Bernadette Delplanque, Robert Gibson, Berthold Koletzko, Alexandre Lapillonne, and Birgitta Strandvik

Lipid quality in infant nutrition: current knowledge and future opportunities

Journal of Pediatric Gastroenterology and Nutrition, 2015; 61(1):8-17

OPEN

Lipid Quality in Infant Nutrition: Current Knowledge and Future Opportunities

*Bernadette Delplanque, †Robert Gibson, ‡Berthold Koletzko, §Alexandre Lapillonne, and ||Birgitta Strandvik

ABSTRACT

Dietary lipids are key for infants to not only meet their high energy needs but also fulfill numerous metabolic and physiological functions critical to their growth, development, and health. The lipid composition of breast milk varies during lactation and according to the mother's diet, whereas the lipid composition of infant formulae varies according to the blend of different fat sources. This report compares the compositions of lipids in breast milk and infant formulae, and highlights the roles of dietary lipids in term and preterm infants and their potential biological and health effects. The major differences between breast milk and formulae lie in a variety of saturated fatty acids (such as palmitic acid, including its structural position) and unsaturated fatty acids (including arachidonic acid and docosahexaenoic acid), cholesterol, and complex lipids. The functional outcomes of these differences during infancy and for later child and adult life are still largely unknown, and some of them are discussed, but there is consensus that opportunities exist for improvements in the qualitative lipid supply to infants through the mother's diet or infant formulae. Furthermore, research is required in several areas, including the needs of term and preterm infants for long-chain polyunsaturated fatty acids, the sites of action and clinical effects of lipid mediators on immunity and inflammation, the role of lipids on metabolic, neurological, and immunological outcomes, and the mechanisms by which lipids act on short- and long-term health.

Key Words: breast milk, cholesterol, complex lipids, fatty acids, infant formulae

(*JPGN* 2015;61: 8–17)

What Is Known

- Lipids are the dominant provider of energy during the early months of life.
- The quality of lipids supplied to infants is of utmost importance for growth, development, and future health.

What Is New

- The lipid composition of breast milk is partly affected by the mother's diet.
- The major differences between breast milk and formulae and within formulae concern saturated fats, polyunsaturated fats, cholesterol, and complex lipids.
- The quality of the lipid supply to infants should be improved by translating the results of current and future research into mothers' diets and into the design of optimized fat blends in formulae.

Breast-feeding is regarded as the best choice for feeding infants (1). The composition of human milk and the physiology of lactation may provide some guidance for feeding infants who receive breast milk substitutes, although similarity of an infant feeding product to human milk in terms of composition alone is not sufficient to support suitability and safety (2). Fats contribute the major portion (45%–55%) of the energy contained in human milk, with a total fat intake of approximately 5.5 kg in a fully breast-fed infant during the first 6 months of life (3). Human milk contains a wide variety of lipid components, some of them being indispensable nutrients, for example, the polyunsaturated fatty acids (PUFAs) and long-chain PUFAs (LC-PUFA) of the n-6 and n-3 series, and the lipid-soluble vitamins. Although their precise functionalities are not yet fully understood, the various lipids provided by human milk are known to modulate gastrointestinal function, lipoprotein metabolism, membrane composition and function, and signaling pathways, thereby markedly affecting infant growth, development, and health (4).

The objectives of this publication are to review the lipid composition of breast milk and of some currently available infant

Received September 20, 2014; accepted April 2, 2015.

From the *Laboratoire de Neuroendocrinologie Moléculaire de la Prise Alimentaire (NMPA), Centre de Neurosciences Université Paris-Sud (CNPS), Orsay, France, the †FOODplus Research Centre, School of Agriculture, Food and Wine, University of Adelaide, Adelaide, Australia, the ‡Division of Metabolic and Nutritional Medicine, Dr von Hauner Children's Hospital. Ludwig-Maximilians-University of Munich, Munich, Germany, the §Necker Enfants Malades Hospital, Paris Descartes University, Paris, France, and the ||Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden.

Address correspondence and reprint requests to Bernadette Delplanque, Universite Paris-Sud, Orsay, France (e-mail: bernadette.delplanque@u-psud.fr).

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (*www.jpgn.org*).

Groupe Lactalis, Laval, France, facilitated this review by providing financial support. The work of Dr Koletzko is carried out with partial financial support from the Commission of the European Communities, the 7th Framework Programme, contract FP7-289346-EARLY NUTRITION and the European Research Council Advanced Grant ERC-2012-AdG, no. 322605 META-GROWTH. This article does not necessarily reflect the views of the Commission and in no way anticipates the future policy in this area.

The authors report no conflicts of interest.

DOI: 10.1097/MPG.0000000000000818

formulae, to highlight the potential functional relevance of differences between breast milk and infant formulae, based on the role of dietary lipids, and the biological and health consequences these differences may have in infancy and in the long term. In addition, new research routes and opportunities for future optimization of lipid supply to infants are identified.

LIPID COMPOSITION OF BREAST MILK AND INFANT FORMULAE

The lipids in human milk and infant formulae are chiefly (in most cases >95%) in the form of triglycerides, that is, 3 fatty acids esterified to a glycerol backbone. Triglycerides are the main form of lipid in commonly occurring oils and fats. Human milk also contains a small proportion of (lyso)phospholipids in which 1 or 2 fatty acids are attached to a glycerol backbone with the third carbon of glycerol linked to a phosphate group and a polar head group such as choline or ethanolamine present.

In human milk, lipid content is far more variable than the other macronutrients. Lipid averages 3.5 to 4.5/100 g in mature milk but changes with the length of time the mother has been breastfeeding, during the course of a day, and increases during an individual feed (5). Mammary alveolar cells produce milk fat globules (Fig. 1), containing a core predominantly consisting of triglycerides (comprising 98%–99% of milk lipids) and small amounts of monoglycerides, diglycerides, and nonesterified fatty acids, surrounded by a milk fat membrane with different phospholipids, esterified cholesterol, glycosylated polypeptides, filaments, mucin, lactadherin, and other components (6,7) (Fig. 1 and Table 1). The specific structure of the milk fat globule, and especially the type of polar lipids at the droplet interface, could influence digestion of lipids (reviewed in (6)).

The fat in most infant formulae used today is based on a mixture of vegetable oils and hence has a much less complex composition than human milk fat. Formulae containing dairy fats were widely used in the first part of the 20th century and are still used in some parts of the world, but their use has diminished. Other sources of lipid used in infant formulae include single cell oils, fractionated lipids and various polar lipids, repeat esterified structured lipids, egg phospholipids, and fish oils. In infant formulae based on vegetable fat, the fat globule is usually smaller than in breast milk, and the phospholipids are provided by emulsifying lecithin, with a wide range of phospholipid species included (usually phosphatidylcholine or inositol). When dairy fats are included as ingredients in formulae, phospholipid content is usually higher, with a wide range of molecular species from different phospholipid classes. Human milk contains 90 to 150 mg/L cholesterol (8), in contrast to no appreciable cholesterol content in vegetable oil–based infant formulae and to approximately 40 mg/L in dairy fat–based infant formulae.

TABLE 1. Lipid classes in mature breast milk

	Content in breast milk	
	Total lipids, % (5)	Mean (minimum–maximum), mg/100 g (6)
Triacylglycerols	98.1–98.8	
Diacylglycerols	0.01–0.7	
Monoacylglycerols	Traces	
Nonesterified fatty acids	0.08–0.4	
Phospholipids	0.26–0.8	23.8 (10.4–38.4)
Phosphatidylinositol		1.1 (0.9–2.3)
Phosphatidylserine		1.4 (1–1.9)
Phosphatidylethanolamine		6.8 (1.98–11.8)
Phosphatidylcholine		6.0 (1.98–9.6)
Sphingomyelin		8.5 (2.7–14.6)
Cholesterol	0.25–0.34	

The properties of milk triglycerides depend on their fatty acid composition. Mature human milk typically contains approximately 34% to 47% saturated fatty acids, mainly palmitic acid (17%–25%), approximately 31% to 43% monounsaturated fatty acids, approximately 12% to 26% n-6 PUFA, and approximately 0.8% to 3.6% n-3 PUFA (Fig. 2 and supplementary digital content 1, *http://links.lww.com/MPG/A473*, and supplementary digital content 2, *http://links.lww.com/MPG/A474*). In infant formulae, the fatty acid composition varies according to the lipid sources used, whose compositions are described in supplementary digital content 3 (*http://links.lww.com/MPG/A475*). Some lipid sources have specific features. For example, palm oil has a high palmitic acid content, with no short- or medium-chain fatty acids, whereas these fatty acids are present in high proportions in coconut oil. Use of different lipid sources therefore translates into differences in contents and proportions of short- and medium-chain fatty acids (Fig. 2A), and in lauric acid (Fig. 2B), myristic acid (Fig. 2C), palmitic acid (Fig. 2D), and oleic acid (Fig. 2E) (see *http://links.lww.com/MPG/A474* for numerical values). Most preterm and some term infant formulae contain medium-chain triglycerides derived from coconut oil.

