

Lipids and infant formulas

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Abstract

The ultimate goal in the design of infant formula is to achieve the outcome seen in breast fed infants. This review of lipids in infant formulas for term infants begins by referring to the lipid composition of human milk, and relates that to differences in lipid digestion and metabolism which exist between breast fed and formula fed infants and which may significantly influence fatty acid bioavailability.

Recommendations are made for the lipid content and fatty acid composition of term infant formulas (especially for lauric, linoleic, α -linolenic, long chain 20 and 22C n-3 and n-6 polyunsaturated fatty acids and the *trans* fatty acids).

Further research is required to define more clearly the long term nutritional, growth and developmental effects of structured lipids in formulas for term infants. More information is required on the differential handling of LCPUFA and other fatty acids at the organ and cellular level. There is a need for large (multi-centre) randomized studies to determine the short and long term functional effects of LCPUFA supplementation. Further research and development is required to determine a commercial source of LCPUFA which is safe, effective and economic. Further information is required on the short and long term effects of cholesterol intake during infancy, and in particular its relationship to LCPUFA metabolism. Long term studies should be initiated to determine the relationship of infant diet (especially saturated fatty acid and cholesterol intake) to the development of cardiovascular disease.

Introduction

Although considerable progress has been made on the design and development of formulas for term infants, it is recognized that the composition of artificial formulas will never attain the unique dynamic qualities of breast milk. It is also appreciated, however, that there continues to be a demand for an alternative to breast milk and a human milk substitute must, as closely as possible, meet the nutritional requirements of the rapidly growing infant.

The lipid component of artificial formulas is not only a major source of energy for the newborn infant (Hamosh *et al.* 1985), but is also a vehicle for the provision of essential fatty acids which, due to their contribution to the structure and function of cellular membranes, influence vital physiological and metabolic processes during infancy and beyond. (Mohrhauer & Holman 1963*a,b*; Clandinin *et al.* 1989; Hernell, 1990; Innis, 1991).

The ultimate goal in the design of infant formula is to achieve the outcome seen in breast fed infants (Department of Health, 1996). This review of lipids in infant formulas for term infants therefore begins by referring to the lipid composition of human milk, and relates that to differences in lipid digestion and metabolism which exist between breast fed and formula

fed infants and which may significantly influence fatty acid bioavailability (Hernell & Bläckberg, 1994b). Finally, specific formulation issues are addressed.

Human milk lipid composition

Content

The lipids in human milk are triacylglycerols (98%), cholesterol (0.4%) and phospholipids (1.3%) (Jensen, 1989; Jensen *et al.* 1990). Lipid content varies with duration of lactation (Dewey *et al.* 1984; Jensen, 1989), with time of day, (Hall, 1979; Harzer *et al.* 1983; Jensen, 1989), and from start to finish of an individual feed (Hyttén, 1954; Hall 1979; Jensen 1989; Koletzko *et al.* 1992b).

Triacylglycerol concentration increases from approximately 30 g/l in colostrum to 35 g/l in transitional milk and 40 g/l in mature milk (Harzer & Bindels, 1987; Jensen, 1989). There is a decrease in milk phospholipid and cholesterol concentrations from 1.1% and 1.3% on day 3 to 0.6% and 0.4% on day 84 respectively (Bitman *et al.* 1984).

Lipid content also alters during a single feed rising from 15–30 g/l in foremilk to 60–70 g/l in hindmilk (Hall, 1979; Jensen, 1989; Koletzko *et al.* 1992b). It has been speculated that this phenomenon is related to adsorption of fat molecules to alveolar surfaces, with displacement occurring only when the gland is nearly empty (Nichols & Nichols, 1983). Whether the increase in lipid content plays a role in the prevention of obesity by acting as a mechanism to regulate intake, as suggested by Hall (1979), remains uncertain. Lipid composition of foremilk of both breasts can differ considerably, with the higher content being in the breast that is sucked second (33 g/l), compared with the breast that is sucked first (24 g/l) (Hall, 1979).

