborg G, Muskiet FAJ. Simultaneous capillary gas chromatographic profiling of medium- and long-chain fatty acid methyl esters with split injection. Correction for injection-related discrimination by the 'bracketing' method. J Chromatogr 1988;434:385-94
31. International Federation of Clinical Chemistry. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. Clin Chim Acta 1987;170:S13-32
32. Crawford MA, Doyle W, Drury P, Lennon A, Costeloe K, Leighfield M. n-6 and n-3 fatty acids during early human development. J Intern Med Suppl 1989;225:159-69
33. Zo eet Nederland (report in Dutch). Results of the Dutch National Food Consumption Survey 1998. Voedingscentrum, The Hague, The Netherlands 1998
34. Smit EN, Woltil HA, Boersma ER, Muskiet FAJ. Low erythrocyte docosahexaenoic acid in malnourished, often breast-fed, Pakistani infants: a matter of concern? Eur J Pediatr 1999;158:525
35. Martinez M. Severe deficiency of docosahexaenoic acid in peroxisomal disorders: a defect of Δ4 desaturation? Neurology 1990;40:1292-8
36. Van den Berg H. Functional vitamin status assessment. Bibl Nutr Dieta 1994;51:142-9
37. SanGiovanni JP, Parra-Cabrera S, Colditz GA, Berkey CS, Dwyer JT. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. Pediatrics 2000;105:1292-8
38. SanGiovanni JP, Berkey CS, Dwyer JT, Colditz GA. Dietary essential fatty acids, long-chain polyunsaturated fatty acids, and visual resolution acuity in healthy fullterm infants: a systematic review. Early Hum Dev 2000;57:165-88
39. Sanders TAB. Polyunsaturated fatty acids in the food chain in Europe. Am J Clin Nutr 2000;71:S176-8
40. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 1985;312:1205-9
41. de Lorgeril M, Renaud S, Mamelle N et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454-9

Summary

The essential fatty acids (EFA) linoleic acid (LA;18:2ω6) and α-linolenic acid (ALA;18:3ω3) cannot be synthesised by the human body and are therefore indispensable components of the diet. Both LA and ALA can be converted into their respective long-chain metabolites, named long chain polyunsaturated fatty acids (LCPUFA; ≥20 carbon atoms and ≥ 3 double bonds). Humans cannot interconvert fatty acids (FA) of the ω3 and ω6 series. The most important LCPUFA of the ω6 series is arachidonic acid (AA; 20:4ω6), while eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3) are the major ω3LCPUFA. AA and DHA are not considered to be essential. They are however regarded as conditionally essential in newborns, especially preterms, since their capacity to convert EFA to LCPUFA seems insufficient to meet their high LCPUFA needs. Analogous to other FA, EFA and LCPUFA are important sources of energy. In addition they are important components of structural lipids and thereby contribute to the regulation of membrane properties like fluidity, flexibility, permeability and modulation of membrane-bound proteins. Some of the C_{20} LCPUFA are precursors of a wide variety of short-lived regulatory hormones, named eicosanoids, which play important roles in processes like inflammatory and anti-viral reactions, endothelial integrity and many more. Polyunsaturated fatty acids (PUFA) are gaining increasing interest as modulators of gene expression by their capacity to act as ligands of peroxisome proliferator activated receptors (PPAR) and to suppress the expression of sterol regulatory element binding proteins (SREBP).

When the fat content of the diet is low, endogenous FA synthesis increases, yielding 16:0, 18:0, 16:1ω7 and 18:1ω9 (oleic acid). Oleic acid, LA and ALA are metabolised by a series of alternating desaturation and elongation steps. The desaturase enzymes show preference for FA of the different series in the order ω3>ω6>ω9. Delta-6-desaturase activity is inhibited by high levels of both its products and precursors and influenced by several dietary factors and circulating hormones. The conventional view is that Δ4-desaturation does not involve another specific desaturase, but is composed of elongation, Δ6-desaturation and retroconversion through β-oxidation **(chapter 1.1)**.

Chapter 1.2 describes the nutritional aspects of EFA and LCPUFA. ALA can be found in green leafy vegetables, nuts and some vegetable oils, while EPA and DHA are found predominantly in fish and fish oil (FO). Most vegetable oils are rich sources of LA, while meat and eggs are the most important sources of AA.

Human milk contains the full range of PUFA, including small amounts of all ω3 and ω6LCPUFA. For many babies this will be the only dietary source of LCPUFA, since

classical formula milks do not contain LCPUFA. The FA in human milk derive from the diet, biosynthesis in the mammary gland and mobilisation from tissue stores. The FA composition of human milk is strongly dependent on the maternal diet and to a smaller extent affected by other factors like time postpartum, gestational age, parity and diseases. Women with high intakes of fat from vegetable origin have high LA contents in their milk, while relatively low LA contents have been found in milk of women on low-fat diets and women consuming diets high in animal fat. DHA levels are much higher in milk of women with high intakes of fish and FO. In contrast, milk AA content seems hardly influenced by the diet and is remarkably similar in omnivores, vegetarians and vegans.

The demand for EFA by the rapidly developing foetus is very high, especially during the last trimester of pregnancy. The two major FA in brain and retina are DHA and AA. The rate of their accretion increases as gestation progresses and accretion continues in the postnatal period up to at least 2 years of age. Maternal FA metabolism is crucial for foetal growth and development, because the foetus is completely dependent on the mother for its EFA, and also predominantly for its LCPUFA supply. After birth the high demands for LCPUFA have to be met by the baby's body stores, the conversion of parent EFA to LCPUFA, and/or intake of pre-formed LCPUFA from breastmilk. A critical period with regard to LCPUFA supply may be the weaning period, since most weaning foods contain only small amounts of LCPUFA. Compared with adults, children are likely to have relatively higher EFA requirements, because of the need for structural lipid synthesis associated with growth. Several committees have provided guidelines for appropriate intakes of the various FA for infants and adults. There are, however, remarkable differences between current recommendations.

Chapter 1.3 describes the (patho)physiological effects of EFA and LCPUFA on e.g. growth and neurological functioning during different stages of life (i.e. the prenatal period, the neonatal period, childhood and adulthood). At birth plasma and red blood cell (RBC) levels of AA and DHA are higher than maternal levels, whereas those of ALA and LA are lower. During the first months of life LCPUFA levels decrease, whereas LA increases, and by the age of around 4 months the child has developed a more or less adult FA pattern.

FA that are thought to be related to growth and development are mainly LA, ALA, AA and DHA. A few studies report impaired growth in (preterm) babies related to LA, AA or DHA status, while others find no correlation between FA status and the various growth parameters. In preterm infants visual functions and neurodevelopment are related to DHA status. Whether this also applies to babies born at term is still controversial. In older children lower DHA levels were observed in boys with attention-deficit hyperactivity disorder (ADHD). Improvement of motor skills was described in dyspraxic children after supplementation with DHA, AA and 18:3ω6. In adults low DHA levels have been found in patients suffering from schizophrenia, depression, dementia, Parkinsonism and other behavioural disorders.

Children suffering from protein energy malnutrition (PEM) appear to have low EFA and LCPUFA status, whereas ω9FA are increased. Clinical symptoms of EFA deficiency (EFAD) include skin changes, impaired resistance to infections, impaired growth rate and transient impaired visual, cognitive and motor skill development. These symptoms are also observed in children with PEM and may indeed be partly caused by low levels of especially LA, AA and DHA. Skin changes can possibly be ascribed to LA deficiency or to lower

levels of 20:3ω6 and AA, which are eicosanoid precursors. The higher infection rate could derive from a depressed immune system caused by, among other factors, reduced eicosanoid precursor levels, increased permeability of skin and gastrointestinal tract due to EFAD, or both. Reduced growth rate could be attributed to low LA, AA and DHA, while effects on neurological development could be partly caused by low DHA.

EFAD in PEM is not only caused by low intakes of EFA and LCPUFA. Also digestion, absorption, transport and metabolism of EFA are impaired. Impaired desaturation may find its origin in deficiencies of protein and one or more micro-nutrients that are involved in desaturation activity. On the other hand EFA expenditure is increased, due to its use as energy source and through lipid peroxidation. EFAD will on its turn perpetuate itself by causing decreased lipid absorption and transport and finally aggravate PEM by impairing lipid absorption and utilisation of dietary calories, all together resulting in a vicious cycle. Locally available vegetable oils could be used to improve EFA status of malnourished children. Fish, eggs and meat are rich sources of DHA and AA, respectively, but may because of high costs 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 EFA and LCPUFA and breastfeeding should therefore also be encouraged for this reason (**chapter 1.4**).

Chapter 2 and chapter 3.1 describe the studies that were carried out in the north of Pakistan. First we investigated whether malnourished children in that area were suffering from EFAD and vitamin E deficiency. For this we determined RBC FA and plasma vitamin E contents in 68 malnourished Pakistani children (ages 4-56 months) and compared the outcome with data from 26 age and sex matched apparently healthy local controls. Patients and controls were recruited from the Nutrition Rehabilitation Centre of the Paediatric Department, Federal Government Services Hospital, Islamabad. RBC FA were determined by capillary gas chromatography with flame ionisation detection. Vitamin E (i.e. α- and γ-tocopherol) was quantified by HPLC with ultraviolet detection. Evaluation with three statistical approaches revealed that both grade 2 and grade 3 malnourished children had decreased RBC ω6FA, and to a lesser extent decreased ω3FA. Increased ω9FA compensated for these decreases. Grade 2 patients had lower plasma vitamin E concentrations. We observed no decrease in the activities of Δ6- and Δ5-desaturase and elongase, as calculated from various FA ratios. The combination of low RBC DHA and a low 22:5ω6/22:4ω6 ratio suggests low Δ4-desaturation activity, which may be due to impaired peroxisomal β-oxidation. EFAD in these children may partly be explained by low EFA intake. Also the EFA content of the consumed breastmilk may be questioned, because more than half of the children were breastfed at the moment of blood collection. Moreover, all children had received breastmilk during their first months of life. Gastrointestinal infections causing impaired lipid absorption, and increased oxidative decomposition of LCPUFA due to vitamin E deficiency may also have contributed to EFAD (**chapter 2.1**).

The encountered differences in EFA status between malnourished children and controls were rather small, which may be explained by the already low EFA status of the controls, compared with western standards. Because of the important role of DHA in neurological development, we were especially concerned about the low DHA levels in both malnourished and control children. Low DHA is probably caused by the minute fish intake in that part of Pakistan. Although breastfed malnourished Pakistani children exhibited

better DHA status than those receiving no human milk, they had much lower DHA levels compared to breastfed infants from The Netherlands and Jerusalem (**chapter 2.2**).

To investigate whether low DHA in the Pakistani children was caused by low DHA in their mother's milk, we analysed the breastmilk FA composition of 8 Pakistani mothers together with the RBC FA composition of their malnourished children. The milk FA composition of Pakistani mothers was compared with previously collected milk FA data of 25 Dutch mothers. We observed that the milk of the Pakistani mothers contained low percentages of all ω3 and most ω6FA, compared with milk of Dutch mothers. Breastmilk EPA and DHA were positively correlated with infant RBC DHA. Milk DHA was also positively correlated with infant RBC AA. It was concluded that DHA status of these malnourished children is indeed strongly dependent on ω3LCPUFA intake from breastmilk. This is especially of importance since none of these children were exclusively breastfed. Prolonged breastfeeding of these malnourished Pakistani children seems therefore not only important for its various other favourable effects, e.g. anti-infective properties, but also as the major source of dietary ω3LCPUFA (**chapter 3.1**).

In an attempt to improve DHA status of malnourished Pakistani children we supplemented 10 children (ages 8-30 months) with one fish oil (FO) capsule (500 mg) per day for 9 weeks. Seven unsupplemented children served as controls. RBC FA were analysed at baseline and study end. We found that FO supplementation increased the mean RBC DHA from 2.3 to 3.3 mol%, without significantly affecting the levels of the ω6LCPUFA. One FO supplemented child with very low initial RBC AA showed a remarkable increase in RBC AA from 4.0 to 13.8 mol%, whereas another child with high AA at baseline exhibited a 30% decrease (from 15.64 to 10.46 mol%). The FO is apparently well absorbed and not exclusively used as a source of energy. As purified FO is quite expensive, the cheaper cod liver oil could be a useful alternative. Moreover, cod liver oil also contains vitamins A and D, which are often deficient in malnourished children. Future efforts should nevertheless also be directed at ω3LCPUFA supplementation of Pakistani women, preferably from early pregnancy onwards. This would prevent both low supply to the foetus and low postnatal supply to the newborn via breastmilk (**chapter 2.3**).

Chapter 3.2 describes a study performed among Palestinian women from 'The Old City' of Jerusalem. We investigated whether supplementation of AA, or a combination of AA and DHA, would affect breastmilk LCPUFA composition. Ten women were supplemented for one week daily with 300 mg AA, while eight women received 300 mg AA plus 110 mg EPA and 400 mg DHA. Eight women served as unsupplemented controls. Milk samples were collected on days 0, 1 and 7. Supplementation with AA alone had no effect on breastmilk AA, but tended to reduce EPA and DHA. Administration of the combination of AA, EPA and DHA tended to increase both milk AA and ω3LCPUFA contents. A larger simultaneous increase of milk AA, DHA and EPA can probably be accomplished by the use of a combination of a lower ω6LCPUFA/ω3LCPUFA ratio and higher AA, EPA and DHA dosages.

For the other studies that concentrated on the milk FA composition we compiled all data on mature human milk samples collected by our research group (n= 465). The collection includes samples from The Netherlands, the Caribbean Region, Jerusalem, Tanzania and Pakistan. Because some investigators stress the uniformity of the human milk FA composition, whereas others point at the large range of the various milk FA, we were

interested in the genuine biological variation (CV_{biol}) of human milk FA. This information is e.g. important for manufacturing of infant formulas, since the human milk composition is still considered to be the 'gold standard' for most nutrients. We calculated the CV_{biol} for the 28 quantitatively most important FA, by using data from the observed variation (CV_{obs}) and the analytical variation (CV_{anal}). We found that the CV_{anal} of the human milk FA was low compared with the genuine inter-individual CV_{biol}. The largest CV_{biol} was observed for the short-term dietary dependent EPA and DHA, and the smallest for FA that derive mainly from adipose tissue, 16:0 and 18:1ω9. The majority of milk FA showed a CV_{biol} between 25 and 40%. We concluded that because of the large CV_{biol} and the many dietary changes in recent history it seems impossible to consider the present human milk FA composition as the 'gold standard' for infant formula (**chapter 3.3**).

In view of the large CV_{biol} of human milk FA we wondered whether the human milk FA composition would comply with current recommendations for formula. For this we projected these recommendations on our human milk FA data. It was found that especially breastmilk from women living in non-western countries did not meet the criteria for 12:0, 14:0 and LA. Other striking discrepancies between recommendations and actual milk FA contents were observed for AA and DHA. These FA are indeed subject to the largest disagreement between recommending committees for infant formula. In addition, we showed that almost all of the quantitatively and qualitatively most important FA are closely related. Based on this observation we propose that a humanised formula FA composition would be any composition that cannot be distinguished from that of human milk on the basis of its balanced FA composition, as e.g. documented via principle component analysis (**chapter 3.4**).

The last chapter describes the assessment of cut-off values for biochemical EFAD and isolated ω3-deficiency. Clinical symptoms of EFAD are rather a-specific and may represent late signs of this condition. Biochemical indicators may be more useful for early detection. We established cut-off values, based on 97.5 percentiles of RBC 20:3ω9, 22:5ω6/22:6ω3 and 22:5ω6/22:4ω6, using data from an apparently healthy omnivorous group and an apparently healthy group with low LCPUFA intake. The cut-off values were validated by their application in an EFAD suspected group of, mostly malnourished, Pakistani children, three paediatric patients with chronic fat-malabsorption and one patient with a peroxisomal disorder. The calculated RBC cut-off values amounted to 0.46 mol% 20:3ω9 for EFAD, 0.22 mol/mol 22:5ω6/22:6ω3 for ω3-marginality, 0.48 mol/mol 22:5ω6/22:6ω3 for ω3-deficiency and 0.33 mol/mol 22:5ω6/22:4ω6 for low ω3FA status. Employment of present cut-off values indicated high prevalence of biochemical EFAD and ω3-deficiency in the Pakistani infants and the patients. In view of lack of toxicity, increasing concern regarding the (long-term) consequences of low micro-nutrient status and the relatively low costs, we propose to use these cut-off values for the decision to initiate dietary (LC)PUFA supplementation, until better concepts have emerged (**chapter 4.1**).