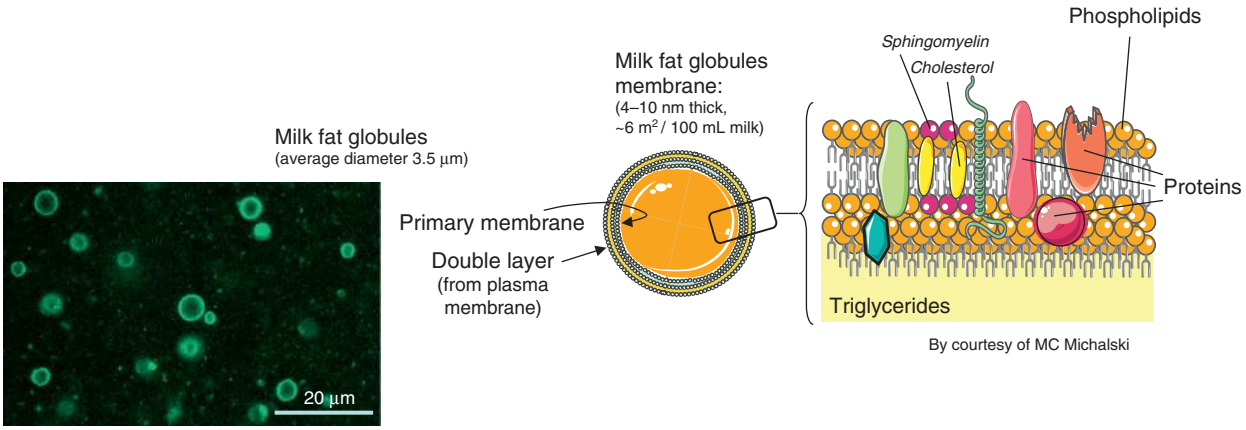

FIGURE 1. Structure of a milk fat globule. Adapted from (5).

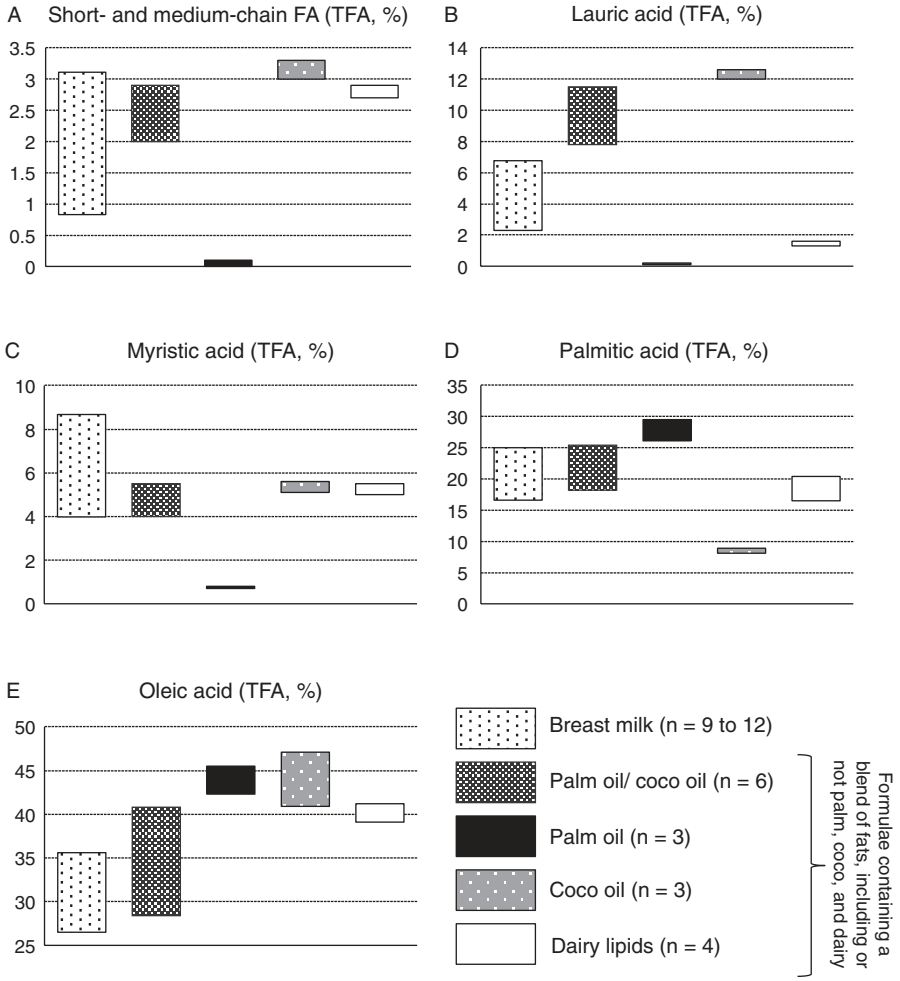

FIGURE 2. Ranges (minimum–maximum) of short- and medium-chain fatty acids (C4:0-C10:0, A), lauric (C12:0, B), myristic (C14:0, C), palmitic (C16:0, D), oleic (C18:1n-9) acid contents, expressed in percentage of total fatty acids in breast milk and infant formulae according to the fat sources used (see *http://links.lww.com/MPG/A474* for numerical values; breast milk data sources are detailed in *http://links.lww.com/MPG/A473*; infant formulae data come from unpublished analyses performed in a certified laboratory). FA = fatty acid; TFA = total fatty acid.

The triglyceride structure (ie, the position of the fatty acids on the glycerol molecule) is also of importance because it has been shown that long-chain saturated fatty acids in the center (sn-2) position are more efficiently digested and absorbed (9). Human milk and bovine milk are rich in the saturated fatty acid palmitic acid (C16:0, approximately 25% of fatty acids), of which approximately 70% of molecules in human milk and 45% in bovine milk, but <20% in most plant oils are esterified in the sn-2 position of triglycerides (10). In infant formulae, this translates into a lower proportion of palmitic acid in the sn-2 position in formulae containing only vegetable oils compared with formulae containing milk fat or β-palmitate (a structured triglyceride with palmitic acid esterified preferentially in the sn-2 position) (11).

The fatty acid composition of human milk lipids is markedly modified by maternal dietary habits. For example, the proportions of the essential PUFA linoleic acid (LA) and α-linolenic acid (ALA) in breast milk depend on the mother's diet and thus vary widely (10%–24% of fatty acids and 0.6%–1.9% of fatty acids, respectively; *http://links.lww.com/MPG/A474*). The increase in maternal intake of LA in the last 60 years is reflected in a significant increase in the LA content of breast milk in the United States. The level of ALA has remained fairly stable during this same period, resulting in a marked increase in the LA:ALA ratio from approximately 6%–8% before 1970 to approximately 14%–16% since 1980 (Fig. 3) (12).

Maternal dietary intake of marine foods (13) is extremely variable and explains the wide range of docosahexaenoic acid (DHA) in breast milk (*http://links.lww.com/MPG/A473*). Human milk in many Western countries has an arachidonic acid (ARA) to DHA ratio of approximately 2:1, which has been mimicked in some infant formulae. Many Asian and Scandinavian breast milk samples, however, have much lower ARA to DHA ratios because of a higher consumption of DHA-rich fish. A minimal part of the LC-PUFA in breast milk derives from endogenous synthesis, which is generally low in humans and particularly low in people who carry less common variants of the genes for the fatty acid desaturating enzymes, *FADS*1 and *FADS*2. Maternal fatty acid desaturase (*FADS*) gene polymorphisms have a significant effect on ARA contents in breast milk but not on DHA, both in early lactation and at 6 months after delivery (14). Human and animal milks always provide preformed ARA; the ARA content in human milk is stable (near 0.5% of fatty acids) around the world despite marked variations in dietary intakes and lifestyles (15). It can fluctuate slightly

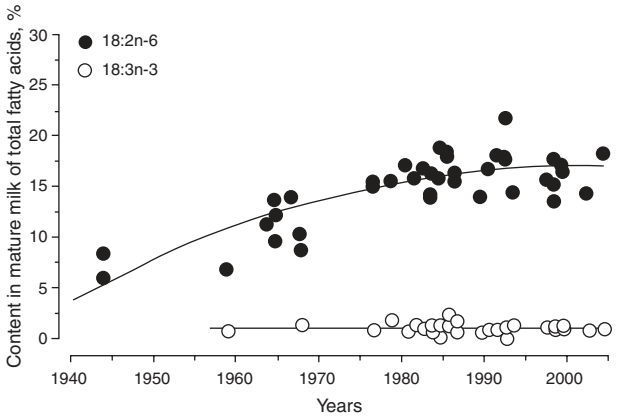

FIGURE 3. LA (C18:2 n-6, black circles) and ALA (C18:3 n-3, open circles) content in mature breast milk of US women. ALA = α-linolenic acid; LA = linoleic acid. Reproduced from (12).

around this value according to maternal intake (16), but ARA is mostly derived from preexisting maternal stores (17).

During the course of the first year of lactation, the content of both LA (200 mg/dL) and ALA (20 mg/dL) in human milk increases by 8% to 38%, whereas the LC-PUFA ARA (15–16 mg/dL) and DHA (7–8 mg/dL) decrease by 32% to 52% (18). PUFA composition of human milk also varies by length of gestation. Preterm milk may contain slightly higher proportions of DHA and of medium- and intermediate-chain length fatty acids than term milk (19). Human milk also contains bile salt–stimulated lipase (BSSL), which increases bioavailability of human milk fat by improving lipolysis. When donor human milk is pasteurized to suppress viral and bacterial activities, this heat treatment inactivates BSSL and changes the structure of the milk fat globule (19).

In accordance with existing regulations, all infant formulae contain LA and ALA but in variable amounts, depending on the blend of fats (*http://links.lww.com/MPG/A474*), which translates into an LA:ALA ratio that can vary from approximately 5 to 12. Some, but not all, formulae contain appreciable amounts of added LC-PUFA, typically from single cell oils, or marine or egg lipids (*http://links.lww.com/MPG/A474*).

Although we have focused on the most abundant lipids in milk and ignored the numerous quantitatively minor lipid components, this short overview illustrates the complexity of the lipid composition and identifies some quantitative differences between breast milk and infant formulae, which may or may not be of importance for childhood health and development.

ROLE OF LIPIDS IN INFANCY AND POTENTIAL RELEVANCE OF DIFFERENCES IN LIPIDS BETWEEN BREAST MILK AND INFANT FORMULAE

Lipids are the dominant provider of energy, contributing 90% of the energy retained by infants during the first 6 months. Lipids are also an efficient source of energy deposition: the energy cost to synthesize and store fat from glucose is 25%, whereas it is only 1% to 4% when lipid is the substrate (20).

Breast-fed infants appear to have a higher fat mass at 3 and 6 months than formulae-fed infants, whereas they tend to have less body fat at later ages (21). Many studies and meta-analyses found breast-feeding associated with a slightly lower risk of obesity or overweight in later life (22,23). It is unclear whether and to which extent the lipid component in infant feeding, however, contributes to these protective effects of breast-feeding (24).

Saturated Fats

Saturated fatty acids not only provide energy but also have structural and metabolic functions. Saturated fatty acids can also be synthesized in humans from nonfat sources or by β-oxidation from unsaturated fatty acids (25).

Saturated fatty acids range in size from 6 to 24 carbons, but the most common in infant diets have 12, 14, 16, and 18 carbon chain lengths (Fig. 2 and *http://links.lww.com/MPG/A474*). Medium-chain–length fatty acids (C8–C10) can be absorbed to a large degree directly into the hepatic portal vein and transported to the liver, where they can be oxidized for energy (26). Medium-chain fatty acids have the potential to limit the oxidation of PUFA and LC-PUFA and to enhance the conversion of PUFA to LC-PUFA (27,28). In preterm infants, because of possible intestinal immaturity, facilitation of fat absorption through the inclusion of medium-chain fatty acids in the diet may be useful, but there is no demonstrated benefit for energy balance or growth (19,29). Adding dietary medium-chain triglycerides, however, has been shown to be beneficial in children with severe fat malabsorption such as intestinal failure because of short bowel syndrome or severe cholestatic liver disease (30).

Most of the saturated 16-carbon fatty acid palmitic acid in breast milk is located in the central position (sn-2 position) of the triglyceride molecule. In palm oil–based formulae, palmitic acid is mainly located at the sn-1 or sn-3 position, impairing absorption of calcium and fat and resulting in insoluble calcium soaps, which negatively influence early bone accretion (31,32). When the sn-2 position of palmitate is duplicated in infant formula by adding repeat esterified β-palmitate, stool consistency and the absorption of palmitic acid and calcium become similar to those seen in breast-fed infants (33). One follow-up study, however, suggested that possible effects on bone do not persist at age 10 years: bone measurements did not differ between children who had been fed a formula with 12% or 50% of the palmitate in the sn-2 position, but the sample size was small and feeding duration was only 12 weeks after birth (34).

Some other features of saturated fatty acids might have physiological or nutritional relevance, such as the presence of palmitic acid in pulmonary surfactant (35). When studied in vitro or in animals, individual saturated fatty acids often exhibit specific properties, such as bactericidal effects for capric acid (36), immunomodulation for arachidic and behenic acids (37), and protein acylation for myristic or palmitic acids (38). These properties may deserve further exploration in humans and infants.

As illustrated in Fig. 2 and in supplementary digital content (*http://links.lww.com/MPG/A474*), infant formulae without palm oil or alternative source of palmitic acid have palmitate levels as low as 8% of the total fatty acids, versus 17% to 25% in breast milk, whereas lauric acid (C12:0) amounts to 12% versus 2% to 7% in breast milk. In formulae with dairy fat, palmitate levels reach 16% to 20%. The physiological and health consequences of these considerable composition changes are not known but would require investigations as palm oil–free formulae are becoming increasingly popular.

Monounsaturated Fatty Acids

Monounsaturated fats are the second most common fatty acids in breast milk and infant formulae. The dominant monounsaturated fatty acids are oleic acid (C18:1n-9) and palmitoleic acid (C16:1n-7). In spite of this abundance, their potential functionalities have not been explored in infants and are of unknown nutritional

relevance. In essential fatty acid deficiency, oleic acid is converted into Mead acid (C20:3n-9), and an inverse relation of ARA and oleic acid has been observed in a human study (39). In rats, the provision of Mead acid reduced leukotriene B4 formation in leukocytes (40). The potential impact of monounsaturated fatty acid supply on the immune system and other functional outcomes in infants remain to be explored.

Although found only at extremely low levels in breast milk and in infant formulae (*http://links.lww.com/MPG/A474*), in the body the 24-carbon nervonic acid (C24:1n-9) is important for myelination and may play a role in brain growth and development; indeed, nervonic acid is the major extremely long chain fatty acid in sphingomyelin, with a dramatic accretion around the time of delivery (41). It has been observed that nervonic acid is 7-fold higher in breast milk of mothers of premature infants than in mature milk of mothers of term infants (42). Nervonic acid, however, may also be endogenously synthesized in newborns, and the relevance of the dietary supply remains speculative.