The main results and conclusions of this thesis may be summarised as follows:

- EFAD and PEM are interrelated. PEM causes EFAD, while EFAD perpetuates itself and aggravates PEM, creating a vicious cycle. Some of the clinical symptoms in PEM, like skin changes, impaired resistance to infections, impaired growth rate and disturbed development, may partly be explained by EFAD. EFA status can be improved by the consumption of locally available vegetable oils, fish, eggs, meat and breastmilk.

- Malnourished Pakistani children suffer from EFAD. Low fish intake and low breastmilk DHA content are responsible for their low DHA status. Low breastmilk DHA is on its turn most probably due to low maternal fish intake. The low DHA status of the children can be improved by FO supplementation. Maternal ω3LCPUFA supplementation, preferably from early pregnancy onwards, would however be a better option in terms of prevention.

- DHA status of malnourished Pakistani children who receive breastmilk is strongly dependent on the ω3LCPUFA content of the milk. Their DHA status is better than that of counterparts receiving no human milk. This provides us with a new argument for encouraging prolonged breastfeeding.

- Although competition between ω3LCPUFA and ω6LCPUFA definitely exists, some of the present studies show that ω3LCPUFA do not necessarily affect ω6LCPUFA status in a negative manner. First, we observed in Pakistani mother-child pairs a positive correlation between the mother's milk DHA and their infant's RBC AA. Second, FO supplementation did not decrease ω6LCPUFA in malnourished children. A decrease occurred only when initial AA was high. And finally, supplementation of AA alone had no effect on milk AA, whereas a combination of AA and DHA increased both milk AA and DHA in well-nourished Palestinian women. A unifying hypothesis may be the existence of a functional ω3/ω6 ratio, with any competition between ω3 and ω6 pointing at a state of non-functional surpluses.

- Most human milk FA are subject to large biological variation, while on the other hand many of the FA are closely related. The large variation sheds doubt on the usefulness of human milk FA contents as the 'gold standard' for formula milk manufacturing. The interrelationships between human milk FA may provide a better basis for future recommendations for humanised formula.

- Biochemical EFA status parameters for the establishment of (sub-clinical) EFAD are likely to be more sensitive and specific than a diagnosis based on clinical symptomatology. Proposed RBC cut-off values are 0.46 mol% 20:3ω9 for EFAD, 0.22 mol/mol 22:5ω6/22:6ω3 for ω3-marginality, 0.48 mol/mol 22:5ω6/22:6ω3 for ω3-deficiency and 0.33 mol/mol 22:5ω6/22:4ω6 for low ω3FA status. These cut-off values may provide us with tools for the decision to initiate dietary (LC)PUFA supplementation, until better concepts have emerged.

Samenvatting

De essentiële vetzuren (Engels: essential fatty acids; EFA) linolzuur (LA;18:2ω6) en α-linoleenzuur (ALA; 18:3ω3) kunnen niet door het menselijk lichaam zelf worden gemaakt en zijn daarom onmisbare onderdelen van de voeding. Zowel LA als ALA kunnen worden omgezet naar hun lange keten metabolieten, lange keten meervoudig onverzadigde vetzuren genaamd (Engels: long chain polyunsaturated fatty acids; LCPUFA). De mens kan vetzuren (FA) van de ω3 en de ω6 series niet in elkaar omzetten. De belangrijkste LCPUFA van de ω6 serie is arachidonzuur (AA; 20:4ω6), terwijl eicosapentaeenzuur (EPA; 20:5ω3) en docosahexaeenzuur (DHA; 22:6ω3) de belangrijkste ω3LCPUFA zijn. AA en DHA worden niet als essentiële FA beschouwd. Ze worden echter gezien als "conditioneel" essentieel in pasgeborenen, met name in prematuren, omdat hun vermogen om EFA om te zetten in LCPUFA onvoldoende lijkt te zijn om te voldoen aan hun hoge LCPUFA behoefte. Net als andere FA, zijn EFA en LCPUFA belangrijke bronnen van energie. Bovendien zijn het belangrijke onderdelen van structurele lipiden en dragen daardoor bij aan de regulatie van membraaneigenschappen zoals vloeibaarheid, flexibiliteit, doorlaatbaarheid en modulatie van membraangebonden eiwitten. Sommige C_{20} LCPUFA zijn voorlopers van een grote verscheidenheid aan “kortlevende” regulerende hormonen, eicosanoïden genaamd, die een belangrijke rol spelen in processen zoals ontstekings- en anti-virale reacties, de integriteit van het endotheel en nog veel meer. Meervoudig onverzadigde FA (Engels: polyunsaturated fatty acids; PUFA) komen steeds meer in de belangstelling als modulatoren van genexpressie vanwege hun vermogen om dienst te doen als liganden van “peroxisome proliferator activated receptors” (PPAR) en als onderdrukkers van de expressie van “sterol regulatory element binding proteins” (SREBP).

Als het vetgehalte van de voeding laag is, stijgt de endogene FA synthese, hetgeen 16:0, 18:0, 16:1ω7 en 18:1ω9 (oliezuur) oplevert. Oliezuur, LA en ALA worden gemetaboliseerd in een serie van afwisselende desaturatie en elongatie stappen. De desaturase enzymen hebben een voorkeur voor FA uit de verschillende series in de volgorde ω3>ω6>ω9. De delta-6-desaturase activiteit wordt onderdrukt door hoge gehaltes van zowel de producten als de voorlopers en wordt beïnvloed door verschillende voedingsfactoren en circulerende hormonen. Het algemeen aanvaarde standpunt is dat er bij Δ4-desaturatie geen ander specifiek desaturase enzym betrokken is, maar dat deze bestaat uit een elongatie, een Δ6-desaturatie, en een retroconversie via β-oxidatie **(hoofdstuk 1.1)**.

Hoofdstuk 1.2 beschrijft de voedingsaspecten van EFA en LCPUFA. ALA bevindt zich in groene bladgroenten, noten en enkele plantaardige oliën, terwijl EPA en DHA met name in vis en visolie gevonden worden. De meeste plantaardige oliën zijn rijk aan LA, terwijl vlees en eieren de belangrijkste bronnen van AA zijn.

Moedermelk bevat het volledige scala aan PUFA, inclusief kleine hoeveelheden van alle ω3- en ω6LCPUFA. Voor veel baby's zal dit de enige voedingsbron van LCPUFA zijn, omdat de meeste "klassieke" flesvoedingen geen LCPUFA bevatten. De FA in moedermelk zijn afkomstig van de voeding, biosynthese in de borstklier en mobilisatie uit weefsel. De FA samenstelling van moedermelk is sterk afhankelijk van de voeding van de moeder en wordt in geringere mate beïnvloed door andere factoren zoals de tijd na de bevalling, zwangerschapsduur, het aantal kinderen en ziektes. Vrouwen met een hoge vet inname van voornamelijk plantaardige afkomst, hebben hoge gehaltes aan LA in hun melk, terwijl relatief lage LA gehaltes gevonden worden in de melk van vrouwen met een vet-arme voeding en van vrouwen die een voeding consumeren met een hoog gehalte aan dierlijk vet. De DHA gehaltes zijn veel hoger in de melk van vrouwen met een hoge vis en visolie inname. Daarentegen wordt het AA gehalte van de melk nauwelijks beïnvloed door de voeding en is verbazingwekkend gelijk in omnivoren, vegetariërs en veganisten.

De EFA behoefte van de zich snel ontwikkelende foetus is erg hoog, met name tijdens het laatste trimester van de zwangerschap. De twee belangrijkste FA in de hersenen en de retina zijn DHA en AA. Hun aanwas snelheid wordt groter naarmate de zwangerschap vordert en deze aanwas gaat in de postnatale periode door tot op tenminste tweejarige leeftijd. Het FA metabolisme van de moeder is cruciaal voor foetale groei en ontwikkeling, omdat de foetus voor zijn/haar EFA voorziening geheel afhankelijk is van de moeder en dit geldt eveneens grotendeels voor zijn/haar LCPUFA voorziening. Na de geboorte moet aan de grote vraag naar LCPUFA voldaan worden door de baby's lichaamsvoorraad, zijn/haar omzetting van de stam EFA naar LCPUFA en/of inname van door de moeder voorgevormde LCPUFA uit moedermelk. Een kritieke periode betreffende de LCPUFA voorziening zou wel eens kunnen zijn de periode van het "spenen" (i.e. de periode waarin de baby naast melk aanvullende voeding krijgt), omdat de meeste voedingsmiddelen die hierbij gebruikt worden slechts geringe hoeveelheden LCPUFA bevatten. Vergeleken met volwassenen hebben kinderen waarschijnlijk een relatief grote behoefte aan EFA, vanwege de behoefte aan de synthese van structurele lipiden die geassocieerd zijn met groei. Verschillende commissies hebben richtlijnen uitgegeven inzake de adequate inname van de verschillende FA voor kleine kinderen en volwassenen. Er bestaan echter opmerkelijke verschillen tussen de huidige aanbevelingen.

Hoofdstuk 1.3 beschrijft de (patho)fysiologische effecten van EFA en LCPUFA op processen zoals groei en neurologisch functioneren gedurende de verschillende stadia van het leven (i.e. de prenatale periode, de neonatale periode, kindertijd en volwassenheid). Bij de geboorte zijn de plasma en rode bloed cel (RBC) gehaltes van AA en DHA hoger dan die van de moeder, terwijl die van ALA en LA lager zijn. Gedurende de eerste levensmaanden dalen de LCPUFA gehaltes, terwijl daarentegen die van LA stijgen, en rond de leeftijd van 4 maanden heeft het kind een min of meer volwassen FA patroon ontwikkeld.

De FA waarvan gedacht wordt dat ze gerelateerd zijn aan groei en ontwikkeling zijn met name LA, ALA, AA en DHA. Enkele studies beschrijven een verslechterde groei in (premature) baby's gerelateerd aan LA, AA of DHA status, terwijl anderen geen correlatie vinden tussen FA status en de verschillende groeiparameters. In premature kinderen zijn visuele functie en neurologische ontwikkeling gerelateerd aan DHA status. Of dit ook geldt voor a-terme kinderen is vooralsnog controversieel. In oudere kinderen zijn lagere DHA gehaltes waargenomen in jongens met het hyperactiviteitssyndroom dat gepaard gaat met

aandachtsstoornissen (“attention-deficit hyperactivity disorders”; ADHD). Verbetering van motorische vaardigheden is beschreven in "dispraktische" kinderen na suppletie met DHA, AA en 18:3ω6. In volwassenen zijn lage DHA gehaltes gevonden in patiënten die lijden aan schizofrenie, depressie, dementie, de ziekte van Parkinson en andere gedragsstoornissen.

Kinderen die lijden aan deficiënte inname van eiwit en calorieën (Engels: protein energy malnutrition, PEM) blijken een lage EFA en LCPUFA status te hebben, terwijl de ω9FA daarentegen verhoogd zijn. Klinische symptomen van EFA deficiëntie (EFAD) omvatten veranderingen van de huid, verminderde weerstand tegen infecties, verlaagde groeisnelheid en een tijdelijk verminderde visuele, cognitieve en motorische ontwikkeling. Deze symptomen worden ook waargenomen in kinderen met PEM en zouden wel eens gedeeltelijk veroorzaakt kunnen worden door lage gehaltes aan met name LA, AA en DHA. Veranderingen van de huid kunnen mogelijk worden toegeschreven aan een LA deficiëntie, of aan de lagere gehaltes van 20:3ω6 en AA, hetgeen voorlopers van eicosanoïden zijn. De hogere infectie frequentie kan een gevolg zijn van een onderdrukt immuunsysteem, veroorzaakt door onder andere verlaagde eicosanoïd voorloper gehaltes, een verhoogde doorlaatbaarheid van de huid en de darm als gevolg van EFAD, of beiden. Een verlaagde groeisnelheid kan worden toegeschreven aan lage LA, AA en DHA gehaltes, terwijl de effecten op de neurologische ontwikkeling gedeeltelijk veroorzaakt zouden kunnen worden door een laag DHA.

EFAD in PEM wordt niet alleen veroorzaakt door een lage inname van EFA en LCPUFA. Ook de vertering, absorptie, transport en metabolisme van FA zijn verlaagd. Verminderde desaturatie vindt mogelijk zijn oorsprong in een deficiëntie van eiwitten en één of meer micronutriënten, die betrokken zijn bij de desaturatie activiteit. Aan de andere kant is het verbruik van EFA verhoogd, als gevolg van het gebruik van EFA als energiebron en via lipiden peroxidatie. EFAD houdt op zijn beurt zichzelf in stand doordat de vetabsorptie en het transport worden verlaagd en kan tenslotte PEM verergeren door een verslechtering van de vetabsorptie en het verbruik van calorieën, bij elkaar resulterend in een vicieuze cirkel. Lokaal verkrijgbare plantaardige oliën zouden gebruikt kunnen worden om de EFA status van ondervoede kinderen te verbeteren. Vis, eieren en vlees zijn rijke bronnen van respectievelijk DHA en AA, doch vanwege hun hoge prijs mogelijk niet geschikt om op grote schaal toe te voegen aan de voeding van ondervoede kinderen in ontwikkelingslanden. Moedermelk is een belangrijke bron van EFA en LCPUFA en borstvoeding zou daarom ook om deze reden bevorderd moeten worden (**hoofdstuk 1.4**).

Hoofdstuk 2 en hoofdstuk 3.1 beschrijven de studies die uitgevoerd zijn in het noorden van Pakistan. Allereerst hebben we gekeken of ondervoede kinderen in dat gebied lijden aan EFAD en vitamine E deficiëntie. Daartoe hebben we de gehaltes aan RBC FA en plasma vitamine E bepaald in 68 ondervoede Pakistaanse kinderen (leeftijd 4-56 maanden) en zijn de resultaten vergeleken met die van 26 in leeftijd en geslacht vergelijkbare ogenschijnlijk gezonde controles. Patiënten en controles werden gerekruteerd in het “Nutrition Rehabilitation Center of the Paediatric Department, Federal Government Services Hospital, Islamabad”. De RBC FA werden bepaald met capillaire gaschromatografie en vlam ionisatie detectie. Vitamine E (i.e. α- en γ-tocopherol) werd gekwantificeerd met HPLC en ultraviolet detectie. Evaluatie door middel van drie statistische benaderingen liet zien dat zowel graad 2 als graad 3 ondervoede kinderen verlaagde RBC ω6FA hadden, en in mindere mate verlaagde ω3FA. Verhoogde ω9FA

compenseerden voor deze verlagingen. Graad 2 patiënten hadden lagere plasma vitamine E concentraties. We vonden geen afname in de activiteiten van Δ6- en Δ5-desaturase of elongase, zoals berekend aan de hand van de verschillende ratio's. De combinatie van een lage RBC DHA en een lage 22:5ω6/22:4ω6 ratio wekt de suggestie van een lage Δ4-desaturatie activiteit, hetgeen het gevolg zou kunnen zijn van een verslechterde peroxisomale β-oxidatie. EFAD in deze kinderen zou gedeeltelijk kunnen worden verklaard door een lage EFA inname. Ook het EFA gehalte van de moedermelk zou in twijfel getrokken kunnen worden, omdat meer dan de helft van de kinderen borstvoeding kreeg op het moment van bloedafname. Bovendien hadden alle kinderen moedermelk gehad in hun eerste levensmaanden. Gastro-intestinale infecties die een verslechterde vetabsorptie veroorzaken, en een verhoogde oxidatieve afbraak van LCPUFA als gevolg van vitamine E deficiëntie zouden ook kunnen hebben bijgedragen aan EFAD (**hoofdstuk 2.1**).