Polyunsaturated Fatty Acids

LA and ALA

Both LA and ALA are essential fatty acids, and they can influence metabolic processes, such as lowering plasma cholesterol. The relative provision of LA and ALA is of importance for the endogenous synthesis of the respective LC-PUFA because these 2 precursor fatty acids compete for desaturases and elongases in the PUFA conversion pathway (Fig. 4) (43). Breast milk contents in LA and ALA vary depending on the maternal intakes of these essential fatty acids and cannot be used as a basis for supply recommendations. Current guidelines for the levels of LA and ALA in infant formulae aim at avoidance of an extremely high LA:ALA ratio, which may reduce ALA conversion to n-3 LC-PUFA (44). The estimated LA requirement of infants to prevent deficiency is approximately 1% energy and that for ALA is approximately 0.5% energy. Considering a certain margin of safety, the amounts of LA and ALA mandated by the European Food Safety Agency (EFSA) for infant formulae are, respectively, 4.5% and 0.5% of energy content, with upper guidance levels set at respectively 10.8% and 0.9% of energy (45). Extremely high levels of LA in formulae may have untoward effects as some of their oxygenated metabolites have proinflammatory functions (46,47). High neonatal LA has been associated with impaired development up to 18 months in preterm infants (48) and with impaired neurodevelopment up to 2 to 3 years of age in term infants (49).

Endogenous Synthesis of LC-PUFA

The question whether it is the total amount of the essential fatty acids or the ratio between LA and ALA that is most important is controversial. Some authors found that, in adults, the absolute amounts of ALA and LA in the diet, but not their ratio, determine ALA conversion (50). Others reported increased eicosapentaenoic

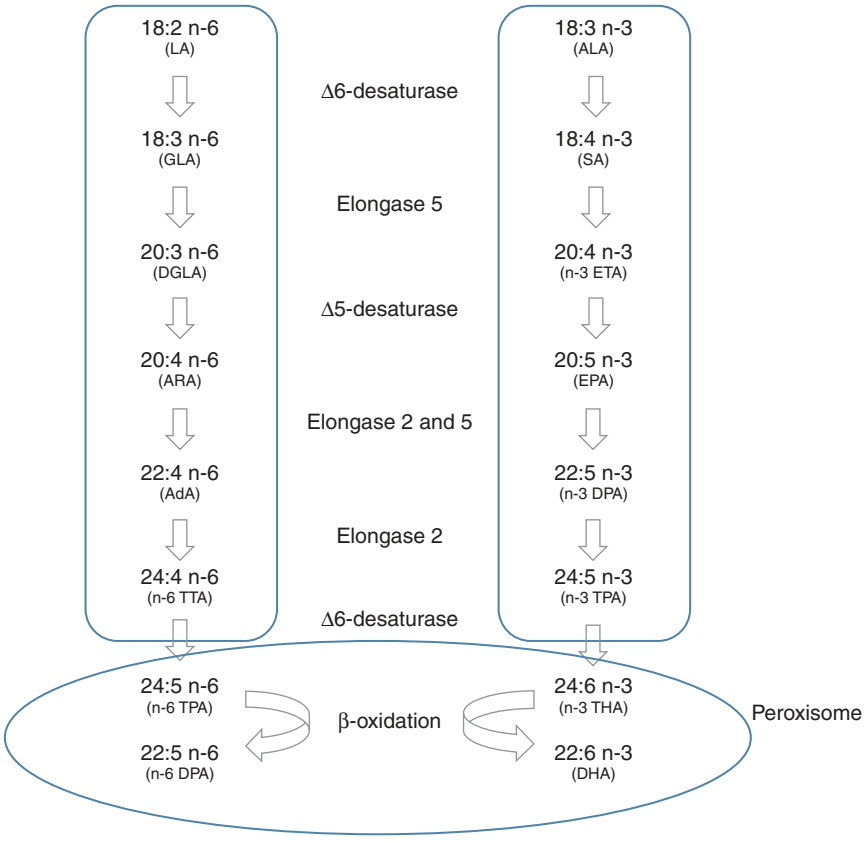

FIGURE 4. Biochemical pathways leading from the LA and ALA to LC-PUFAs. AdA = adrenic acid; ALA = α-linolenic acid; ARA = arachidonic acid; (D)GLA = (di-homo) γ-linolenic acid; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; ETA = eicosatetraenoic acid; LA = linoleic acid; LC-PUFA = long-chain polyunsaturated fatty acid; SA = stearidonic acid; TFA = total fatty acid; THA = tetrahexaenoic acid; TPA = tetraeicosapentaenoic acid; TTA = tetracosatetraenoic acid.

acid (EPA) concentration when LA was decreased relatively to ALA (51).

The LA-ALA contents and ratios in breast milk and different formulae vary (*http://links.lww.com/MPG/A474*), which may influence an infant's conversion of ALA and LA to their respective LC-PUFAs. In term infants, an infant formula with a 5:1 ratio of LA to ALA induced higher DHA concentrations in plasma and erythrocyte phospholipids than a 10:1 formula with the same LA content (16% of total fatty acids) (52). This study supports the concept that the relative intakes of LA and ALA may affect n-3 LC-PUFA synthesis and tissue levels in infants. The effects and safety of formulae with low LA:ALA ratios, however, may need further evaluation because infants fed from birth with a formula with a 4.8 LA:ALA ratio had a lower body weight at 4 months (but not at 6 months), compared with formulae with higher ratios resulting from lower ALA concentrations (43).

In young rats, a dairy/vegetable fat blend diet providing 1.5% and 14% of total fatty acids as ALA and LA, respectively, induced higher plasma, erythrocyte, and brain DHA levels than a pure vegetable fat blend diet with extremely similar ALA and LA contents (1.5% and 16% of total fatty acids) (53,54). In a small trial in term infants, a formula containing evaporated bovine milk providing reduced intakes of LA and ALA (2.3% and 0.8% of total fatty acids, respectively) led to higher DHA levels than vegetable oil–based formulae providing 30% of total fatty acids as LA and 5% as ALA (55).

In formula-fed preterm infants, DHA synthesis was 6 times lower at 7 months of age than at 1 month, whereas ARA synthesis decreased by half (56); estimates of the mean endogenous synthesis rate suggest that endogenous synthesis of LC-PUFAs in preterm infants is insufficient to meet their requirements defined by the normal fetal accretion rate (19,29).

Role of DHA

The nervous system is especially rich in DHA. DHA accumulation in nervous tissues starts in utero and proceeds at high rates during the first 2 postnatal years, when the growth and differentiation of the central nervous system are most rapid. Breast milk–derived DHA is readily incorporated into infant brains: breast-fed infants have a greater proportion of DHA in their erythrocytes and brain cortex relative to those fed formula. During infancy, cortex DHA increases in breast-fed but not formula-fed infants (57).

Many studies have compared the supplementation of formulae with DHA with feeding human breast milk, which is relatively rich in LC-PUFA. In term infants, the addition of DHA to infant formula has not been consistently shown to have benefits in visual, neural, or growth outcomes (58). The strongest evidence for the role of DHA on development comes from the studies performed in preterm infants. DHA supplementation has consistently demonstrated better visual development of preterm infants compared with nonsupplemented formulae and appears related to improvements in more global measures of development, without any adverse effects (19,26). Following clinical interventions with DHA doses similar to human milk lipid contents (0.3% of total fatty acids), more studies have demonstrated that DHA doses of approximately 1% of total fatty acids were related to further improvements in neurodevelopment at 18 months in girls (not boys) born preterm (59). These DHA doses in breast milk were also linked to a reduced incidence of bronchopulmonary dysplasia in preterm infants (60).

Adequate growth is an important indicator of health and well being in infants. Trials designed to test the effect of LC-PUFA supplementation in term infants were subjected to a systematic review, which included 14 studies and 1846 infants (61). No significant effect of LC-PUFA supplementation on infant weight, length, or head circumference was found, at any assessment age, and whatever the source of LC-PUFA supplementation (phospholipid or triacylglycerol). Many of the included studies, however, were neither designed nor powered to detect growth effects, and the included interventions were extremely heterogeneous. Furthermore, long-term effects have not been extensively studied, although some studies suggest long-term effects of early DHA supply on growth and body composition (62).

In preterm infants, early studies reported failure to thrive after feeding fish oil–supplemented formula (63,64), and the effect of fish oil without provision of ARA on the growth of preterm infants has been controversial since then. A large randomized controlled trial including 657 infants born at <33 weeks showed that a 1% dose of DHA had no adverse effect on weight or head circumference up to 18 months corrected age compared with standard feeding practice (0.2%–0.3% DHA), ARA being present at similar levels (0.4%) in both the groups. In fact, preterm infants fed higher DHA were significantly (0.7 cm) longer at 18 months corrected age (65). Thus, there are no major safety concerns regarding the current levels of LC-PUFA provided to preterm infants, whereas there are indications that they can be beneficial.

Long-lasting benefits of DHA status in infancy for brain and immune development and health have been recently found in studies that revealed exciting breast-feeding–gene interactions. A marked protective effect of prolonged breast-feeding against physician-diagnosed asthma was found up to the age of 10 years in children with a genotype *FADS* gene cluster resulting in low LC-PUFA synthesis, whereas there was no significant effect in children homozygous for the major genetic allele (66). Similarly, higher IQ results at the age of 8 years were reported in previously breast-fed children. The benefit of receiving breast milk, providing preformed LC-PUFA, was more than 4 IQ points greater in those children with *FADS*2 genetic variants that lead to low LC-PUFA synthesis (67). A benefit of early LC-PUFA status on brain development was also shown in a randomized clinical trial in which breast-feeding women received supplementation with 200 mg DHA per day or placebo during the first 4 months of lactation. Improved psychomotor development was observed at 2.5 years of age and enhanced sustained attention at the age of 5 years (68). Study results, however, are heterogeneous, and recent meta-analyses both on term (69) and preterm infants (70) did not find conclusive evidence for long-term benefits of DHA supplementation (60).

Because adequate DHA status is important for infant development, it has been recommended to include DHA in infant formulae at levels approximately 0.10% to 0.18% of energy (71). More recently, the EFSA proposed an increased recommendation setting a minimum–maximal range of 0.18% to 0.45% energy (45). A cause and effect relationship has been recognized by the EFSA between infant DHA intake levels of 0.3% of total fatty acids intake and visual function achieved at 12 months (72). Higher DHA intakes have been recently recommended for preterm infants, who have higher requirements regarding neurodevelopment (19,26). Mothers who consumed 3.45 g of n-3 LC-PUFA from salmon, weekly from the 20th gestational week, delivered neonates with higher cord blood concentrations in EPA and DHA compared with nonsupplemented mothers (73). A similar supplementation with 1.2 g/day from the 15th week of gestation until 4 months of lactation resulted in increased concentrations of n-3 LC-PUFA in breast milk (74). It is thus recommended that pregnant and breast-feeding women should consume preformed n-3 LC-PUFA providing an average intake of ≥200 mg DHA/day (13,71).

Role of ARA

An ongoing challenge is to fully understand the role of ARA, which is consistently found in breast milk and in some but not all

infant formulae. Brain ARA increases rapidly in late gestation and in the first year of life, but the regulation of its accumulation and the potential impact of ARA in the infant diet are not fully understood. The brains of breast-fed infants contain no more ARA than those of infants fed infant formula without DHA and ARA (57). An inverse correlation, however, has been observed in plasma and red blood cells of newborns between LA and ARA levels (48). This is a general phenomenon, which is also seen in adults (75,76). The potential of such a negative relationship on the ARA cascade in the neonatal period is unknown, but some studies found an association between low ARA and less growth (63,77), reflecting experimental data that found ARA had a stimulatory effect on cell growth (64,78).

Whether or not the provision of preformed dietary ARA in infancy is of high importance remains controversial (45,79). The appropriateness of an ARA:DHA ratio of 2:1 in infant formulae has been questioned (61). The balance between ARA and DHA might be of importance because it might contribute to LC-PUFA deposition in the growing brain (80). A recent recommendation from EFSA (45) considers that there is no necessity to set a specific minimum content of ARA or EPA or a specific ratio for DHA:ARA, whereas the Food and Agriculture Organization has defined an adequate intake for ARA as 0.2% to 0.3% of energy (which translates into 0.4% to 0.6% fatty acids) (71) and, based on a systematic data analysis (79), an international expert consultation advised infant minimum intakes of ARA and DHA of 140 and 100 mg/day, respectively. Future research should further explore DHA and ARA needs in infancy.

Other LC-PUFA and Other PUFA

Breast milk and dairy fats contain low levels of several other LC-PUFA, including EPA and n-3 docosapentaenoic acid (*http://links.lww.com/MPG/A473*). EPA has antithrombotic activities in adults, and, together with other n-3 LC-PUFA, serves as a precursor of metabolites with anti-inflammatory properties (81). Breast milk also contains extremely small amounts of n-3 stearidonic acid, which may be more efficiently transformed into EPA and DHA than ALA (82). No data are available concerning their potential physiological functions in infants, and requirements for these fatty acids have not been defined (71).

LC-PUFAs are ligands to nuclear transcription factors and have been shown, in vitro and in animals, to influence gene expression. In rodents, diets rich in saturated fatty acids and poor in PUFA provided during pregnancy and/or lactation have been shown to influence obesity development and glucose/insulin homeostasis in the offspring when they became adults (83). Recent data in humans suggest similar long-term influences on adiposity (84), insulin resistance (85) and neurodevelopment (49), which might be because of epigenetic mechanisms and require further research, especially in light of the changes in breast milk composition of PUFA during the latest decades and the potential for transgenerational transfer.

Synthesis and Roles of Cholesterol

Cholesterol is endogenously synthesized, and dietary sources are animal lipids and mammalian milks (86). Cholesterol is the substrate for the synthesis of bile acids, lipoproteins, vitamin D, and hormones. It also acts by stabilizing the structure of cellular membranes and is incorporated into brain lipids mainly during the first months of life (87). The balance and interaction between DHA and cholesterol might modulate membrane rafts and functions of channels, enzymes, and receptors associated with membranes, but clinical consequences in infants are not known.

The higher cholesterol concentration of human milk is most likely the reason for the higher blood levels of cholesterol and low-density lipoprotein cholesterol levels in breast-fed infants compared with formula-fed infants (88). Several studies in formula-fed infants have assessed the possible risks and benefits of various levels of cholesterol. Breast-fed infants show a 3-fold lower fractional synthesis rate of cholesterol than infants fed formulae containing extremely low levels of cholesterol (89), suggesting that dietary cholesterol intake, in addition to other possible factors, may modulate cholesterol metabolism in infancy. Other trials in infants reported similar short-term effects, which usually disappear after 18 months (86,90,91). Lasting effects were reported in meta-analyses of studies on the association of breast-feeding with modestly but significantly reduced concentrations of total cholesterol and low-density lipoprotein cholesterol in adults. A greater difference (0.15 mmol/L) was observed for exclusive rather than partial breast-feeding, suggesting that exclusive breast-feeding of 30% of infants could reduce population prevalence of cardiovascular disease by 5% (92). Indeed, the longitudinal study of 87,252 nurses born in the first half of the 20th century found breast-feeding associated with a 10% risk reduction for cardiovascular disease (93).

Cholesterol supplementation in preterm infants did not influence vitamin D metabolism or endogenous cholesterol synthesis (94). Addition of dairy fat in infant formulae has been reported not only to increase LC-PUFA levels in red blood cells (95–97), which may be because of cholesterol, but also to increase other components of dairy fat, such as myristic acid (98). At this time, conclusive evidence on the potential benefits of adding different sources of cholesterol to infant formulae is still lacking.

Role of Complex Lipids

Mammalian milks contain phospholipids plasmalogens, glycerophospholipids, and sphingolipids (including ceramides and gangliosides) at levels accounting for 0.2% to 1% of total lipids (approximately 10 to 40 mg/100 mL). Phospholipids (Table 1) have a structural role as constituents of the biomolecular membranes that surround all cells and organelles in the body, and they modify cellular metabolism and other functions. Complex lipids play important roles in signal transmission and cell recognition, which have been suggested to interact with the physiology of the brain, gut, and skin. Recently, the results of a randomized trial of sphingomyelin-enriched formula were reported showing a positive association with neurobehavioral development of low-birth-weight infants (99). Gangliosides have been reported to reduce proinflammatory signaling in the intestine (100) and protect the bowel in an infant model of necrotizing enterocolitis (101). Gangliosides make up 10% of the total lipid mass in the brain and are highly concentrated in the cerebral cortex of the brain's gray matter. It has therefore been proposed that complex lipids should be included by enriching infant formula using dairy sources (102). Complex lipids in the milk fat globule appear to provide anti-infective protection to breast-fed infants (3) or to young children receiving a fat-globule–enriched dairy product (103). A recent randomized intervention study feeding infants with a standard formula based on vegetable oil or a formula with an added dairy lipid fraction enriched in bovine milk fat globule membranes reported a benefit of the latter on mental development at 1 year (104), which should stimulate further research on the use of dairy fat in infant formulae.