De aangetroffen verschillen in EFA status tussen ondervoede kinderen en controles waren vrij gering, hetgeen mogelijk verklaard kan worden door de reeds lage EFA status van de controles, vergeleken met westerse maatstaven. Vanwege de belangrijke rol van DHA in de neurologische ontwikkeling, waren we in het bijzonder bezorgd over de lage waarden van DHA in zowel ondervoede als controle kinderen. De lage DHA wordt vermoedelijk veroorzaakt door de marginale visinname in dat deel van Pakistan. Ofschoon borstgevoede ondervoede Pakistaanse kinderen een betere DHA status hadden dan degenen die geen moedermelk kregen, hadden ze veel lagere DHA waarden vergeleken met borstgevoede kinderen uit Nederland en Jeruzalem (**hoofdstuk 2.2**).

Om te bepalen of het lage DHA in de Pakistaanse kinderen veroorzaakt werd door een laag DHA in de melk van hun moeders, analyseerden we de moedermelk FA samenstelling van 8 Pakistaanse moeders samen met de RBC FA samenstelling van hun ondervoede kinderen. De melk FA samenstelling van de Pakistaanse moeders werd vergeleken met reeds eerder verzamelde melk FA gegevens van 25 Nederlandse moeders. We zagen dat de melk van de Pakistaanse moeders lage percentages van alle ω3 en van de meeste ω6FA bevatten, vergeleken met melk van Nederlandse moeders. Moedermelk EPA en DHA waren positief gecorreleerd met RBC DHA van het kind. DHA in de melk was ook positief gecorreleerd met RBC AA van het kind. We concludeerden dat de DHA status van deze ondervoede kinderen inderdaad sterk afhankelijk is van de ω3LCPUFA inname via moedermelk. Dit is met name van belang omdat geen van de kinderen exclusief borstgevoed was. Een langere borstvoedingsperiode van deze ondervoede Pakistaanse kinderen lijkt daarom niet alleen belangrijk vanwege de verschillende andere gunstige effecten, bijvoorbeeld een verminderde kans op infecties, maar ook vanwege de belangrijke voedingsbron van ω3LCPUFA (**hoofdstuk 3.1**).

In een poging om de DHA status van ondervoede Pakistaanse kinderen te verbeteren suppleerden we 10 kinderen (leeftijd 8-30 maanden) met één visolie (FO) capsule (500 mg) per dag gedurende 9 weken. Zeven niet-gesuppleerde kinderen dienden als controles. RBC FA werden geanalyseerd aan het begin en het eind van de studie. We vonden dat FO suppletie het gemiddelde RBC DHA verhoogde van 2,3 naar 3,3 mol%, zonder dat het de ω6LCPUFA gehaltes significant aantaste. Eén FO gesuppleerd kind met een zeer laag initieel RBC AA, liet een opmerkelijke verhoging zien in RBC AA van 4,0 naar 13,8 mol%, terwijl daarentegen een ander kind met een hoog initieel AA een 30% afname liet zien (van 15.64 tot 10.46 mol%). De FO wordt blijkbaar goed geabsorbeerd en niet alleen gebruikt als een energiebron. Omdat gezuiverde FO vrij duur is, zou de goedkopere

levertraan een bruikbaar alternatief kunnen zijn. Bovendien bevat levertraan eveneens de vitamines A en D, waarin ondervoede kinderen vaak deficiënt zijn. Toekomstige pogingen zouden niettemin eveneens gericht moeten zijn op ω3LCPUFA suppletie van Pakistaanse vrouwen, bij voorkeur vanaf de vroege zwangerschap. Dit zou zowel een lage aanvoer naar de foetus als naar de pasgeborene via moedermelk kunnen voorkomen (**hoofdstuk 2.3**).

Hoofdstuk 3.2 beschrijft een studie onder Palestijnse vrouwen uit "De oude stad" van Jeruzalem. We onderzochten of suppletie met AA, of een combinatie van AA en DHA, de moedermelk LCPUFA samenstelling zou beïnvloeden. Tien vrouwen werden gedurende één week dagelijks gesuppleerd met 300 mg AA, terwijl acht vrouwen 300 mg AA plus 110 mg EPA en 400 mg DHA kregen. Acht vrouwen dienden als niet-gesuppleerde controles. Melkmonsters werden verzameld op dag 0, 1 en 7. Suppletie met AA alleen had geen effect op moedermelk AA, maar had de neiging om EPA en DHA te verlagen. Toediening van de combinatie van AA, EPA en DHA had de neiging om zowel de AA als de ω3LCPUFA gehaltes van de melk te verhogen. Een grotere gelijktijdige verhoging van AA, EPA en DHA kan mogelijk bereikt worden door het gebruik van een combinatie van een lagere ω6LCPUFA/ω3LCPUFA ratio en hogere AA, EPA and DHA doseringen.

Voor de andere studies die zich richten op de melk FA samenstelling hebben we alle gegevens samengevoegd van de mature moedermelkmonsters die verzameld zijn door onze onderzoeksgroep (n=465). De collectie omvatte monsters uit Nederland, het Caraïbische gebied, Jeruzalem, Tanzania en Pakistan. Omdat sommige onderzoekers de uniformiteit van moedermelk benadrukken, terwijl anderen daarentegen duiden op het grote bereik van de verschillende melk FA, waren wij geïnteresseerd in de werkelijke biologische variatie (CV_{biol}) van moedermelk FA. Deze informatie is bijvoorbeeld van belang voor de productie van flesvoeding, omdat moedermelk nog steeds gezien wordt als de "gouden standaard" voor the meeste nutriënten. We berekenden de CV_{biol} voor de 28 kwantitatief meest belangrijke FA, door gebruik te maken van gegevens van de gevonden variatie (CV_{obs}) en de analytische variatie (CV_{anal}). We vonden dat de CV_{anal} van de moedermelk FA laag was vergeleken met de werkelijke inter-individuele CV_{biol}. De grootste CV_{biol} werd gevonden voor de korte-termijn voedingsafhankelijke EPA en DHA, en de kleinste voor FA die voornamelijk afkomstig zijn uit het vetweefsel, 16:0 en 18:1ω9. Het merendeel van de FA liet een CV_{biol} zien tussen de 25 en 40%. We concludeerden dat het, vanwege de grote CV_{biol} en de vele veranderingen in de voeding in de recente geschiedenis, onmogelijk lijkt om de huidige moedermelk FA samenstelling als de "gouden standaard" voor flesvoeding te beschouwen (**hoofdstuk 3.3**).

Vanwege de grote CV_{biol} van moedermelk vroegen we ons af of de moedermelk FA samenstelling zou voldoen aan de huidige aanbevelingen voor flesvoeding. Hiervoor projecteerden we deze aanbevelingen op onze moedermelk FA data. Gevonden werd dat met name de moedermelk van vrouwen die in niet-westerse landen wonen niet voldeed aan de criteria voor 12:0, 14:0 en LA. Andere opvallende verschillen tussen de aanbevelingen en de werkelijke FA gehaltes werden waargenomen voor AA en DHA. Deze FA vertonen inderdaad de grootste verschillen tussen aanbevelingen van de diverse commissies voor flesvoeding. Bovendien bleek dat bijna alle kwantitatief en kwalitatief belangrijkste FA sterk aan elkaar gerelateerd zijn. Gebaseerd op deze waarneming stellen we voor dat een "gehumaniseerde" flesvoeding FA samenstelling elke samenstelling zou moeten zijn die niet kan worden onderscheiden van moedermelk op grond van zijn gebalanceerde FA

samenstelling, zoals bijvoorbeeld gedocumenteerd via een “principale component analyse” (**hoofdstuk 3.4**).

Het laatste hoofdstuk beschrijft de vaststelling van afkapwaarden voor een biochemische EFAD en een geïsoleerde ω3-deficientie. Klinische symptomen van EFAD zijn tamelijk aspecifiek en vertegenwoordigen mogelijk de late verschijnselen van deze aandoening. Biochemische indicatoren zouden beter bruikbaar kunnen zijn voor een vroege opsporing. We bepaalden afkapwaarden, gebaseerd op 97.5 percentielen van RBC 20:3ω9, 22:5ω6/22:6ω3 en 22:5ω6/22:4ω6, door gebruik te maken van gegevens van een ogenschijnlijk gezonde groep omnivoren en een ogenschijnlijk gezonde groep met een lage LCPUFA inname. De afkapwaarden werden gevalideerd door hun toepassing in een van EFAD verdachte groep, bestaande uit voornamelijk ondervoede, Pakistaanse kinderen, drie pediatrische patiënten met chronische vet-malabsorptie en een patiënt met een peroxisomale afwijking. De berekende RBC afkapwaarden waren 0,46 mol% 20:3ω9 voor EFAD, 0,22 mol/mol 22:5ω6/22:6ω3 voor ω3-marginaliteit, 0,48 mol/mol 22:5ω6/22:6ω3 voor ω3-deficientie en 0.,3 mol/mol 22:5ω6/22:4ω6 voor een lage ω3FA status. Toepassing van de huidige afkapwaarden wees op een hoge prevalentie van biochemische EFAD en ω3-deficientie in de Pakistaanse kinderen en in de patiënten. Vanwege de afwezigheid van toxiciteit, groeiende bezorgdheid over de (lange termijn) consequenties van een lage micronutriënt status en relatief lage kosten, stellen we voor deze afkapwaarden te gebruiken voor de beslissing om te beginnen met (LC)PUFA voedingssuppletie, totdat zich betere concepten voordoen (**hoofdstuk 4.1**).

De belangrijkste resultaten en conclusies van dit proefschrift kunnen als volgt worden samengevat:

- EFAD en PEM zijn aan elkaar gerelateerd. PEM veroorzaakt EFAD, terwijl EFAD zichzelf in stand houdt en PEM verergert, hetgeen een vicieuze cirkel veroorzaakt. Sommige van de klinische symptomen in PEM, zoals veranderingen van de huid, verminderde weerstand tegen infecties, verlaagde groeisnelheid en verstoorde ontwikkeling, zouden mogelijk gedeeltelijk kunnen worden verklaard door EFAD. De EFA status kan verbeterd worden door de consumptie van lokaal verkrijgbare plantaardige oliën, vis, eieren, vlees en moedermelk.

- Ondervoede Pakistaanse kinderen lijden aan EFAD. Een lage visinname en lage moedermelk DHA gehaltes zijn verantwoordelijk voor hun lage DHA status. Het lage moedermelk DHA is op zijn beurt hoogst waarschijnlijk het gevolg van een lage visinname door de moeder. De lage DHA status van de kinderen kan verbeterd worden door FO suppletie. Suppletie van de moeder met ω3LCPUFA, bij voorkeur vanaf de vroege zwangerschap, zou in termen van preventie echter een betere optie zijn.

- De DHA status van ondervoede Pakistaanse kinderen die moedermelk krijgen is sterk afhankelijk van het ω3LCPUFA gehalte van de melk. Hun DHA status is beter dan die van tegenhangers die geen moedermelk krijgen. Dit voorziet ons van een nieuw argument om een langere borstvoedingsperiode aan te bevelen.

- Ofschoon er geen twijfel bestaat over het bestaan van competitie tussen ω3LCPUFA en ω6LCPUFA, laten enkele van de huidige studies zien dat ω3LCPUFA de ω6LCPUFA status niet noodzakelijkerwijs negatief beïnvloedt. Ten eerste zagen we in

Pakistaanse moeder-kind paren een positieve correlatie tussen het moedermelk DHA en het RBC AA van hun kind. Ten tweede, FO suppletie verlaagde ω6LCPUFA niet in ondervoede kinderen. Een verlaging trad slechts op indien het initieel AA hoog was. En tenslotte, suppletie met AA alleen had geen effect op melk AA, terwijl daarentegen een combinatie van AA en DHA zowel het melk AA als DHA verhoogde in goed gevoede Palestijnse vrouwen. Een unificerende hypothese zou kunnen zijn het bestaan van een functionele ω3/ω6 ratio, waarbij elke competitie tussen ω3 en ω6 wijst op een toestand van niet-functionele overschotten.

- De meeste moedermelk FA zijn onderhevig aan een grote biologische variatie, terwijl aan de andere kant veel van de FA nauw aan elkaar gerelateerd zijn. De grote variatie zaait twijfel over de bruikbaarheid van moedermelk als “gouden standaard” voor de productie van flesvoeding. Mogelijk voorzien de interrelaties tussen de moedermelk FA in een betere basis voor toekomstige aanbevelingen voor een “gehumaniseerde” flesvoeding.

- Biochemische EFA status parameters voor de bepaling van (sub-klinische) EFAD zijn waarschijnlijk gevoeliger en specifieker dan een diagnostiek gebaseerd op klinische symptomatologie. De voorgestelde RBC afkapwaarden zijn 0,46 mol% 20:3ω9 voor EFAD, 0,22 mol/mol 22:5ω6/22:6ω3 voor ω3-marginaliteit, 0,48 mol/mol 22:5ω6/22:6ω3 voor ω3-deficientie en 0,33 mol/mol 22:5ω6/22:4ω6 voor een lage ω3FA status. Deze afkapwaarden kunnen ons voorzien van handvaten in de beslissing om te starten met (LC)PUFA suppletie van de voeding, totdat zich betere concepten voordoen.

Summary for the layman

Fatty acids do not taste sour.

The fats we eat every day are actually composed of acids; so called fatty acids (FAs). They don't taste sour because they are bound to another molecule. Together with that molecule they form a fat molecule, giving our food that wonderful oily taste. FAs come in many shapes and forms; there are 200 or more of them. The different roles that FAs play in the body are still barely understood by scientists. FAs are known to be crucial in the development of the nervous system (like the brain and the nerves) and in the protection against viruses and bacteria. There are three distinct series of FAs called ω3, ω6 and ω9 (pronounce as omega 3, omega 6 and omega 9). With some help (enzymes etc) the human body can manufacture the more complicated FAs from the simpler FAs in the series. The ω3 and ω6 series start with a FA that cannot be manufactured by the body; the so-called Essential Fatty Acids (EFAs). They are so important because we can only get them from certain food items; food items that are not part of the diet in many developing countries. In the womb the baby receives these EFAs through the placenta. After birth, babies get them from human milk.
Soy oil and fish (oil) are rich in ω3 FAs. Before and after birth, these FAs play an important role in neural development of the young infant. In the long run these FAs are known to prevent cardiovascular diseases and hypertension. ω6 FAs are found in corn oil, sunflower oil, meat and eggs and are important for the growth of the human body.

This thesis focuses on different aspects of EFA deficiency in malnutrition in mothers and children in developing countries. Information was collected in Pakistan and Jerusalem when we lived there as a family. This information pertains to the health status of mothers and children, their eating habits, the FA composition of breastmilk and of blood of children. For comparison, the thesis uses additional information, collected by others scientists, about mothers and children in developed countries like the Netherlands, and developing countries like Tanzania and the Caribbean.

In the introductory and longest chapter, the thesis summarizes everything there is to know about EFAs. It contains some 350 references to the work of other scientists. The last part of this chapter shows that there is a relationship between EFA deficiency and malnutrition: malnutrition causes EFA deficiency, which in turn aggravates malnutrition.

Chapter 2 analyses the **FA status of malnourished children**. It turns out that malnourished Pakistani children indeed have very low levels of ω3 and ω6 in their blood. Also their body's ability to make complicated FAs from simpler ones seems to be impaired. This is explained by the fact that they get little (healthy) food to eat and drink. Also their mother's

milk does not contain a lot of ω3 and ω6 either. Many of the children also suffer from gastrointestinal infections leading to a decrease in the body's ability to absorb nutrients from food. In addition these children also suffer from vitamin E deficiency; without Vitamin E, EFAs disintegrate before the body can use them.
The author also finds that breastfeeding does play a positive role: breastfed Pakistani children have higher EFA levels than their non-breastfed friends. Nevertheless, breastfeeding in itself is not enough: most of these breastfed Pakistani kids had lower levels of ω3 and ω6 than healthy breastfed Dutch kids. Therefore, breastfeeding is recommended but it needs to be combined with a healthy diet of mothers during lactation and even pregnancy.
In an attempt to increase their ω3 status, a number of Pakistani children were given fish oil capsules for a period of time. Fish oil is rich in DHA (a more complicated EFA in the ω3 series). The thesis finds that the EFA status of these children actually improved as a result. The supplementation had a stunning effect on the growth of a 21-months old girl. At the start of the experiment she was 5.5 kilogrammes and the one with the lowest EFA levels of all. After 9 weeks of supplementation she had gained 1.8 kilogrammes of weight!

Chapter 3 deals with the **EFA composition of breastmilk**. The thesis proves that the EFA status of Pakistani kids depends strongly on the quality of their mother's milk, even when they grow older and start eating other things on the side. Therefore, the thesis recommends that mothers who do not get enough EFAs from their diets be given EFA supplements like fish oil and cod liver oil, preferably already from early pregnancy. A second conclusion is that even when the breastmilk is low in EFAs, breastfed kids do better than formula-fed kids. The thesis thus recommends breastfeeding and continued breastfeeding even when children are older and eat more solid foods.
In order to influence the EFA composition of human milk, a number of breastfeeding mothers were given supplements of different EFAs. Indeed, the EFA composition of their breastmilk improved as a result. By varying the proportions of different EFAs, it is expected that even better results could be achieved.
In a complicated statistical study, the thesis finds that the EFA composition of breastmilk differs highly between individual women and also between different countries and cultures. There is no such thing as the 'best' composition of breastmilk. Also, the thesis argues, there is no base for claims by infant formula producers that their formula closely resembles the real thing.
Finally, in this chapter the EFA content of infant formulas is analysed and compared with breastmilk samples from different countries. Infant formulas are subject to international standards that define how much of what has to be included. As far as EFAs are concerned, the thesis shows that the composition of formulas does not reflect that of breastmilk at all. It recommends that international standards should not only concern themselves with absolute quantities of FAs but should pay more attention to the relative proportions of FAs in formulas.

The last chapter of the thesis attempts to **define what constitutes EFA deficiency**. In other words, when do we call a person EFA deficient? The thesis proposes a cut-off value at which 2.5% of healthy Dutch persons falls into the 'EFA deficient' category. At that same cut-off value, the thesis finds that one out of every three Pakistani children is EFA deficient and for these children the thesis proposes EFA supplementation of their diets.

Samenvatting voor de leek

Vetzuren smaken niet zuur.

Het vet dat we dagelijks eten bestaat eigenlijk uit zuren; zogenaamde vetzuren (Engels: Fatty Acids; FAs). Ze smaken niet zuur omdat ze gebonden zijn aan een ander molecuul. Samen met dat molecuul vormen ze een vetmolecuul, wat ons eten die heerlijke vettige smaak geeft. FAs zijn er in vele soorten en maten; er bestaan op zijn minst 200 verschillende. Wetenschappers begrijpen nog maar nauwelijks de verschillende rollen die FAs spelen in het lichaam. Het is bekend dat FAs cruciaal zijn voor de ontwikkeling van het zenuwstelsel (zoals de hersenen en de zenuwen) en in de bescherming tegen virussen en bacteria. Er zijn drie verschillende series van FAs, genaamd ω3, ω6 and ω9 (spreek uit als: omega 3, omega 6 and omega 9). Met enige hulp (van bv. enzymen) kan het lichaam meer gecompliceerde FAs maken van de meer simpele in die serie. De ω3 en ω6 series beginnen met een FA dat niet door het lichaam zelf gemaakt kan worden; de zogenaamde essentiële vetzuren (EFAs). Deze zijn zo belangrijk omdat we ze alleen uit bepaalde voedingsmiddelen kunnen verkrijgen; producten die geen deel uitmaken van de voeding in veel ontwikkelingslanden. Voor de geboorte krijgt de baby deze EFAs via de placenta en na de geboorte via moedermelk.
Soyaolie en vis(olie) zijn rijk aan ω3 FAs. Voor en na de geboorte spelen deze FAs een belangrijke rol in de neurologische ontwikkeling van het jonge kind. Op de lange termijn hebben deze FAs een preventief effect op hartinfarcten en hoge bloeddruk. ω6 FAs kunnen worden gevonden in maisolie, zonnebloemolie, vlees en eieren en zijn belangrijk voor de groei.
Dit proefschrift belicht verschillende aspecten van EFA tekort van ondervoede moeders en kinderen in ontwikkelingslanden. Informatie is verzameld in Pakistan en Jeruzalem toen we daar als familie woonden. Deze informatie heeft betrekking op de gezondheidstoestand van moeders en kinderen, hun eetgewoonten, de FA samenstelling van moedermelk en van het bloed van de kinderen. Ter vergelijking wordt aanvullende informatie gebruikt, verzameld door andere wetenschappers, over moeders en kinderen in ontwikkelde landen zoals Nederland, en ontwikkelingslanden zoals Tanzania en het Caribisch gebied.
Het eerste en langste hoofdstuk van het proefschrift vat alles samen wat er over EFAs te weten valt. Het bevat zo'n 350 referenties naar werk van andere onderzoekers. Het laatste deel van dit hoofdstuk betoogt dat er een verband is tussen ondervoeding en EFA tekort: ondervoeding veroorzaakt EFA tekort, wat op zijn beurt ondervoeding verergert.

Hoofdstuk 2 analyseert de **FA status van ondervoede kinderen**. Het blijkt dat ondervoede Pakistaanse kinderen inderdaad lage gehaltes van ω3 en ω6 in hun bloed hebben. Ook de capaciteit van het lichaam om van de simpele de meer gecompliceerde FAs te maken, is verslechterd. Dit kan worden verklaard door het feit dat deze kinderen weinig (gezond) voedsel te eten en te drinken krijgen. Bovendien bevat de melk van hun moeders ook niet veel ω3 en ω6. Veel van deze kinderen hebben last van darminfecties, wat de opname van voedingsstoffen uit eten verlaagt. Ook hebben ze vaak een vitamine E tekort; zonder

vitamine E vallen EFAs uiteen voordat het lichaam ze kan gebruiken.
Het proefschrift vindt ook dat borstvoeding een positieve rol speelt: Borstgevoede Pakistaanse kinderen hebben hogere EFA gehaltes dan hun niet-borstgevoede vriendjes. Desondanks is borstvoeding op zichzelf niet genoeg: de meeste van de borstgevoede Pakistaanse kinderen hadden lagere gehaltes aan EFA dan gezonde borstgevoede Nederlandse kinderen. Daarom wordt borstvoeding aanbevolen, maar het moet ook gecombineerd worden met een gezond dieet van de moeder tijdens periode van borstvoeding en zelfs al tijdens de zwangerschap.
In een poging om hun ω3 status te verhogen, werd aan een aantal Pakistaanse kinderen voor een bepaalde periode visolie capsules gegeven. Visolie is rijk aan DHA (een meer gecompliceerd FA in de ω3 serie). De auteur constateert dat de EFA status van deze kinderen als gevolg hiervan verbeterde. De suppletie had een verbazend effect op de groei van een 21 maanden oud meisje: Aan het begin van de studie woog ze 5,5 kg en had ze de laagste EFA gehaltes van allemaal. Na negen weken was ze 1,8 kg zwaarder!

Hoofdstuk 3 behandelt de **EFA samenstelling van moedermelk**. Het boekje bewijst dat de EFA status van Pakistaanse kinderen sterk afhankelijk is van de kwaliteit van de melk van hun moeder, zelfs als ze ouder worden en er andere dingen naast gaan eten. Daarom doet het proefschrift de aanbeveling dat moeders die een lage inname van EFA uit voeding hebben, EFA supplementen krijgen zoals visolie en levertraan, bij voorkeur al vanaf vroeg in de zwangerschap. Een tweede conclusie is dat zelfs als de moedermelk laag is in EFAs, borstgevoede kinderen het beter doen dan flesgevoede. Borstvoeding wordt dus aanbevolen, ook voor een langere periode als de kinderen ouder worden en meer ander voedsel gaan eten.
Om de EFA samenstelling van borstvoeding te beïnvloeden, kreeg een aantal moeders een supplement van verschillende EFAs. Als gevolg hiervan verbeterde inderdaad de EFA samenstelling van hun melk. Door de verhoudingen van de verschillende EFAs te veranderen, zouden mogelijk nog betere resultaten kunnen worden bereikt.
In een gecompliceerde statistische studie concludeert het proefschrift dat de EFA samenstelling van moedermelk erg verschilt tussen vrouwen en ook tussen verschillende landen en culturen. Er bestaat niet zoiets als ‘de beste’ samenstelling van moedermelk. Het boekje betoogt ook dat er geen basis is voor claims door flessenmelkfabrikanten die stellen dat hun voeding sterk lijkt op moedermelk.
Ten slotte wordt in dit hoofdstuk de EFA samenstelling van flessenmelk geanalyseerd en vergeleken met moedermelk uit verschillende landen. Flessenmelk is onderhevig aan internationale standaarden die bepalen hoeveel van wat erin mag of moet zitten. Voor wat betreft EFAs laat dit proefschrift zien dat de samenstelling van flessenmelk bepaald niet die van moedermelk weerspiegelt. Er wordt de aanbeveling gedaan dat internationale standaarden niet alleen zouden moeten letten op absolute hoeveelheden van FAs, maar ook aandacht moeten geven aan de onderlinge verhoudingen van FAs in flessenmelk.

In het laatste hoofdstuk van dit boekje wordt een poging gedaan om **EFA tekort te definiëren**. Met andere woorden: wanneer noemen we iemand EFA deficiënt? Het proefschrift stelt een afkapwaarde voor waarbij 2,5% van gezonde Nederlandse personen in de EFA deficiënt categorie vallen. Bij deze afkapwaarde blijkt dat van elke drie Pakistaanse kinderen er één EFA deficiënt is. Voor deze kinderen wordt aanvulling van hun dieet met EFAs aangeraden.

Dankwoord (Acknowledgement)

My strength is in You Lord
My hope is in You Lord
All of my life is in You

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Lieve Janthomas, zonder jou had de inhoud van dit boekje er heel anders uitgezien. Ik denk niet dat er veel proefschriften zijn waarbij de partner zo’n grote invloed heeft gehad op de beschreven onderzoeken. Of ik je moet bedanken voor plaatsen zoals Pakistan, Palestine en Bosnie en Herzegovina weet ik niet. Mijn voorkeur gaat nog steeds uit naar Zuid-Oost-Azië en Zuid-Amerika. Voor de praktische hulp bij de lay-out van dit boekje en je kritische vragen ben ik je wel erg dankbaar. Je bent een schat (eindelijk plaats voor een niet objectieve opmerking).
Lieve Joep, Roos, Olivier en Pip, kan ik nu ook piano spelen??

Curriculum Vitae

Ella Smit werd geboren op 30 januari 1966 in Middelburg. Ze is getrouwd met Janthomas Hiemstra en heeft vier kinderen: Joep, Roos, Olivier en Pip. De eerste 5 jaar van haar leven bracht ze door op een binnenvaartschip (waarschijnlijk de reden waardoor ze nu nog steeds de voorkeur geeft aan de aanduidingen ‘stuurboord’ en ‘bakboord’ boven respectievelijk ‘rechts’ en ‘links’). In 1984 behaalde ze haar VWO diploma aan de Prof. Dr. S. Greydanusschool in Zwolle en een paar maanden later begon ze met de studie biologie aan de Rijksuniversiteit Groningen. Van juni tot december 1988 deed ze afstudeeronderzoek in Jakarta, Indonesië voor de vakgroep voeding. Na dit "buitenland-experiment" was ze ervan overtuigd dat ze, als de mogelijkheid zich voordeed, wilde wonen en werken in het buitenland. Het zou echter nog 3 jaar duren voor het zover was. In die tijd was ze verbonden aan de vakgroep voeding en in de zomer van 1991 rondde ze haar studie medische biologie af. Aan het eind van dat jaar verhuisde ze naar Islamabad, Pakistan. Daar werden kontakten gelegd met het Federal Government Services Hospital en werd, onder begeleiding van Prof. Dr. E.R. Boersma en Dr. F.A.J. Muskiet (destijds nog geen professor) in de vorm van een KAP (klein ambassade project), de basis voor dit proefschrift gelegd. Na tweeënhalf jaar verhuisde ze naar Jerusalem waar het onderzoek in ‘The Old City’ en Hebron werd voortgezet. Vier jaar later verhuisde ze naar Sarajevo, Bosnië en Herzegovina, waar het overgrote deel van dit proefschrift op papier werd gezet. Sinds juli 2001 woont ze op de Malediven waar ze momenteel werkzaam is in ‘the Ministry of Health’ en doorgaat met onderzoek naar de vetzuursamenstelling van rode bloedcellen van ondervoede en gezonde kinderen en van moedermelk. Het betreft hier een populatie met een voeding die eenzijdig is en voor een belangrijk deel bestaat uit vis (tonijn).

Appendix A. Breastmilk fatty acid compositions in different populations

A1. Milk FA of Dutch women

A2. Milk FA of Caribbean women

A3. Milk FA of Palestinian women

A4. Milk FA of Tanzanian women

A5. Milk FA of Pakistani women from the Islamabad area

Appendix A1. Milk FA of Dutch women, n=222*

	Mean	SD	Median	Min	Max	P2.5	P97.5
6:0	0.31	0.06	0.30	0.17	0.52	0.21	0.45
8:0	0.67	0.10	0.66	0.45	0.94	0.49	0.85
10:0	2.74	0.54	2.73	1.58	4.27	1.78	3.85
12:0	8.37	2.50	8.20	2.91	15.75	4.69	14.09
14:0	8.14	1.91	7.89	3.68	14.21	5.05	12.50
16:0	23.03	2.23	23.21	14.45	28.82	18.39	26.93
18:0	7.23	0.88	7.18	4.84	9.68	5.58	8.83
20:0	0.22	0.05	0.21	0.03	0.37	0.14	0.34
22:0	0.10	0.03	0.10	0.05	0.21	0.06	0.18
24:0	0.07	0.02	0.07	0.03	0.16	0.04	0.12
18:3ω3	1.10	0.33	1.02	0.64	2.71	0.68	1.90
20:5ω3	0.05	0.03	0.05	0.00	0.29	0.00	0.14
22:5ω3	0.12	0.03	0.12	0.08	0.24	0.08	0.17
22:6ω3	0.21	0.10	0.19	0.09	0.84	0.11	0.42
LCPω3	0.38	0.14	0.36	0.20	1.33	0.22	0.75
ω3	1.49	0.35	1.42	0.90	3.08	1.01	2.40
14:1ω5	0.37	0.11	0.37	0.03	0.69	0.17	0.61
ω5	0.37	0.11	0.37	0.03	0.69	0.17	0.61
18:2ω6	13.35	4.05	12.84	6.01	28.21	7.45	23.36
18:3ω6	0.10	0.04	0.09	0.03	0.20	0.04	0.18
20:2ω6	0.31	0.08	0.31	0.17	0.57	0.18	0.48
20:3ω6	0.35	0.08	0.33	0.18	0.78	0.23	0.50
20:4ω6	0.37	0.07	0.37	0.21	0.62	0.24	0.54
22:4ω6	0.07	0.02	0.07	0.04	0.16	0.05	0.12
22:5ω6	0.03	0.01	0.03	0.00	0.08	0.00	0.05
LCPω6	1.14	0.19	1.11	0.71	1.72	0.80	1.55
ω6	14.58	4.14	14.04	7.22	29.53	8.53	24.63
16:1ω7	2.42	0.66	2.33	0.76	4.99	1.33	3.85
18:1ω7	3.19	0.75	3.13	1.57	5.34	1.78	4.78
ω7	5.61	1.09	5.50	2.66	9.40	3.83	7.90
18:1ω9	26.58	2.83	26.49	19.06	34.47	20.98	32.20
20:1ω9	0.38	0.07	0.37	0.22	0.69	0.26	0.57
20:3ω9	0.05	0.02	0.05	0.00	0.09	0.00	0.08
24:1ω9	0.05	0.03	0.04	0.00	0.46	0.02	0.08
ω9	27.05	2.87	26.99	19.48	35.11	21.35	32.76
SAFA	50.89	4.66	50.96	39.25	63.37	41.87	59.96
MCSAFA	20.23	4.77	19.89	9.19	35.20	12.53	30.22
MUFA	32.99	3.37	33.04	22.20	44.74	26.47	39.31
PUFA	16.12	4.24	15.53	8.41	32.15	9.69	26.36
ω3/ω6	0.11	0.03	0.10	0.04	0.28	0.05	0.18
LCPω3/LCPω6	0.35	0.15	0.31	0.17	1.50	0.19	0.70
20:3ω9/20:4ω6	0.13	0.04	0.13	0.00	0.23	0.00	0.21
18:2ω6/18:3ω3	12.69	4.38	11.81	4.77	30.55	6.83	23.92
20:4ω6/22:6ω3	2.01	0.58	2.04	0.30	3.75	0.80	3.02