CONCLUSIONS

Dietary lipids have a wide range of biological actions beyond the provision of energy and are essential for infants' growth, development, and health. Lipids in breast milk are extremely complex and diverse, and their physiological roles are not yet fully understood. Evidence continues to accumulate that the quality of

dietary lipids provided to infants has a marked impact on health outcomes.

There is thus some opportunity for improving the quality of the lipid intake of breast-fed infants by modifying the dietary supply of women during pregnancy and lactation, either in the general population or following targeted approaches. Although the lipid composition of infant formulae has been amended over time, currently available products continue to markedly differ in their lipid composition and structure from breast milk; most of these differences might be of importance for infant health and development, including, but not restricted to those in LC-PUFA, the nature and position in the triglyceride molecule of saturated fatty acids or medium- and short-chain fatty acids, cholesterol, and complex lipids.

Changes in the lipid composition of infant formulae should take advantage of the increasing knowledge and must be based on solid scientific evidence, exploring biological effects and evaluating clinical outcomes. When elaborating infant formulae, the ultimate challenge is to approximate the biochemical and clinical outcomes of breast-feeding, rather than simply mimicking the composition of human milk.

Significant improvements have been achieved in the last few years not only in research methodologies and understanding of biology, but also toward optimal usage of raw materials in the manufacture of the fat blends used in formulae. This combined progress provides opportunities to explore and evaluate optimized lipid nutrition in infants, with the aim of improving health in both the short and long terms.

RESEARCH PERSPECTIVES

Although we are gaining knowledge about lipid nutritional requirements and functions, more research is required. There are many areas of primary research but the most relevant aim to improve both understanding of underlying mechanisms and the short- and long-term clinical outcomes of infant nutrition. The latter should be addressed via adequately powered clinical trials, conducted with high methodological quality standards to achieve reliable conclusions. Future research should aim to

1. Refine the understanding of the needs of preterm and term infants for LC-PUFA and other fatty acids, including the use of dose-response studies and in-depth mechanistic studies
2. Explore the effects of lipids on innate and acquired immunity and inflammation in term and preterm infants as well as on new and promising areas, such as pulmonary function
3. Delineate the metabolic response to dietary lipids in infants, the associated pathways and the productions of mediators and signaling molecules, as well as related effects in infants
4. Investigate the effects of dairy lipids or dairy lipid fractions supplied to infants on immediate and later functional outcomes, including neurological (including behavioral and mental health) and immunological (including risk of infection) effects
5. Assess the long-term impact of dietary fatty acids in infants (such as LA, ARA, saturated and monounsaturated fatty acids, and LC-PUFA, and ratios of the different fatty acids) on growth, body composition, lipid metabolism, and insulin resistance
6. Unravel the role of lipids in epigenetic genome modification (DNA methylation, histone acetylase, and microRNA) and the resulting long-term effects, such as propensity for obesity or type 2 diabetes.

Acknowledgments: The authors thank Prof Carlo Agostoni (University of Milano, Italy) for helpful discussions and suggestions about the topic of this review and Dr Charlotte Baudry (Lactalis R&D) for scientific support and review of milk analysis.

REFERENCES

1. WHO. *Global Strategy for Infant and Young Child Feeding*. Geneva: WHO; 2003.
2. Koletzko B, Shamir R, Ashwell M. Quality and safety aspects of infant nutrition. *Ann Nutr Metab* 2012;60:179–84.
3. Koletzko B, Agostoni C, Bergmann R, et al. Physiological aspects of human milk lipids and implications for infant feeding: a workshop report. *Acta Paediatr* 2011;100:1405–15.
4. Krohn K, Demmelmair H, Koletzko B. Macronutrient requirements for growth: fats and fatty acids. In: Duggan C, Watkins JB, Walker WA, eds. *Nutrition in Pediatrics: Basic Science, Clinical, Applications.* Toronto: BC Decker; 2008.
5. Keating EM, Curtis BA, Slusher TM. Maternal milk volume and breast milk expression: implications for diet and nutrition in infants. In: Zibadi S, Watson RR, Preedy VR, eds. *Handbook of Dietary and Nutritional Aspects of Human Breast Milk.* Wageningen: Wageningen Academic Publishers; 2013:193–213.
6. Michalski MC. Lipids and milk fat globule properties in human milk. In: Zibadi S, Watson RR, Preedy VR, eds. *Handbook of dietary and nutritional aspects of human breast milk.* Wageningen: Wageningen Academic Publishers; 2013:315–34.
7. Giuffrida F, Cruz-Hernandez C, Fluck B, et al. Quantification of phospholipids classes in human milk. *Lipids* 2013;48:1051–8.
8. Kamelska AM, Pietrzak-Fiecko R, Bryl K. Determination of cholesterol concentration in human milk samples using attenuated total reflectance Fourier transform infrared spectroscopy. *J Appl Spectrosc* 2013;80:148–52.
9. Innis SM. Dietary triacylglycerol structure and its role in infant nutrition. *Adv Nutr* 2011;2:275–83.
10. Bracco U. Effect of triglyceride structure on fat absorption. *Am J Clin Nutr* 1994;60 (6 suppl):1002S–9.
11. Gonzalez HF, Vicentin D, Giumelli O, et al. Profile of triacylglycerols and percentage of palmitic acid at the sn-2 in breast milk substitutes [Article in Spanish]. *Arch Argent Pediatr* 2012;110:227–30.
12. Ailhaud G, Massiera F, Weill P, et al. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Prog Lipid Res* 2006;45:203–36.
13. Brenna JT, Lapillonne A. Background paper on fat and fatty acid requirements during pregnancy and lactation. *Ann Nutr Metab* 2009; 55:97–122.
14. Lattka E, Rzehak P, Szabo E, et al. Genetic variants in the *FADS* gene cluster are associated with arachidonic acid concentrations of human breast milk at 1.5 and 6 mo postpartum and influence the course of milk dodecanoic, tetracosenoic, and trans-9-octadecenoic acid concentrations over the duration of lactation. *Am J Clin Nutr* 2011;93:382–91.
15. Brenna JT, Varamini B, Jensen RG, et al. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am J Clin Nutr* 2007;85:1457–64.
16. Weseler AR, Dirix CE, Bruins MJ, et al. Dietary arachidonic acid dose-dependently increases the arachidonic acid concentration in human milk. *J Nutr* 2008;138:2190–7.
17. 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.
18. Koletzko B, Rodriguez-Palmero M, Demmelmair H, et al. Physiological aspects of human milk lipids. *Early Hum Dev* 2001;65 (suppl): S3–18.
19. Lapillonne A, Groh-Wargo S, Gonzalez CH, et al. Lipid needs of preterm infants: updated recommendations. *J Pediatr* 2013;162 (3 suppl):S37–47.
20. Flatt JP. Use and storage of carbohydrate and fat. *Am J Clin Nutr* 1995;61 (4 suppl):952S–9S.
21. Gale C, Logan KM, Santhakumaran S, et al. Effect of breastfeeding compared with formula feeding on infant body composition: a systematic review and meta-analysis. *Am J Clin Nutr* 2012;95:656–69.
22. Arenz S, Ruckerl R, Koletzko B, et al. Breast-feeding and childhood obesity-a systematic review. *Int J Obes Relat Metab Disord* 2004; 28:1247–56.
23. Weng SF, Redsell SA, Swift JA, et al. Systematic review and meta-analyses of risk factors for childhood overweight identifiable during infancy. *Arch Dis Child* 2012;97:1019–26.