*: Milk of 99 women collected at postnatal day 14, of 98 women at day 42 and of 25 women at day 89, resulting in a total of 222 samples.
Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MCSAFA: 6:0-14:0; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix A2. Milk FA of Caribbean women, n=159*

	Mean	SD	Median	Min	Max	P2.5	P97.5
6:0	0.19	0.08	0.17	0.03	0.48	0.07	0.35
8:0	0.69	0.22	0.67	0.24	1.76	0.32	1.22
10:0	3.63	0.84	3.62	0.57	6.15	2.11	5.35
12:0	14.22	4.44	13.82	4.12	34.90	6.90	22.86
14:0	11.91	3.82	11.54	4.09	26.00	5.80	19.55
16:0	21.09	2.49	20.89	14.29	29.21	16.61	25.68
18:0	5.49	1.24	5.45	2.14	8.77	2.98	8.20
20:0	0.22	0.10	0.20	0.07	0.91	0.10	0.46
22:0	0.10	0.05	0.09	0.00	0.34	0.04	0.20
24:0	0.08	0.04	0.07	0.00	0.31	0.03	0.14
18:3ω3	0.72	0.30	0.67	0.27	2.00	0.32	1.54
20:5ω3	0.05	0.05	0.05	0.00	0.36	0.00	0.15
22:5ω3	0.14	0.05	0.13	0.00	0.31	0.06	0.27
22:6ω3	0.38	0.23	0.33	0.09	1.63	0.13	1.00
LCPω3	0.57	0.28	0.52	0.16	1.68	0.22	1.40
ω3	1.08	0.53	0.98	0.16	2.79	0.38	2.35
14:1ω5	0.24	0.10	0.23	0.05	0.52	0.11	0.47
ω5	0.24	0.10	0.23	0.05	0.52	0.11	0.47
18:2ω6	11.74	3.85	11.26	3.51	25.94	6.18	19.83
18:3ω6	0.08	0.04	0.07	0.00	0.23	0.03	0.16
20:2ω6	0.36	0.14	0.32	0.08	0.99	0.17	0.66
20:3ω6	0.38	0.09	0.38	0.20	0.68	0.22	0.57
20:4ω6	0.51	0.14	0.50	0.19	0.99	0.30	0.86
22:4ω6	0.13	0.06	0.12	0.00	0.50	0.06	0.30
22:5ω6	0.05	0.03	0.05	0.00	0.18	0.00	0.11
LCPω6	1.44	0.39	1.40	0.59	3.25	0.85	2.33
ω6	13.26	4.08	12.80	4.14	27.36	7.20	22.15
16:1ω7	2.70	0.96	2.58	0.89	5.89	1.22	4.96
18:1ω7	3.06	0.92	2.98	0.79	7.63	1.54	4.76
ω7	5.76	1.43	5.55	1.96	10.34	3.43	9.18
18:1ω9	21.53	4.27	21.38	7.17	34.59	14.15	29.77
20:1ω9	0.41	0.15	0.38	0.06	1.10	0.20	0.76
20:3ω9	0.06	0.03	0.06	0.00	0.20	0.00	0.14
24:1ω9	0.06	0.04	0.05	0.00	0.27	0.00	0.14
ω9	22.06	4.32	21.84	7.28	35.63	14.52	30.26
SAFA	57.61	7.33	57.47	37.78	85.43	44.23	70.88
MCSAFA	30.63	8.72	30.62	9.90	67.92	16.47	48.50
MUFA	28.00	5.02	28.06	9.54	42.91	18.50	38.09
PUFA	14.39	4.08	13.93	5.03	27.82	8.07	23.08
ω3/ω6	0.09	0.05	0.09	0.01	0.27	0.02	0.22
LCPω3/LCPω6	0.42	0.29	0.35	0.10	2.48	0.18	1.10
20:3ω9/20:4ω6	0.11	0.07	0.11	0.00	0.47	0.00	0.27
18:2ω6/18:3ω3	15.50	4.33	14.53	9.13	32.91	9.82	25.79
20:4ω6/22:6ω3	1.64	0.67	1.60	0.15	3.83	0.35	2.98

*: Includes samples from Antigua (n=23), Belize (n=10), Curaçao (n=47), Dominica (n=17), St. Lucia (n=12), St. Vincent (n=30), Surinam (n=20). 18:3ω3 was not analysed in the samples from Curaçao.
Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MCSAFA: 6:0-14:0; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix A3. Milk FA of Palestinian women, n=63

	Mean	SD	Median	Min	Max	P2.5	P97.5
6:0	0.34	0.11	0.32	0.07	0.69	0.13	0.54
8:0	0.62	0.21	0.57	0.11	1.30	0.30	1.14
10:0	2.94	0.92	2.80	0.75	5.58	1.28	5.25
12:0	10.06	3.27	9.67	2.14	16.53	4.16	16.01
14:0	8.27	3.05	7.98	1.57	15.93	3.07	14.68
16:0	19.25	3.14	18.97	12.68	28.19	13.79	26.80
18:0	4.99	0.97	4.93	2.57	8.11	3.56	6.79
20:0	0.15	0.03	0.14	0.08	0.23	0.09	0.22
22:0	0.08	0.02	0.07	0.02	0.13	0.04	0.12
24:0	0.06	0.02	0.06	0.03	0.13	0.03	0.10
18:3ω3	1.01	0.37	0.97	0.46	2.01	0.52	1.76
20:5ω3	0.04	0.03	0.04	0.00	0.16	0.00	0.13
22:5ω3	0.11	0.04	0.10	0.05	0.23	0.05	0.17
22:6ω3	0.18	0.07	0.16	0.08	0.49	0.09	0.33
LCPω3	0.33	0.12	0.32	0.13	0.78	0.16	0.65
ω3	1.33	0.42	1.25	0.73	2.43	0.76	2.26
14:1ω5	0.14	0.07	0.12	0.04	0.36	0.04	0.28
ω5	0.14	0.07	0.12	0.04	0.36	0.04	0.28
18:2ω6	17.73	4.08	16.57	10.48	30.03	11.96	25.51
18:3ω6	0.16	0.07	0.15	0.00	0.33	0.06	0.31
20:2ω6	0.31	0.09	0.28	0.16	0.63	0.18	0.58
20:3ω6	0.44	0.12	0.42	0.22	0.78	0.26	0.69
20:4ω6	0.49	0.10	0.48	0.28	0.81	0.34	0.74
22:4ω6	0.10	0.03	0.10	0.05	0.20	0.06	0.16
22:5ω6	0.04	0.02	0.04	0.00	0.12	0.02	0.07
LCPω6	1.38	0.26	1.34	0.91	2.11	0.96	2.04
ω6	19.27	4.15	18.18	11.99	31.35	13.37	27.36
16:1ω7	1.92	0.80	1.79	0.65	4.21	0.83	3.85
18:1ω7	1.90	0.56	1.74	0.73	4.00	1.13	2.98
ω7	3.82	1.07	3.73	1.65	6.51	2.05	6.25
18:1ω9	28.33	4.71	28.14	18.46	40.05	21.12	38.63
20:1ω9	0.27	0.06	0.26	0.15	0.54	0.17	0.38
20:3ω9	0.05	0.02	0.05	0.00	0.09	0.02	0.08
24:1ω9	0.05	0.02	0.05	0.02	0.10	0.02	0.09
ω9	28.69	4.74	28.61	18.78	40.45	21.43	39.00
SAFA	46.75	6.01	46.48	34.49	58.98	35.88	57.17
MCSAFA	22.23	6.94	21.74	4.65	35.25	9.44	34.89
MUFA	32.60	4.97	33.18	21.85	46.60	24.80	42.60
PUFA	20.65	4.28	19.90	12.93	32.24	14.62	29.54
ω3/ω6	0.07	0.02	0.07	0.03	0.13	0.03	0.11
LCPω3/LCPω6	0.24	0.07	0.24	0.10	0.42	0.12	0.39
20:3ω9/20:4ω6	0.10	0.04	0.09	0.00	0.20	0.05	0.15
18:2ω6/18:3ω3	19.62	7.79	17.24	9.02	40.85	10.01	39.14
20:4ω6/22:6ω3	3.09	1.04	2.88	1.60	5.71	1.69	5.38

Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MCSAFA: 6:0-14:0; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix A4. Milk FA of Tanzanian women, n=11

	Mean	SD	Median	Min	Max	P2.5	P97.5
6:0	0.35	0.09	0.35	0.21	0.54	0.21	0.51
8:0	0.87	0.16	0.86	0.66	1.26	0.68	1.18
10:0	3.72	0.68	3.83	2.17	4.84	2.45	4.70
12:0	19.31	5.89	19.87	11.28	28.43	11.62	27.87
14:0	15.21	5.93	13.22	7.75	27.61	7.99	26.20
16:0	19.69	3.78	18.57	14.16	28.65	15.04	27.42
18:0	3.25	1.01	3.63	1.08	4.44	1.41	4.36
20:0	0.09	0.02	0.10	0.05	0.12	0.06	0.12
22:0	0.05	0.02	0.05	0.00	0.07	0.01	0.07
24:0	0.04	0.02	0.05	0.00	0.07	0.01	0.06
18:3ω3	0.88	0.41	0.82	0.44	1.84	0.47	1.69
20:5ω3	0.16	0.34	0.06	0.00	1.18	0.01	0.91
22:5ω3	0.12	0.06	0.11	0.00	0.21	0.02	0.20
22:6ω3	0.21	0.09	0.17	0.10	0.40	0.11	0.38
LCPω3	0.48	0.38	0.40	0.10	1.54	0.15	1.31
ω3	1.37	0.51	1.17	0.54	2.16	0.65	2.15
14:1ω5	0.20	0.08	0.18	0.10	0.36	0.11	0.35
ω5	0.20	0.08	0.18	0.10	0.36	0.11	0.35
18:2ω6	12.54	5.13	12.47	5.15	23.56	5.43	21.71
18:3ω6	0.10	0.05	0.10	0.05	0.22	0.05	0.20
20:2ω6	0.24	0.09	0.23	0.11	0.39	0.11	0.38
20:3ω6	0.33	0.08	0.32	0.18	0.50	0.20	0.48
20:4ω6	0.50	0.12	0.49	0.31	0.71	0.33	0.69
22:4ω6	0.10	0.02	0.10	0.07	0.12	0.07	0.12
22:5ω6	0.05	0.02	0.06	0.00	0.07	0.01	0.07
LCPω6	1.22	0.23	1.30	0.73	1.46	0.77	1.45
ω6	13.86	5.32	13.92	5.92	25.06	6.27	23.20
16:1ω7	2.25	1.02	1.94	1.28	5.07	1.30	4.46
18:1ω7	2.08	1.29	1.48	0.50	4.53	0.56	4.28
ω7	4.33	1.83	3.96	1.83	7.75	1.88	7.42
18:1ω9	17.44	5.15	17.31	7.83	23.94	9.13	23.59
20:1ω9	0.16	0.06	0.15	0.06	0.26	0.07	0.25
20:3ω9	0.05	0.02	0.05	0.03	0.08	0.03	0.08
24:1ω9	0.01	0.02	0.00	0.00	0.04	0.00	0.04
ω9	17.66	5.21	17.64	7.95	24.23	9.26	23.88
SAFA	62.57	9.12	63.89	50.47	76.47	50.81	75.97
MCSAFA	39.46	11.61	39.72	23.91	59.14	24.76	57.58
MUFA	22.14	6.70	22.37	9.95	30.86	11.44	30.65
PUFA	15.28	5.54	15.57	7.12	26.11	7.32	24.52
ω3/ω6	0.11	0.04	0.11	0.04	0.19	0.05	0.18
LCPω3/LCPω6	0.38	0.24	0.32	0.11	1.06	0.14	0.91
20:3ω9/20:4ω6	0.11	0.04	0.11	0.04	0.19	0.05	0.18
18:2ω6/18:3ω3	16.33	10.19	13.55	6.04	42.89	6.72	38.32
20:4ω6/22:6ω3	2.69	1.09	2.24	1.52	4.77	1.57	4.55

Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MCSAFA: 6:0-14:0; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix A5. Milk FA of Pakistani women from the Islamabad area, n=10

	Mean	SD	Median	Min	Max	P2.5	P97.5
6:0	0.30	0.09	0.32	0.16	0.42	0.16	0.41
8:0	0.53	0.16	0.46	0.28	0.77	0.32	0.76
10:0	2.41	0.60	2.28	1.43	3.32	1.55	3.30
12:0	9.54	2.15	10.03	5.05	11.87	5.44	11.77
14:0	10.67	2.71	10.99	4.94	14.04	5.73	13.96
16:0	28.12	4.31	27.94	18.99	34.36	20.37	34.08
18:0	5.39	1.32	5.20	3.97	7.70	4.01	7.65
20:0	0.16	0.03	0.17	0.12	0.19	0.12	0.19
22:0	0.07	0.02	0.07	0.05	0.11	0.05	0.11
24:0	0.06	0.02	0.06	0.03	0.09	0.03	0.09
18:3ω3	0.49	0.48	0.34	0.25	1.84	0.25	1.54
20:5ω3	0.03	0.03	0.02	0.00	0.09	0.00	0.08
22:5ω3	0.07	0.04	0.05	0.04	0.14	0.04	0.14
22:6ω3	0.08	0.05	0.06	0.03	0.19	0.03	0.18
LCPω3	0.18	0.10	0.14	0.09	0.38	0.09	0.36
ω3	0.67	0.55	0.53	0.37	2.21	0.37	1.86
14:1ω5	0.15	0.06	0.13	0.07	0.27	0.08	0.25
ω5	0.15	0.06	0.13	0.07	0.27	0.08	0.25
18:2ω6	9.99	4.59	8.73	7.13	22.71	7.20	19.99
18:3ω6	0.06	0.04	0.05	0.00	0.12	0.01	0.11
20:2ω6	0.18	0.07	0.16	0.14	0.37	0.14	0.33
20:3ω6	0.23	0.08	0.21	0.15	0.36	0.16	0.36
20:4ω6	0.29	0.09	0.26	0.20	0.44	0.20	0.44
22:4ω6	0.06	0.01	0.06	0.04	0.08	0.04	0.08
22:5ω6	0.02	0.02	0.02	0.00	0.05	0.00	0.04
LCPω6	0.78	0.23	0.69	0.57	1.30	0.58	1.22
ω6	10.82	4.80	9.35	7.78	24.10	7.88	21.31
16:1ω7	2.42	1.15	2.23	1.20	5.02	1.23	4.68
18:1ω7	4.02	1.39	4.05	2.22	5.80	2.23	5.78
ω7	6.44	1.62	6.17	4.35	9.31	4.42	9.06
18:1ω9	24.34	2.14	24.19	21.22	28.94	21.56	28.27
20:1ω9	0.25	0.03	0.25	0.21	0.32	0.21	0.31
20:3ω9	0.06	0.01	0.06	0.03	0.07	0.03	0.07
24:1ω9	0.04	0.02	0.04	0.02	0.07	0.02	0.07
ω9	24.68	2.16	24.53	21.52	29.31	21.87	28.62
SAFA	57.24	4.87	57.75	47.33	62.78	48.48	62.57
MCSAFA	23.44	4.92	25.36	12.77	27.79	13.75	27.74
MUFA	31.21	3.16	30.93	26.31	35.77	26.69	35.64
PUFA	11.55	5.34	9.96	8.22	26.36	8.35	23.20
ω3/ω6	0.06	0.02	0.05	0.04	0.09	0.04	0.09
LCPω3/LCPω6	0.23	0.10	0.20	0.11	0.44	0.12	0.41
20:3ω9/20:4ω6	0.20	0.05	0.20	0.11	0.29	0.12	0.28
18:2ω6/18:3ω3	24.87	6.87	26.90	12.37	33.94	13.55	33.67
20:4ω6/22:6ω3	4.24	1.94	4.23	1.65	7.78	1.77	7.40

Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MCSAFA: 6:0-14:0; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B. Erythrocyte fatty acid compositions in different populations

B1. RBC FA of Dutch adults

B2. RBC FA of Dutch vegans

B3. RBC FA of 3.5 year old Dutch children

B4. RBC FA of well-nourished, breastfed, Palestinian babies from 'The Old City' of Jerusalem

B5. RBC FA of Pakistani children from the Islamabad area

B6. RBC FA of malnourished Pakistani children from the Islamabad area

B7. RBC FA of well-nourished Pakistani children from the Islamabad area

B8. RBC FA of breastfed Pakistani children from the Islamabad area

B9. RBC FA of non-breastfed Pakistani children from the Islamabad area

B10. RBC FA of Dutch LBW infants fed human milk

B11. RBC FA of Dutch LBW infants fed formula with LCP

B12. RBC FA of Dutch LBW infants fed formula without LCP

Appendix B1. RBC FA of Dutch adults							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (years)	35.0	8.9	37	22	61	22.0	51.7
Weight (kg)*	71.7	10.5	70	56	88	55.0	88.0
Length (m)*	1.80	0.08	1.77	1.69	1.95	1.69	1.95
BMI (kg/m2)*	22.2	2.7	22.1	18.1	29.1	17.6	27.5
Sex (m/f)	29/34						
14:0	0.43	0.08	0.43	0.30	0.63	0.26	0.54
16:0	23.80	0.78	23.77	22.50	25.80	22.08	25.30
18:0	16.46	0.66	16.41	15.18	18.19	14.97	17.78
20:0	0.44	0.06	0.44	0.36	0.56	0.07	0.53
22:0	1.86	0.18	1.85	1.50	2.27	1.37	2.17
24:0	4.56	0.38	4.62	3.88	5.44	3.78	5.19
26:0	0.23	0.04	0.23	0.17	0.33	0.16	0.30
18:3ω3	0.19	0.05	0.18	0.13	0.34	0.00	0.28
20:5ω3	0.47	0.19	0.44	0.23	1.26	0.22	0.93
22:5ω3	1.89	0.29	1.85	1.40	2.69	1.29	2.46
22:6ω3	3.77	0.86	3.75	2.29	6.37	2.02	5.45
LCPω3	6.14	1.03	6.17	4.39	9.93	3.99	8.28
ω3	6.33	1.02	6.38	4.59	9.93	4.26	8.44
18:2ω6	10.42	1.32	10.28	8.26	13.23	7.39	13.03
18:3ω6	0.04	0.05	0.03	0.00	0.15	0.00	0.14
20:2ω6	0.27	0.05	0.26	0.18	0.46	0.17	0.42
20:3ω6	1.60	0.25	1.57	1.23	2.19	1.17	2.14
20:4ω6	13.92	1.07	13.87	12.15	16.69	11.70	15.91
22:4ω6	2.78	0.43	2.77	1.99	3.76	1.64	3.53
22:5ω6	0.50	0.10	0.50	0.35	0.79	0.33	0.70
LCPω6	18.81	1.27	18.73	16.42	21.91	15.93	21.42
ω6	29.51	1.56	29.31	26.48	33.16	25.07	31.87
16:1ω7	0.25	0.09	0.23	0.14	0.44	0.14	0.43
18:1ω7	1.77	0.25	1.76	1.36	2.38	1.25	2.23
ω7	1.82	0.24	1.84	1.40	2.38	1.33	2.31
18:1ω9	10.62	0.67	10.76	9.16	11.95	8.88	11.48
20:1ω9	0.22	0.05	0.22	0.14	0.44	0.14	0.31
20:3ω9	0.26	0.08	0.25	0.13	0.43	0.11	0.42
22:3ω9	0.09	0.03	0.08	0.05	0.16	0.04	0.14
24:1ω9	3.39	0.42	3.37	2.65	4.44	2.48	4.29
ω9	14.56	0.87	14.48	13.05	16.17	12.10	15.93
SAFA	47.78	0.61	47.76	46.68	49.17	46.59	48.88
MUFA	16.07	0.95	16.10	14.44	17.87	13.32	17.54
PUFA	36.15	0.98	36.13	34.39	38.34	33.65	37.72
P/S	0.76	0.03	0.76	0.72	0.80	0.69	0.80
ω3/ω6	0.22	0.05	0.21	0.15	0.40	0.13	0.31
LCPω3/LCPω6	0.33	0.07	0.32	0.22	0.59	0.20	0.48
20:3ω9/20:4ω6	0.02	0.01	0.02	0.01	0.03	0.01	0.03
22:5ω6/22:4ω6	0.18	0.03	0.18	0.13	0.27	0.14	0.24
22:5ω6/22:6ω3	0.14	0.04	0.13	0.05	0.25	0.07	0.22
20:4ω6/22:6ω3	3.91	1.06	3.74	2.04	6.97	2.53	6.45

*: weight, length, BMI, 18:3ω6, 16:1ω7: n=15.

Abbreviations: BMI: body mass index: weight/lenght^2 ; LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3. SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B2. RBC FA of Dutch vegans, n=12							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (years)*	37.8	11.8	32.0	25	57	25.8	56.5
Weight (kg)**	64.0	6.7	63.5	54	78	55.1	75.7
Length (m)**	1.76	0.07	1.76	1.68	1.90	1.68	1.88
BMI (kg/m2)**	20.6	2.0	20.2	18.0	24.1	18.2	23.8
Sex (m/f)	8/4						
14:0	0.38	0.16	0.36	0.19	0.83	0.20	0.73
16:0	21.08	6.72	22.93	0.12	25.18	5.93	25.00
18:0	17.46	1.50	17.20	15.79	21.60	15.93	20.70
20:0	0.42	0.07	0.41	0.32	0.56	0.33	0.55
22:0	1.78	0.31	1.70	1.32	2.57	1.38	2.41
24:0	4.79	0.69	4.54	4.08	6.07	4.11	6.06
26:0	0.29	0.04	0.30	0.23	0.33	0.24	0.33
18:3ω3	0.13	0.11	0.16	0.00	0.30	0.00	0.30
20:5ω3	0.22	0.20	0.27	0.00	0.49	0.00	0.49
22:5ω3	3.74	0.76	3.71	2.32	4.92	2.48	4.81
22:6ω3	2.04	0.87	1.59	0.98	3.63	1.06	3.46
LCPω3	6.00	0.97	5.79	4.89	8.33	4.94	8.00
ω3	6.13	1.04	5.82	5.09	8.61	5.13	8.29
18:2ω6	11.61	1.67	11.20	10.17	16.28	10.19	15.36
18:3ω6	0.05	0.04	0.06	0.00	0.11	0.00	0.10
20:2ω6	0.42	0.13	0.41	0.22	0.71	0.23	0.66
20:3ω6	1.60	0.84	1.56	0.19	3.09	0.19	2.96
20:4ω6	14.24	1.09	14.22	12.40	16.53	12.55	16.16
22:4ω6	1.60	0.73	1.59	0.64	2.96	0.65	2.80
22:5ω6	0.55	0.14	0.54	0.30	0.73	0.32	0.72
LCPω6	17.99	2.00	18.02	15.66	23.32	15.72	22.10
ω6	30.07	3.30	29.28	27.54	40.11	27.74	37.56
16:1ω7	0.21	0.13	0.18	0.04	0.43	0.05	0.42
18:1ω7	1.55	0.26	1.48	1.20	1.93	1.21	1.92
ω7	1.76	0.32	1.68	1.26	2.32	1.32	2.28
18:1ω9	12.05	0.83	12.40	10.81	13.38	10.86	13.19
20:1ω9	0.30	0.09	0.29	0.20	0.53	0.20	0.48
20:3ω9	0.24	0.06	0.24	0.14	0.33	0.15	0.32
22:3ω9	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24:1ω9	3.25	1.36	3.68	0.20	4.33	0.31	4.27
ω9	15.84	1.25	15.73	13.35	17.41	13.70	17.39
SAFA	46.21	4.52	47.66	32.07	48.56	35.85	48.51
MUFA	17.36	1.13	17.05	15.26	18.93	15.61	18.88
PUFA	36.44	4.02	35.43	33.83	49.01	34.02	45.48
P/S	0.81	0.23	0.75	0.71	1.53	0.71	1.32
ω3/ω6	0.20	0.03	0.20	0.16	0.27	0.17	0.26
LCPω3/LCPω6	0.33	0.05	0.33	0.27	0.46	0.27	0.43
20:3ω9/20:4ω6	0.02	0.00	0.02	0.01	0.02	0.01	0.02
22:5ω6/22:4ω6	0.43	0.27	0.34	0.15	1.01	0.17	0.96
22:5ω6/22:6ω3	0.33	0.18	0.30	0.10	0.68	0.11	0.64
20:4ω6/22:6ω3	8.12	3.02	9.07	3.56	12.63	3.86	12.33

*: age: n=11; **weight, length, BMI: n=8.

Abbreviations: BMI: body mass index: weight/lenght^2 ; LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B3. RBC FA of 3.5 year old Dutch children, n=33							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (years)	3.5	0.0	3.5	3.5	3.5	3.5	3.5
Weight (kg)*	15.6	2.0	15.5	11.7	21.3	12.7	18.7
Length (cm)**	100.9	4.0	100.5	90.5	109.0	94.4	108.6
BMI (kg/m2)*	15.3	1.4	15.0	13.5	20.3	13.7	18.2
Sex (m/f)	17/16						
14:0	0.46	0.12	0.43	0.32	0.84	0.32	0.75
16:0	23.72	0.55	23.72	22.48	25.00	22.62	24.74
18:0	16.97	0.52	16.91	15.85	18.19	16.06	18.14
20:0	0.46	0.05	0.46	0.34	0.61	0.38	0.55
22:0	2.14	0.17	2.15	1.81	2.67	1.85	2.40
24:0	4.50	0.31	4.44	3.77	5.16	4.03	5.14
26:0	0.22	0.09	0.21	0.02	0.68	0.13	0.35
18:3ω3	0.19	0.04	0.19	0.09	0.27	0.11	0.25
20:5ω3	0.36	0.13	0.34	0.20	0.84	0.20	0.66
22:5ω3	1.84	0.24	1.83	1.42	2.39	1.44	2.31
22:6ω3	2.91	0.47	2.87	2.01	3.94	2.11	3.88
LCPω3	5.11	0.65	5.04	3.76	6.86	3.86	6.13
ω3	5.30	0.66	5.27	3.88	7.11	4.01	6.32
18:2ω6	10.33	1.34	10.10	7.79	13.46	8.39	12.95
20:2ω6	0.25	0.05	0.25	0.16	0.37	0.17	0.35
20:3ω6	1.64	0.30	1.59	1.21	2.29	1.22	2.20
20:4ω6	14.35	0.68	14.37	12.59	15.26	12.97	15.26
22:4ω6	2.91	0.37	2.95	2.10	3.73	2.26	3.63
22:5ω6	0.67	0.10	0.66	0.50	1.01	0.50	0.84
LCPω6	19.57	0.79	19.60	17.53	20.79	17.99	20.72
ω6	30.15	1.17	30.16	28.47	33.96	28.69	32.65
18:1ω7	1.55	0.19	1.52	1.12	2.16	1.29	1.98
ω7	1.55	0.19	1.52	1.12	2.16	1.29	1.98
18:1ω9	10.80	0.64	10.83	9.03	11.90	9.39	11.88
20:1ω9	0.22	0.05	0.22	0.13	0.39	0.14	0.35
20:3ω9	0.28	0.07	0.27	0.13	0.51	0.17	0.46
22:3ω9	0.08	0.04	0.07	0.04	0.20	0.04	0.17
24:1ω9	3.14	0.35	3.14	2.54	3.80	2.58	3.79
ω9	14.53	0.87	14.53	12.27	16.00	12.63	15.92
SAFA	48.47	0.69	48.61	46.63	50.00	46.83	49.88
MUFA	15.72	0.89	15.66	13.42	17.14	13.82	17.13
PUFA	35.81	0.90	35.80	34.00	38.14	34.40	37.81
P/S	0.74	0.02	0.74	0.69	0.80	0.69	0.79
ω3/ω6	0.18	0.03	0.17	0.11	0.25	0.13	0.22
LCPω3/LCPω6	0.26	0.04	0.26	0.18	0.36	0.20	0.34
20:3ω9/20:4ω6	0.02	0.01	0.02	0.01	0.04	0.01	0.03
22:5ω6/22:4ω6	0.23	0.03	0.23	0.17	0.29	0.16	0.29
22:5ω6/22:6ω3	0.24	0.06	0.23	0.16	0.40	0.15	0.37
20:4ω6/22:6ω3	5.06	0.88	4.91	3.76	7.08	3.66	7.00

*: Weight, BMI, n=30; ** length, n=32. Abbreviations: BMI: body mass index: weight/lenght^2; LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B4. RBC FA of well-nourished, breastfed, Palestinian babies from 'The Old City' of Jerusalem, n=31							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (months)*	3.2	1.7	3	1	6	1.0	6.0
Weight (kg)	5.78	1.13	6.04	3.99	7.55	3.95	7.53
Weight for age (%)*	100	9	99	85	124	84	119
Sex (m/f)	10/21						
14:0	0.63	0.16	0.59	0.41	1.07	0.34	0.98
16:0	24.41	1.23	24.20	22.80	29.00	22.61	27.82
18:0	17.64	1.32	17.45	16.12	22.08	16.01	21.66
20:0	0.60	0.09	0.59	0.48	0.89	0.43	0.80
22:0	1.55	0.15	1.54	1.32	1.92	1.31	1.85
24:0	4.20	0.44	4.14	3.48	4.97	3.45	4.96
26:0	0.27	0.09	0.27	0.16	0.49	0.14	0.45
18:3ω3	0.11	0.05	0.12	0.02	0.25	0.02	0.19
20:5ω3	0.17	0.07	0.16	0.06	0.30	0.04	0.28
22:5ω3	1.10	0.34	1.21	0.46	1.63	0.35	1.56
22:6ω3	4.44	0.64	4.51	2.95	5.40	2.48	5.30
LCPω3	5.71	0.87	5.81	3.89	6.95	2.88	6.85
ω3	5.82	0.88	5.96	3.99	7.06	2.90	6.94
18:2ω6	8.51	1.25	8.37	6.72	12.04	6.35	11.14
20:2ω6	0.32	0.07	0.32	0.19	0.46	0.18	0.43
20:3ω6	1.85	0.45	1.71	1.28	3.11	1.16	2.85
20:4ω6	13.78	1.45	13.96	10.06	16.14	9.16	15.97
22:4ω6	2.96	0.49	2.84	2.24	4.49	2.23	3.93
22:5ω6	1.00	0.30	0.90	0.69	1.65	0.69	1.63
LCPω6	19.60	1.81	19.97	14.41	21.97	13.72	21.72
ω6	28.43	2.34	28.90	21.32	30.65	20.29	30.43
18:1ω7	1.61	0.32	1.52	1.22	2.51	1.15	2.39
ω7	1.61	0.32	1.52	1.22	2.51	1.15	2.39
18:1ω9	10.36	0.83	10.33	8.82	12.43	8.67	11.87
20:1ω9	0.20	0.05	0.19	0.13	0.32	0.09	0.30
20:3ω9	0.31	0.10	0.32	0.16	0.53	0.15	0.50
22:3ω9	0.23	0.04	0.23	0.17	0.28	0.16	0.28
24:1ω9	3.88	0.53	3.80	2.96	5.00	2.86	4.68
ω9	14.84	1.13	14.99	13.15	17.92	12.65	16.71
SAFA	49.31	2.66	48.72	46.56	58.80	45.71	57.52
MUFA	16.05	1.28	16.15	14.13	19.07	14.09	18.64
PUFA	34.65	2.87	35.32	25.87	37.25	23.83	36.89
P/S	0.71	0.08	0.72	0.44	0.78	0.42	0.78
ω3/ω6	0.20	0.03	0.20	0.14	0.25	0.14	0.24
LCPω3/LCPω6	0.29	0.04	0.30	0.21	0.35	0.20	0.35
20:3ω9/20:4ω6	0.02	0.01	0.02	0.01	0.04	0.01	0.04
22:5ω6/22:4ω6	0.34	0.08	0.32	0.24	0.58	0.24	0.50
22:5ω6/22:6ω3	0.23	0.09	0.20	0.13	0.46	0.14	0.43
20:4ω6/22:6ω3	3.14	0.33	3.01	2.71	4.08	2.72	3.85

*: Weight, weight for age, n=29. Weight for age based on figures from National Centre of Health Statistics, USA.
Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B5. RBC FA of Pakistani children from the Islamabad area, n=116							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (months)	18.8	12.6	15.0	2	60	3.9	54.3
Weight (kg)	7.2	2.4	6.9	3.3	17.0	3.7	13.6
Weight for age (%)*	67	15	63	37	111	41	102
Sex (m/f)	57/59						
14:0	0.53	0.18	0.51	0.22	1.36	0.28	0.96
16:0	25.56	2.47	25.25	18.69	36.53	22.17	32.96
18:0	16.29	1.32	16.09	12.74	21.45	14.43	20.71
20:0	0.44	0.11	0.43	0.25	1.26	0.30	0.62
22:0	1.77	0.32	1.78	0.90	2.90	1.07	2.36
24:0	4.35	0.69	4.26	2.60	6.59	3.03	5.57
26:0	0.27	0.10	0.25	0.14	1.10	0.16	0.47
18:3ω3	0.19	0.12	0.17	0.00	1.20	0.04	0.36
20:5ω3	0.30	0.22	0.24	0.00	1.41	0.06	0.96
22:5ω3	1.50	0.53	1.39	0.31	3.86	0.59	2.75
22:6ω3	2.45	0.82	2.43	0.41	5.20	1.04	3.99
LCPω3	4.24	1.09	4.30	0.77	7.89	1.84	6.54
ω3	4.43	1.13	4.45	0.77	8.36	2.02	6.79
18:2ω6	8.35	1.89	8.50	4.06	13.62	4.47	12.19
20:2ω6	0.22	0.11	0.21	0.00	1.05	0.09	0.45
20:3ω6	1.55	0.36	1.54	0.52	2.70	0.83	2.17
20:4ω6	13.78	2.33	14.23	3.54	17.41	5.77	16.29
22:4ω6	2.80	0.66	2.86	0.43	4.24	0.98	3.87
22:5ω6	1.02	0.30	1.00	0.13	2.16	0.32	1.50
LCPω6	19.15	3.20	19.81	4.79	24.05	8.33	22.75
ω6	27.71	4.14	28.82	9.27	34.47	14.14	32.42
18:1ω7	1.83	0.46	1.81	1.01	2.97	1.09	2.79
ω7	1.83	0.46	1.81	1.01	2.97	1.09	2.79
18:1ω9	11.96	1.72	11.63	8.69	18.02	9.25	15.53
20:1ω9	0.25	0.10	0.24	0.12	1.14	0.16	0.39
20:3ω9	0.53	0.42	0.38	0.11	2.59	0.15	1.53
22:3ω9	0.22	0.18	0.17	0.00	0.80	0.00	0.71
24:1ω9	3.85	0.55	3.76	2.39	5.27	2.85	4.97
ω9	16.81	2.42	16.13	12.15	25.45	13.32	22.64
SAFA	49.21	3.61	48.39	43.85	66.66	46.36	62.67
MUFA	17.88	1.98	17.55	13.71	24.94	14.80	21.93
PUFA	32.90	4.50	33.88	11.98	38.79	17.83	37.32
P/S	0.68	0.11	0.70	0.18	0.88	0.28	0.80
ω3/ω6	0.16	0.05	0.16	0.06	0.36	0.09	0.27
LCPω3/LCPω6	0.22	0.06	0.21	0.11	0.43	0.14	0.38
20:3ω9/20:4ω6	0.04	0.04	0.03	0.01	0.22	0.01	0.15
22:5ω6/22:4ω6	0.37	0.08	0.36	0.20	0.71	0.22	0.56
22:5ω6/22:6ω3	0.44	0.15	0.43	0.16	0.95	0.21	0.75
20:4ω6/22:6ω3	6.18	2.03	5.68	2.99	13.09	3.62	11.69

*: Weight for age based on figures from National Centre of Health Statistics, USA. Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B6. RBC FA of malnourished Pakistani children from the Islamabad area, n=90							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (months)	19.2	11.3	16.5	4	56	4.1	51.3
Weight (kg)	6.6	1.7	6.5	3.3	11.0	3.5	9.8
Weight for age (%)*	59	8	61	37	72	40	70
Sex (m/f)	45/45						
14:0	0.54	0.19	0.51	0.22	1.36	0.28	1.00
16:0	25.75	2.72	25.36	18.69	36.53	21.77	34.55
18:0	16.32	1.40	16.06	13.88	21.45	14.54	20.88
20:0	0.44	0.12	0.42	0.25	1.26	0.29	0.60
22:0	1.75	0.34	1.78	0.90	2.90	1.00	2.34
24:0	4.38	0.71	4.26	2.60	6.59	3.08	5.97
26:0	0.28	0.11	0.27	0.15	1.10	0.17	0.49
18:3ω3	0.19	0.13	0.18	0.00	1.20	0.01	0.41
20:5ω3	0.30	0.23	0.24	0.00	1.41	0.06	1.06
22:5ω3	1.49	0.55	1.39	0.31	3.86	0.46	2.70
22:6ω3	2.33	0.82	2.33	0.41	5.20	0.69	3.73
LCPω3	4.12	1.16	4.20	0.77	7.89	1.63	6.73
ω3	4.32	1.19	4.39	0.77	8.36	1.89	6.92
18:2ω6	8.17	1.92	8.34	4.06	13.53	4.43	11.96
20:2ω6	0.22	0.12	0.20	0.00	1.05	0.09	0.36
20:3ω6	1.51	0.38	1.52	0.52	2.70	0.73	2.16
20:4ω6	13.53	2.54	14.05	3.54	17.41	5.06	16.17
22:4ω6	2.76	0.70	2.80	0.43	4.24	0.88	3.96
22:5ω6	0.98	0.29	0.98	0.13	1.68	0.30	1.45
LCPω6	18.79	3.49	19.62	4.79	24.05	7.02	22.76
ω6	27.18	4.44	28.46	9.27	34.47	13.70	32.40
18:1ω7	1.81	0.46	1.78	1.01	2.97	1.07	2.75
ω7	1.81	0.46	1.78	1.01	2.97	1.07	2.75
18:1ω9	12.25	1.78	11.95	8.69	18.02	9.14	16.03
20:1ω9	0.26	0.11	0.25	0.15	1.14	0.16	0.39
20:3ω9	0.56	0.45	0.38	0.11	2.59	0.15	1.57
22:3ω9	0.23	0.18	0.18	0.00	0.80	0.00	0.77
24:1ω9	3.94	0.55	3.89	2.57	5.27	2.97	5.05
ω9	17.23	2.49	16.62	12.15	25.45	13.41	22.74
SAFA	49.47	4.03	48.49	43.85	66.66	45.98	64.69
MUFA	18.24	2.02	17.83	13.71	24.94	14.93	21.94
PUFA	32.29	4.88	33.50	11.98	38.79	15.57	37.49
P/S	0.66	0.12	0.69	0.18	0.88	0.24	0.80
ω3/ω6	0.16	0.05	0.15	0.06	0.36	0.08	0.29
LCPω3/LCPω6	0.22	0.06	0.21	0.11	0.43	0.14	0.39
20:3ω9/20:4ω6	0.04	0.04	0.03	0.01	0.22	0.01	0.16
22:5ω6/22:4ω6	0.36	0.08	0.35	0.20	0.62	0.22	0.53
22:5ω6/22:6ω3	0.45	0.16	0.43	0.16	0.95	0.22	0.77
20:4ω6/22:6ω3	6.39	2.13	5.83	2.99	13.09	3.88	11.71

*: Weight for age based on figures from National Centre of Health Statistics, USA. Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA.

Appendix B7. RBC FA of well-nourished Pakistani children from the Islamabad area, n=26							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (months)	17.5	16.5	13	2	60	2.3	52.5
Weight (kg)	9.3	3.2	8.7	3.8	17.0	4.5	15.7
Weight for age (%)*	91.3	8.7	91	76	111	78.5	110.4
Sex (m/f)	14/12						
14:0	0.49	0.12	0.51	0.28	0.80	0.31	0.72
16:0	24.88	1.11	24.93	23.11	28.42	23.36	27.26
18:0	16.19	0.99	16.14	12.74	17.80	14.22	17.72
20:0	0.47	0.08	0.46	0.31	0.68	0.35	0.63
22:0	1.83	0.27	1.79	1.29	2.44	1.40	2.37
24:0	4.25	0.62	4.24	2.74	5.47	3.05	5.33
26:0	0.21	0.05	0.20	0.14	0.34	0.14	0.33
18:3ω3	0.17	0.08	0.17	0.07	0.35	0.08	0.33
20:5ω3	0.30	0.19	0.24	0.13	0.94	0.14	0.84
22:5ω3	1.50	0.48	1.40	0.62	2.91	0.81	2.47
22:6ω3	2.86	0.68	2.90	1.72	4.04	1.81	4.03
LCPω3	4.66	0.71	4.58	3.37	5.85	3.62	5.82
ω3	4.84	0.75	4.80	3.49	6.12	3.74	6.06
18:2ω6	8.94	1.69	8.83	5.67	13.62	5.94	12.09
20:2ω6	0.25	0.08	0.25	0.12	0.48	0.12	0.46
20:3ω6	1.65	0.27	1.60	1.25	2.36	1.26	2.23
20:4ω6	14.64	1.03	14.68	12.34	16.94	12.76	16.38
22:4ω6	2.94	0.47	3.05	2.13	3.85	2.15	3.81
22:5ω6	1.14	0.31	1.08	0.78	2.16	0.79	1.90
LCPω6	20.38	1.29	20.46	17.10	22.75	17.48	22.37
ω6	29.57	1.96	29.97	23.49	32.46	25.05	32.28
18:1ω7	1.92	0.48	1.85	1.09	2.90	1.13	2.68
ω7	1.92	0.48	1.85	1.09	2.90	1.13	2.68
18:1ω9	10.97	1.03	10.86	9.44	14.72	9.61	13.33
20:1ω9	0.22	0.05	0.22	0.12	0.34	0.13	0.32
20:3ω9	0.45	0.28	0.37	0.14	1.32	0.20	1.27
22:3ω9	0.17	0.14	0.14	0.00	0.53	0.00	0.49
24:1ω9	3.53	0.43	3.48	2.39	4.53	2.68	4.33
ω9	15.35	1.44	15.04	13.33	20.36	13.37	18.34
SAFA	48.32	1.15	48.07	46.43	51.23	46.84	50.88
MUFA	16.65	1.23	16.67	14.10	19.60	14.58	18.91
PUFA	35.03	1.41	35.29	31.37	37.27	32.08	37.05
P/S	0.73	0.04	0.73	0.63	0.80	0.64	0.79
ω3/ω6	0.17	0.03	0.16	0.11	0.26	0.12	0.24
LCPω3/LCPω6	0.23	0.04	0.22	0.17	0.32	0.17	0.31
20:3ω9/20:4ω6	0.03	0.02	0.03	0.01	0.10	0.01	0.09
22:5ω6/22:4ω6	0.39	0.09	0.37	0.26	0.71	0.28	0.61
22:5ω6/22:6ω3	0.41	0.11	0.40	0.21	0.67	0.21	0.61
20:4ω6/22:6ω3	5.43	1.42	5.10	3.54	8.99	3.57	8.03

*: Weight for age based on figures from National Centre of Health Statistics, USA. Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B8. RBC FA of breastfed Pakistani children from the Islamabad area, n=67

	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (months)	14.3	8.2	13.0	2	42	3.2	33.4
Weight (kg)	6.7	1.6	6.5	3.5	10.8	3.8	10.2
Weight for age (%)*	69	17	64	37	111	45	105
Sex (m/f)	31/36						
14:0	0.55	0.19	0.53	0.22	1.36	0.33	1.12
16:0	25.54	2.53	25.31	18.69	36.53	21.73	32.02
18:0	16.32	1.26	16.10	14.85	21.45	14.91	20.18
20:0	0.46	0.13	0.45	0.25	1.26	0.29	0.63
22:0	1.73	0.30	1.75	0.90	2.33	1.14	2.26
24:0	4.32	0.61	4.27	2.60	5.48	3.03	5.39
26:0	0.27	0.12	0.25	0.15	1.10	0.17	0.42
18:3ω3	0.17	0.15	0.16	0.00	1.20	0.00	0.34
20:5ω3	0.25	0.15	0.21	0.05	0.94	0.09	0.63
22:5ω3	1.39	0.43	1.34	0.31	2.91	0.53	2.21
22:6ω3	2.76	0.77	2.81	0.41	5.20	1.31	4.01
LCPω3	4.40	0.97	4.43	0.77	6.80	2.21	6.07
ω3	4.57	1.00	4.61	0.77	6.96	2.28	6.31
18:2ω6	8.50	1.48	8.66	5.67	12.23	5.75	11.01
20:2ω6	0.24	0.12	0.22	0.12	1.05	0.12	0.48
20:3ω6	1.50	0.35	1.51	0.52	2.36	0.80	2.05
20:4ω6	14.09	2.15	14.51	4.04	16.94	7.47	16.39
22:4ω6	2.88	0.58	2.91	0.81	4.24	1.57	3.91
22:5ω6	1.09	0.31	1.09	0.25	2.16	0.50	1.70
LCPω6	19.57	2.94	20.10	5.66	24.05	10.53	22.77
ω6	28.31	3.54	29.18	12.26	34.47	17.48	32.39
18:1ω7	1.96	0.46	1.93	1.09	2.97	1.14	2.91
ω7	1.96	0.46	1.93	1.09	2.97	1.14	2.91
18:1ω9	11.36	1.21	11.09	9.11	14.72	9.34	13.73
20:1ω9	0.24	0.12	0.22	0.15	1.14	0.15	0.35
20:3ω9	0.41	0.23	0.35	0.11	1.32	0.14	1.20
22:3ω9	0.17	0.11	0.14	0.00	0.53	0.00	0.49
24:1ω9	3.81	0.53	3.72	2.57	5.10	2.91	4.93
ω9	15.98	1.59	15.79	12.96	20.36	13.37	19.53
SAFA	49.19	3.45	48.62	43.85	66.66	45.67	58.78
MUFA	17.34	1.52	17.35	14.10	21.45	14.67	20.63
PUFA	33.46	4.12	34.18	13.16	38.79	20.43	37.38
P/S	0.69	0.11	0.71	0.20	0.88	0.35	0.80
ω3/ω6	0.16	0.03	0.16	0.06	0.26	0.10	0.24
LCPω3/LCPω6	0.22	0.04	0.22	0.14	0.33	0.15	0.33
20:3ω9/20:4ω6	0.03	0.02	0.02	0.01	0.10	0.01	0.08
22:5ω6/22:4ω6	0.38	0.08	0.37	0.21	0.71	0.27	0.56
22:5ω6/22:6ω3	0.16	0.06	0.15	0.08	0.50	0.09	0.29
20:4ω6/22:6ω3	5.45	1.41	5.21	2.99	9.85	3.57	8.36