24. Koletzko B, von Kries R, Monasterolo RC, et al. Infant feeding and later obesity risk. *Adv Exp Med Biol* 2009;646:15–29.
25. Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. *Curr Opin Clin Nutr Metab Care* 2002; 5:127–32.
26. Lapillonne A. Lipids. In: Koletzko B, Poindexter B, Uauy R, eds. *Nutritional Care of Preterm Infants. Scientific Basis and Practical Guidelines.* Basel: Karger; 2014:82–98.
27. Lehner F, Demmelmair H, Roschinger W, et al. Metabolic effects of intravenous LCT or MCT/LCT lipid emulsions in preterm infants. *J Lipid Res* 2006;47:404–11.
28. Rodriguez M, Funke S, Fink M, et al. Plasma fatty acids and [13C]linoleic acid metabolism in preterm infants fed a formula with medium-chain triglycerides. *J Lipid Res* 2003;44:41–8.
29. Lapillonne A. Enteral and parenteral lipid requirements of preterm infants. *World Rev Nutr Diet* 2014;110:82–98.
30. Goulet O, Olieman J, Ksiazyk J, et al. Neonatal short bowel syndrome as a model of intestinal failure: physiological background for enteral feeding. *Clin Nutr* 2013;32:162–71.
31. Koo WW, Hammami M, Margeson DP, et al. Reduced bone mineralization in infants fed palm olein-containing formula: a randomized, double-blinded, prospective trial. *Pediatrics* 2003;111 (5 pt 1):1017–23.
32. Leite ME, Lasekan J, Baggs G, et al. Calcium and fat metabolic balance, and gastrointestinal tolerance in term infants fed milk-based formulas with and without palm olein and palm kernel oils: a randomized blinded crossover study. *BMC Pediatr* 2013;13:215.
33. Carnielli VP, Luijendijk IH, van Beek RH, et al. Effect of dietary triacylglycerol fatty acid positional distribution on plasma lipid classes and their fatty acid composition in preterm infants. *Am J Clin Nutr* 1995;62:776–81.
34. Fewtrell MS, Kennedy K, Murgatroyd PR, et al. Breast-feeding and formula feeding in healthy term infants and bone health at age 10 years. *Br J Nutr* 2013;110:1061–7.
35. Schmidt R, Meier U, Markart P, et al. Altered fatty acid composition of lung surfactant phospholipids in interstitial lung disease. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L1079–85.
36. Bergsson G, Arnfinnsson J, Steingrimsson O, et al. Killing of Gram-positive cocci by fatty acids and monoglycerides. *APMIS* 2001;109: 670–8.
37. Tollin M, Bergsson G, Kai-Larsen Y, et al. Vernix caseosa as a multi-component defence system based on polypeptides, lipids and their interactions. *Cell Mol Life Sci* 2005;62:2390–9.
38. Rioux V, Pedrono F, Legrand P. Regulation of mammalian desaturases by myristic acid: N-terminal myristoylation and other modulations. *Biochim Biophys Acta* 2011;1811:1–8.
39. Hostmark AT, Haug A. Percentages of oleic acid and arachidonic acid are inversely related in phospholipids of human sera. *Lipids Health Dis* 2013;12:106.
40. Cleland LG, Gibson RA, Neumann MA, et al. Dietary (n-9) eicosatrienoic acid from a cultured fungus inhibits leukotriene B4 synthesis in rats and the effect is modified by dietary linoleic acid. *J Nutr* 1996;126:1534–40.
41. Martinez M, Mougan I. Fatty acid composition of human brain phospholipids during normal development. *J Neurochem* 1998;71: 2528–33.
42. Ntoumani E, Strandvik B, Sabel KG. Nervonic acid is much lower in donor milk than in milk from mothers delivering premature infants—of neglected importance? *Prostaglandins Leukot Essent Fatty Acids* 2013;89:241–4.
43. 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.
44. Koletzko B, Bhutta ZA, Cai W, et al. Compositional requirements of follow-up formula for use in infancy: recommendations of an international expert group coordinated by the Early Nutrition Academy. *Ann Nutr Metab* 2013;62:44–54.
45. EFSA NDA Panel. Scientific Opinion on the essential composition of infant and follow-on formulae. *EFSA J* 2014;12:3760.
46. Innis SM. Dietary lipids in early development: relevance to obesity, immune and inflammatory disorders. *Curr Opin Endocrinol Diabetes Obes* 2007;14:359–64.
47. Ramsden CE, Ringel A, Feldstein AE, et al. Lowering dietary linoleic acid reduces bioactive oxidized linoleic acid metabolites in humans. *Prostaglandins Leukot Essent Fatty Acids* 2012;87:135–41.
48. Sabel KG, Strandvik B, Petzold M, et al. Motor, mental and behavioral developments in infancy are associated with fatty acid pattern in breast milk and plasma of premature infants. *Prostaglandins Leukot Essent Fatty Acids* 2012;86:183–8.
49. Bernard JY, De Agostini M, Forhan A, et al. The dietary n6:n3 fatty acid ratio during pregnancy is inversely associated with child neurodevelopment in the EDEN mother-child cohort. *J Nutr* 2013;143: 1481–8.
50. Goyens PL, Spilker ME, Zock PL, et al. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. *Am J Clin Nutr* 2006;84:44–53.
51. Liou YA, King DJ, Zibrik D, et al. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. *J Nutr* 2007;137:945–52.
52. Makrides M, Neumann MA, Jeffrey B, et al. A randomized trial of different ratios of linoleic to alpha-linolenic acid in the diet of term infants: effects on visual function and growth. *Am J Clin Nutr* 2000;71:120–9.
53. Du Q, Martin JC, Agnani G, et al. Dairy fat blends high in alpha-linolenic acid are superior to n-3 fatty-acid–enriched palm oil blends for increasing DHA levels in the brains of young rats. *J Nutr Biochem* 2012;23:1573–82.
54. Delplanque B, Du Q, Agnani G, et al. A dairy fat matrix providing alpha-linolenic acid (ALA) is better than a vegetable fat mixture to increase brain DHA accretion in young rats. *Prostaglandins Leukot Essent Fatty Acids* 2013;88:115–20.
55. Courage ML, McCloy UR, Herzberg GR, et al. Visual acuity development and fatty acid composition of erythrocytes in full-term infants fed breast milk, commercial formula, or evaporated milk. *J Dev Behav Pediatr* 1998;19:9–17.
56. Carnielli VP, Simonato M, Verlato G, et al. Synthesis of long-chain polyunsaturated fatty acids in preterm newborns fed formula with long-chain polyunsaturated fatty acids. *Am J Clin Nutr* 2007;86:1323–30.
57. 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.
58. Campoy C, Escolano-Margarit MV, Anjos T, et al. Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. *Br J Nutr* 2012;107 (suppl 2):S85–106.
59. Makrides M, Gibson RA, McPhee AJ, et al. Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. *JAMA* 2009;301:175–82.
60. Manley BJ, Makrides M, Collins CT, et al. High-dose docosahexaenoic acid supplementation of preterm infants: respiratory and allergy outcomes. *Pediatrics* 2011;128:e71–7.
61. Makrides M, Gibson RA, Udell T, et al. Supplementation of infant formula with long-chain polyunsaturated fatty acids does not influence the growth of term infants. *Am J Clin Nutr* 2005;81: 1094–101.
62. Pedersen L, Lauritzen L, Brasholt M, et al. Polyunsaturated fatty acid content of mother's milk is associated with childhood body composition. *Pediatr Res* 2012;72:631–6.
63. Carlson SE. Arachidonic acid status of human infants: influence of gestational age at birth and diets with very long chain n-3 and n-6 fatty acids. *J Nutr* 1996;126 (4 suppl):1092S–8.
64. Lapillonne A, Carlson SE. Polyunsaturated fatty acids and infant growth. *Lipids* 2001;36:901–11.
65. Collins CT, Makrides M, Gibson RA, et al. Pre- and post-term growth in pre-term infants supplemented with higher-dose DHA: a randomised controlled trial. *Br J Nutr* 2011;105:1635–43.
66. Standl M, Sausenthaler S, Lattka E, et al. *FADS* gene cluster modulates the effect of breastfeeding on asthma. Results from the GINIplus and LISAplus studies. *Allergy* 2012;67:83–90.
67. Steer CD, Davey Smith G, Emmett PM, et al. FADS2 polymorphisms modify the effect of breastfeeding on child IQ. *PLoS One* 2010;5: e11570.