*: Weight for age based on figures from National Centre of Health Statistics, USA. Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B9. RBC FA of non-breastfed Pakistani children from the Islamabad area, n=46							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (months)	25.85	14.85	24.00	4.00	60.00	7.13	56.00
Weight (kg)	7.97	3.04	7.80	3.30	17.00	3.56	14.85
Weight for age (%)*	63.02	13.09	63.00	40.00	104.00	41.25	92.75
Sex (m/f)	25/21						
14:0	0.51	0.16	0.48	0.27	0.95	0.27	0.91
16:0	25.66	2.42	24.98	22.95	35.11	23.27	32.40
18:0	16.20	1.41	16.03	12.74	20.94	13.94	20.45
20:0	0.43	0.08	0.42	0.29	0.72	0.31	0.59
22:0	1.82	0.37	1.87	0.97	2.90	1.11	2.43
24:0	4.43	0.77	4.25	3.07	6.59	3.12	6.21
26:0	0.27	0.07	0.26	0.15	0.50	0.16	0.49
18:3ω3	0.21	0.08	0.19	0.10	0.47	0.12	0.42
20:5ω3	0.36	0.28	0.29	0.00	1.41	0.07	1.17
22:5ω3	1.61	0.63	1.44	0.41	3.86	0.68	3.12
22:6ω3	1.99	0.68	2.04	0.48	4.03	1.11	3.17
LCPω3	3.97	1.23	3.83	1.55	7.89	1.88	7.02
ω3	4.17	1.27	4.04	1.85	8.36	2.06	7.44
18:2ω6	8.17	2.42	8.14	4.06	13.62	4.39	13.36
20:2ω6	0.20	0.08	0.20	0.00	0.45	0.08	0.36
20:3ω6	1.60	0.38	1.61	0.69	2.70	0.85	2.18
20:4ω6	13.28	2.56	13.83	3.54	17.41	6.11	15.67
22:4ω6	2.68	0.73	2.79	0.43	3.73	1.03	3.66
22:5ω6	0.91	0.27	0.91	0.13	1.44	0.31	1.41
LCPω6	18.47	3.52	19.24	4.79	23.13	8.77	21.90
ω6	26.84	4.87	27.75	9.27	34.00	14.47	32.45
18:1ω7	1.67	0.40	1.58	1.01	2.50	1.05	2.49
ω7	1.67	0.40	1.58	1.01	2.50	1.05	2.49
18:1ω9	12.81	2.00	12.64	8.69	18.02	9.23	16.38
20:1ω9	0.26	0.06	0.25	0.12	0.40	0.16	0.39
20:3ω9	0.70	0.56	0.44	0.14	2.59	0.17	2.16
22:3ω9	0.28	0.22	0.20	0.00	0.80	0.00	0.79
24:1ω9	3.94	0.55	3.81	2.86	5.27	3.02	5.06
ω9	17.99	2.95	17.69	12.15	25.45	13.35	24.01
SAFA	49.31	3.98	48.06	46.43	66.60	46.48	61.94
MUFA	18.70	2.29	18.34	13.71	24.94	15.00	22.43
PUFA	32.00	5.01	33.00	11.98	38.29	18.40	37.22
P/S	0.66	0.13	0.69	0.18	0.80	0.30	0.79
ω3/ω6	0.16	0.06	0.15	0.08	0.36	0.09	0.34
LCPω3/LCPω6	0.22	0.07	0.20	0.11	0.43	0.14	0.41
20:3ω9/20:4ω6	0.06	0.05	0.03	0.01	0.22	0.01	0.19
22:5ω6/22:4ω6	0.35	0.09	0.33	0.20	0.62	0.22	0.51
22:5ω6/22:6ω3	0.49	0.18	0.46	0.21	0.95	0.21	0.80
20:4ω6/22:6ω3	7.25	2.36	6.59	3.68	13.09	4.27	11.91

*: Weight for age based on figures from National Centre of Health Statistics, USA. Abbreviations: LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B10. RBC FA of Dutch LBW infants fed human milk, n=96*							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (days)	21.88	11.27	21.00	5.00	46.00	7.00	44.00
Age at birth (weeks)	35.69	2.37	35.07	30.14	41.00	32.18	40.48
Sex (m/f)	17/15						
14:0	0.79	0.47	0.63	0.35	3.23	0.36	2.18
16:0	25.75	1.75	25.46	22.75	31.07	23.11	29.52
18:0	17.30	1.13	17.03	15.59	20.85	15.87	20.35
20:0	0.59	0.07	0.59	0.44	0.82	0.47	0.75
22:0	1.37	0.14	1.35	1.11	1.83	1.16	1.68
24:0	4.44	0.44	4.36	3.55	5.80	3.81	5.49
26:0	0.39	0.06	0.39	0.24	0.56	0.28	0.53
18:3ω3	0.11	0.03	0.10	0.04	0.23	0.05	0.17
20:5ω3	0.20	0.06	0.20	0.07	0.39	0.10	0.36
22:5ω3	0.65	0.22	0.61	0.34	1.22	0.35	1.14
22:6ω3	4.12	0.67	4.17	2.19	5.32	2.55	5.21
LCPω3	4.97	0.84	5.12	2.73	6.82	3.13	6.35
ω3	5.08	0.85	5.21	2.81	6.97	3.23	6.48
18:2ω6	7.40	1.29	7.64	4.55	10.73	5.14	9.84
20:2ω6	0.27	0.05	0.26	0.16	0.38	0.18	0.37
20:3ω6	2.09	0.36	2.01	1.40	3.09	1.52	2.84
20:4ω6	13.68	1.60	13.92	8.32	16.94	9.92	16.54
22:4ω6	2.95	0.46	2.94	2.05	3.98	2.10	3.78
22:5ω6	1.10	0.23	1.07	0.66	1.74	0.77	1.69
LCPω6	19.81	1.99	20.23	13.09	23.73	14.89	22.94
ω6	27.48	2.70	28.28	18.85	33.30	20.74	31.20
18:1ω7	2.12	0.37	2.07	1.28	3.25	1.51	2.98
ω7	2.12	0.37	2.07	1.28	3.25	1.51	2.98
18:1ω9	9.95	0.78	10.05	8.00	11.65	8.27	11.11
20:1ω9	0.20	0.04	0.19	0.12	0.34	0.12	0.29
20:3ω9	0.58	0.21	0.56	0.23	1.55	0.27	1.17
22:3ω9	0.30	0.09	0.30	0.13	0.59	0.16	0.48
24:1ω9	3.66	0.35	3.63	2.94	4.57	3.00	4.43
ω9	14.68	1.06	14.68	12.36	16.69	12.53	16.36
SAFA	50.64	2.77	49.75	46.65	59.19	47.12	57.35
MUFA	15.92	1.04	15.94	13.52	18.17	13.66	17.80
PUFA	33.44	3.11	34.42	22.64	38.21	25.57	37.33
P/S	0.67	0.09	0.69	0.38	0.80	0.45	0.78
ω3/ω6	0.18	0.03	0.19	0.13	0.24	0.14	0.23
LCPω3/LCPω6	0.25	0.04	0.25	0.16	0.35	0.18	0.32
20:3ω9/20:4ω6	0.04	0.02	0.04	0.02	0.13	0.02	0.10
22:5ω6/22:4ω6	0.37	0.05	0.37	0.26	0.54	0.28	0.51
22:5ω6/22:6ω3	0.27	0.08	0.26	0.14	0.49	0.16	0.45
20:4ω6/22:6ω3	3.38	0.51	3.30	2.47	4.96	2.63	4.67

*: 32 babies were studied at 3 occasions (days 10, 20 and 42), resulting in a total number of samples of 96.
Abbreviations: BMI: body mass index: weight/lenght^2; LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B11. RBC FA of Dutch LBW infants fed formula with LCP, n=78*							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (days)	19.6	9.6	21.0	2	35	7.0	35.0
Age at birth (weeks)	36.7	3.4	36.9	31	42	31.3	41.5
Sex (m/f)	15/11						
14:0	0.51	0.18	0.51	0.29	1.60	0.30	0.68
16:0	27.10	1.75	26.88	24.52	32.84	24.68	31.48
18:0	16.19	0.99	15.93	13.94	18.89	14.72	18.44
20:0	0.60	0.07	0.59	0.43	0.77	0.46	0.76
22:0	1.34	0.09	1.32	1.06	1.71	1.14	1.57
24:0	4.57	0.53	4.54	2.89	7.15	3.61	5.57
26:0	0.41	0.06	0.41	0.22	0.67	0.28	0.52
18:3ω3	0.09	0.03	0.08	0.04	0.30	0.04	0.14
20:5ω3	0.46	0.28	0.38	0.13	1.16	0.17	1.07
22:5ω3	0.61	0.22	0.60	0.23	1.14	0.28	1.01
22:6ω3	4.18	0.78	4.37	2.17	5.95	2.38	5.81
LCPω3	5.25	1.18	5.41	2.63	8.15	2.94	7.34
ω3	5.34	1.19	5.53	2.67	8.26	3.02	7.43
18:2ω6	8.06	1.11	8.17	5.60	11.50	6.04	10.02
20:2ω6	0.22	0.04	0.22	0.14	0.38	0.15	0.31
20:3ω6	2.05	0.34	1.97	1.38	3.28	1.45	3.09
20:4ω6	11.68	1.44	11.78	7.57	14.90	8.95	14.47
22:4ω6	2.79	0.51	2.70	1.92	4.58	1.99	3.78
22:5ω6	1.08	0.20	1.03	0.64	2.00	0.68	1.89
LCPω6	17.61	1.92	17.51	13.31	21.89	13.79	20.83
ω6	25.89	1.93	26.16	20.27	29.11	20.36	28.77
18:1ω7	1.89	0.23	1.79	1.32	3.40	1.40	3.03
ω7	1.89	0.23	1.79	1.32	3.40	1.40	3.03
18:1ω9	11.38	1.14	11.30	9.03	15.10	9.11	13.57
20:1ω9	0.22	0.05	0.21	0.12	0.37	0.13	0.33
20:3ω9	0.58	0.21	0.51	0.22	1.29	0.30	1.20
22:3ω9	0.28	0.09	0.27	0.12	0.61	0.14	0.52
24:1ω9	3.71	0.62	3.76	1.37	4.99	2.77	4.83
ω9	16.17	1.53	16.13	12.73	19.86	13.03	19.33
SAFA	50.72	2.60	50.26	46.53	59.85	47.00	56.40
MUFA	17.19	1.47	16.90	13.92	21.60	14.18	20.55
PUFA	32.09	2.55	32.89	23.83	36.03	24.79	35.14
P/S	0.64	0.08	0.66	0.42	0.76	0.44	0.74
ω3/ω6	0.21	0.04	0.21	0.13	0.32	0.13	0.29
LCPω3/LCPω6	0.30	0.08	0.30	0.17	0.51	0.19	0.45
20:3ω9/20:4ω6	0.05	0.01	0.04	0.02	0.14	0.03	0.11
22:5ω6/22:4ω6	0.38	0.05	0.37	0.31	0.54	0.31	0.51
22:5ω6/22:6ω3	0.27	0.06	0.26	0.14	0.47	0.15	0.46
20:4ω6/22:6ω3	2.92	0.63	2.76	1.70	4.48	1.91	4.21

*: 25 babies were studied at 3 occasions, 1 at 1 and 1 at 2 (days 10, 20 and 42), resulting in a total number of samples of 78.

Abbreviations: BMI: body mass index: weight/lenght^2; LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA

Appendix B12. RBC FA of Dutch LBW infants fed formula without LCP, n=243*							
	Mean	SD	Median	Min	Max	P2.5	P97.5
Age (days)	23.9	13.4	20.0	7	50	8.0	46.0
Age at birth (weeks)	35.8	2.3	36.0	29	40	32.0	40.0
Sex (m/f)	46/35						
14:0	0.66	0.32	0.59	0.25	3.27	0.35	1.65
16:0	25.64	1.13	25.51	22.89	29.27	23.50	27.80
18:0	15.27	0.61	15.32	13.65	17.22	14.16	16.40
20:0	0.54	0.06	0.54	0.37	0.69	0.43	0.66
22:0	1.29	0.15	1.28	0.92	1.87	1.00	1.55
24:0	4.42	0.55	4.39	2.96	6.08	3.43	5.42
26:0	0.43	0.07	0.43	0.26	0.65	0.31	0.59
18:3ω3	0.13	0.03	0.13	0.07	0.27	0.09	0.19
20:5ω3	0.16	0.04	0.15	0.08	0.32	0.10	0.24
22:5ω3	0.54	0.15	0.52	0.27	1.72	0.33	0.84
22:6ω3	3.54	0.62	3.49	2.19	5.49	2.42	4.77
LCPω3	4.23	0.62	4.20	2.90	6.25	3.18	5.66
ω3	4.36	0.62	4.31	3.04	6.35	3.34	5.76
18:2ω6	9.06	1.40	9.17	5.95	13.40	6.42	11.53
20:2ω6	0.23	0.05	0.22	0.13	0.39	0.14	0.35
20:3ω6	1.91	0.36	1.85	1.28	3.58	1.41	2.75
20:4ω6	12.81	1.21	12.73	10.04	16.22	10.66	15.50
22:4ω6	2.98	0.44	2.96	2.01	4.51	2.31	3.94
22:5ω6	1.13	0.20	1.11	0.71	1.63	0.75	1.52
LCPω6	18.82	1.36	18.72	15.15	22.44	16.37	21.42
ω6	28.11	1.06	28.09	24.68	30.49	26.03	30.05
18:1ω7	1.96	0.24	1.93	1.40	2.58	1.54	2.47
ω7	1.96	0.24	1.93	1.40	2.58	1.54	2.47
18:1ω9	12.06	1.16	12.01	9.00	14.71	10.02	14.08
20:1ω9	0.27	0.08	0.27	0.10	0.48	0.15	0.44
20:3ω9	0.64	0.21	0.60	0.25	1.43	0.32	1.15
22:3ω9	0.33	0.10	0.32	0.11	0.67	0.14	0.57
24:1ω9	4.01	0.57	3.99	2.56	6.64	2.96	5.31
ω9	17.31	1.54	17.34	12.15	20.91	14.50	19.83
SAFA	48.25	1.43	48.32	44.67	53.80	45.43	51.12
MUFA	18.31	1.47	18.26	13.96	22.06	15.88	20.76
PUFA	33.44	1.06	33.47	29.41	36.05	31.35	35.38
P/S	0.69	0.03	0.69	0.55	0.78	0.64	0.76
ω3/ω6	0.17	0.26	0.15	0.11	4.21	0.12	0.21
LCPω3/LCPω6	0.23	0.03	0.22	0.16	0.33	0.17	0.30
20:3ω9/20:4ω6	0.05	0.01	0.05	0.02	0.10	0.03	0.08
22:5ω6/22:4ω6	0.38	0.06	0.37	0.22	0.55	0.27	0.50
22:5ω6/22:6ω3	0.33	0.07	0.32	0.15	0.51	0.18	0.48
20:4ω6/22:6ω3	3.71	0.60	3.70	2.46	5.64	2.63	5.01

*: 81 babies were studied at 3 occasions (days 10, 20 and 42), resulting in a total number of samples of 243.
Abbreviations: BMI: body mass index: weight/lenght^2; LCP: long chain polyunsaturated fatty acid, C≥20, double bonds ≥ 3; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P/S: PUFA/SAFA