68. Jensen CL, Voigt RG, Llorente AM, et al. Effects of early maternal docosahexaenoic acid intake on neuropsychological status and visual acuity at five years of age of breast-fed term infants. *J Pediatr* 2010;157:900–5.
69. Simmer K, Patole SK, Rao SC, et al. Long-chain polyunsaturated fatty acid supplementation in infants born at term. *Cochrane Database Syst Rev* 2011:CD000376.
70. Schulzke SM, Patole SK, Simmer K, et al. Long-chain polyunsaturated fatty acid supplementation in preterm infants. *Cochrane Database Syst Rev* 2011:CD000375.
71. FAO. Fats and fatty acids in human nutrition. Report of an expert consultation. *FAO Food Nutr Pap* 2008;91:63–76.
72. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). DHA and ARA and visual development: scientific substantiation of a health claim related to docosahexaenoic acid (DHA) and arachidonic acid (ARA) and visual development pursuant to Article14 of Regulation (EC) No 1924/2006. *EFSA J* 2009;941:1–14.
73. Noakes PS, Vlachava M, Kremmyda LS, et al. Increased intake of oily fish in pregnancy: effects on neonatal immune responses and on clinical outcomes in infants at 6 mo. *Am J Clin Nutr* 2012;95:395–404.
74. Much D, Brunner S, Vollhardt C, et al. Breast milk fatty acid profile in relation to infant growth and body composition: results from the INFAT study. *Pediatr Res* 2013;74:230–7.
75. Liou AY, Innis SM. Dietary linoleic acid has no effect on arachidonic acid, but increases n-6 eicosadienoic acid, and lowers dihomo-gamma-linolenic and eicosapentaenoic acid in plasma of adult men. *Prostaglandins Leukot Essent Fatty Acids* 2009;80:201–6.
76. Friesen RW, Innis SM. Linoleic acid is associated with lower long-chain n-6 and n-3 fatty acids in red blood cell lipids of Canadian pregnant women. *Am J Clin Nutr* 2010;91:23–31.
77. Koletzko B, Braun M. Arachidonic acid and early human growth: is there a relation? *Ann Nutr Metab* 1991;35:128–31.
78. Sellmayer A, Koletzko B. Long-chain polyunsaturated fatty acids and eicosanoids in infants—physiological and pathophysiological aspects and open questions. *Lipids* 1999;34:199–205.
79. Koletzko B, Boey C, Campoy C, et al. Current information and Asian perspectives on long-chain polyunsaturated fatty acids in pregnancy, lactation, and infancy: systematic review and practice recommendations from an early nutrition academy workshop. *Ann Nutr Metab* 2014;65:49–80.
80. Novak EM, Dyer RA, Innis SM. High dietary omega-6 fatty acids contribute to reduced docosahexaenoic acid in the developing brain and inhibit secondary neurite growth. *Brain Res* 2008;1237:136–45.
81. Dalli J, Colas RA, Serhan CN. Novel n-3 immunoresolvents: structures and actions. *Sci Rep* 2013;3:1940.
82. Lemke SL, Maki KC, Hughes G, et al. Consumption of stearidonic acid-rich oil in foods increases red blood cell eicosapentaenoic acid. *J Acad Nutr Diet* 2013;113:1044–56.
83. Palsdottir V, Wickman A, Andersson N, et al. Postnatal deficiency of essential fatty acids in mice results in resistance to diet-induced obesity and low plasma insulin during adulthood. *Prostaglandins Leukot Essent Fatty Acids* 2011;84:85–92.
84. de Vries PS, Gielen M, Rizopoulos D, et al. Association between polyunsaturated fatty acid concentrations in maternal plasma phospholipids during pregnancy and offspring adiposity at age 7: the MEFAB cohort. *Prostaglandins Leukot Essent Fatty Acids* 2014;91:81–5.
85. Rump P, Popp-Snijders C, Heine RJ, et al. Components of the insulin resistance syndrome in seven-year-old children: relations with birth weight and the polyunsaturated fatty acid content of umbilical cord plasma phospholipids. *Diabetologia* 2002;45:349–55.
86. Bayley TM, Alasmi M, Thorkelson T, et al. Longer term effects of early dietary cholesterol level on synthesis and circulating cholesterol concentrations in human infants. *Metabolism* 2002;51:25–33.
87. Kinney HC, Karthigasan J, Borenshteyn NI, et al. Myelination in the developing human brain: biochemical correlates. *Neurochem Res* 1994;19:983–96.
88. Shamir R, Nganga A, Berkowitz D, et al. Serum levels of bile salt–stimulated lipase and breast feeding. *J Pediatr Endocrinol Metab* 2003;16:1289–94.
89. Wong WW, Hachey DL, Insull W, et al. Effect of dietary cholesterol on cholesterol synthesis in breast-fed and formula-fed infants. *J Lipid Res* 1993;34:1403–11.
90. Makrides M, Gibson RA. The role of fats in the lifecycle stages: pregnancy and the first year of life. *Med J Aust* 2002;176 (suppl): S111–2.
91. Demmers TA, Jones PJ, Wang Y, et al. Effects of early cholesterol intake on cholesterol biosynthesis and plasma lipids among infants until 18 months of age. *Pediatrics* 2005;115:1594–601.
92. Owen CG, Whincup PH, Kaye SJ, et al. Does initial breastfeeding lead to lower blood cholesterol in adult life? A quantitative review of the evidence. *Am J Clin Nutr* 2008;88:305–14.
93. Rich-Edwards JW, Stampfer MJ, Manson JE, et al. Breastfeeding during infancy and the risk of cardiovascular disease in adulthood. *Epidemiology* 2004;15:550–6.
94. Picaud JC, Boucher P, Lapillonne A, et al. Influence of dietary cholesterol on vitamin D metabolism in formula-fed preterm neonates. *J Pediatr Gastroenterol Nutr* 2002;35:180–4.
95. Katoku Y, Yamada M, Yonekubo A, et al. Effect of the cholesterol content of a formula on the lipid compositions of plasma lipoproteins and red blood cell membranes in early infancy. *Am J Clin Nutr* 1996;64:871–7.
96. Van Biervliet JP, Vinaimont N, Vercaemst R, et al. Serum cholesterol, cholesteryl ester, and high-density lipoprotein development in newborn infants: response to formulas supplemented with cholesterol and gamma-linolenic acid. *J Pediatr* 1992;120 (4 pt 2):S101–8.
97. Agostoni C, Riva E, Bellu R, et al. Effects of diet on the lipid and fatty acid status of full-term infants at 4 months. *J Am Coll Nutr* 1994;13:658–64.
98. Rioux V, Catheline D, Bouriel M, et al. Dietary myristic acid at physiologically relevant levels increases the tissue content of C20:5 n-3 and C20:3 n-6 in the rat. *Reprod Nutr Dev* 2005;45:599–612.
99. Tanaka K, Hosozawa M, Kudo N, et al. The pilot study: sphingomyelin-fortified milk has a positive association with the neurobehavioural development of very low birth weight infants during infancy, randomized control trial. *Brain Dev* 2013;35:45–52.
100. Miklavcic JJ, Schnabl KL, Mazurak VC, et al. Dietary ganglioside reduces proinflammatory signaling in the intestine. *J Nutr Metab* 20122012(280286.
101. Schnabl KL, Larsen B, Van Aerde JE, et al. Gangliosides protect bowel in an infant model of necrotizing enterocolitis by suppressing proinflammatory signals. *J Pediatr Gastroenterol Nutr* 2009;49:382–92.
102. Billeaud C, Puccio G, Saliba E, et al. Safety and tolerance evaluation of milk fat globule membrane–enriched infant formulas: a randomized controlled multicenter non-inferiority trial in healthy term infants. *Clin Med Insights Pediatr* 2014;8:51–60.
103. Veereman-Wauters G, Staelens S, Rombaut R, et al. Milk fat globule membrane (INPULSE) enriched formula milk decreases febrile episodes and may improve behavioral regulation in young children. *Nutrition* 2012;28:749–52.
104. Timby N, Domellof E, Hernell O, et al. Neurodevelopment, nutrition, and growth until 12 mo of age in infants fed a low-energy, low-protein formula supplemented with bovine milk fat globule membranes: a randomized controlled trial. *Am J Clin Nutr* 2014;99:860–8.