Lipid content changes throughout the day with peak levels occurring at different times of the day, depending upon the population studied. In Western women, the milk fat content rises during the early part of the day, peaks at about 18.00 hours and then declines; the reason for this pattern is not known (Jensen, 1989). Total milk lipid content is not significantly influenced by maternal diet (Hamosh & Bitman, 1992), but diet can alter individual fatty acid concentrations.

Triacylglycerols

Triacylglycerols, which are composed of one molecule of glycerol esterified with three fatty acids, are synthesized primarily in the rough endoplasmic reticulum of the mammary alveolar cell by the reaction of fatty acyl CoA with glycerol 3-phosphate (Hansen *et al.* 1986). The mammary cell specific enzyme, thioesterase II, terminates fatty acid synthesis at chain lengths of 16 carbons or less (Thompson & Smith, 1985) and thus only short and medium chain fatty acids are synthesized *de novo* in the breast. Long chain fatty acids are derived from the diet or from the mobilization of fat depots (Nichols & Nichols, 1983; Dils, 1989). Dietary triacylglycerols are transported to the mammary gland as components of chylomicra or lipoprotein particles. The endothelial enzyme, lipoprotein lipase, hydrolyses the triacylglycerols to fatty acids and monoacylglycerols which are then transported into the alveolar cells (Scow & Chernick, 1987; Hamosh *et al.* 1970). Lipids mobilized from fat depots are largely transported in plasma as fatty acids bound to albumin.

Fatty Acids

More than 200 fatty acids have been identified in human milk, with seven fatty acids representing 90 wt % of total fat (van Beusekom *et al.* 1993)—oleic, palmitic, lauric, linoleic, myristic, stearic, and capric acids (Table 1). Milk triacylglycerols are mainly composed of long chain fatty acids (only 7% of the fatty acids are medium chain, C6–12), and saturated fats constitute 50.1% with unsaturated fats accounting for 48.5% of total lipid (Department of Health and Social Security, 1981). The fatty acid composition of the triacylglycerol can vary with maternal diet, length of gestation, duration of lactation and parity (Koletzko *et al.* 1988; Prentice *et al.* 1989).

Studies from Europe, Africa and Australia, have demonstrated that levels of essential polyunsaturated fatty acids (PUFA) linoleic (18:2 n-6) and α -linolenic (18:3 n-3) acid in human milk are influenced by the maternal diet of lactating women but the longer chain polyunsaturated fatty acid concentrations (LCPUFA) are less affected (Gibson & Kneebone, 1981; Koletzko *et al.* 1988, 1992a). Moreover, the n-6 and n-3 LCPUFA in milk were found not to be related to their respective precursors, linoleic and α -linolenic acids (Gibson & Kneebone, 1984; Koletzko *et al.* 1988). In contrast, n-6 and n-3 LCPUFA correlated with each other, which may reflect a protective metabolic mechanism that provides the breast fed infant with a balanced ratio of the two LCPUFA (Koletzko, 1992a). The average ratio of n-6 to n-3 was 2.7 for European milks and 2.2 for African milks. However, it has been shown that if the dietary intake of preformed n-3 LCPUFA is significantly increased, this is reflected by an increase in n-3 LCPUFA concentrations in the milk (Gibson & Kneebone, 1981; Harris *et al.* 1984; Kneebone *et al.* 1985; Carlson *et al.* 1986; Innis & Kuhnlein, 1988; Koletzko *et al.* 1988; Innis *et al.* 1990; Sanders & Reddy, 1992). More recently a direct correlation was demonstrated between the level of docosahexaenoic acid (DHA) in breast milk and DHA in the maternal diet (Makrides *et al.* 1996).

Human milk fatty acid composition remains constant during a single feed and during the day, but is influenced by duration of gestation and lactation (Hall, 1979; Harzer *et al.* 1983; Jensen, 1989). As lactation advances, human milk medium chain fatty acid contents, notably lauric (12:0) and myristic (14:0) acids increase, mainly at the expense of oleic (18:1 n-9) and palmitic (16:0) acids (Read & Sarrif, 1965; Bitman *et al.* 1983; Lammi-Keefe & Jensen, 1984; Boersma *et al.* 1991). The increase in medium chain fatty acid content suggests an

Table 1. Fatty Acid Nomenclature

Capric	10:0
Lauric	12:0
Myristic	14:0
Palmitic	16:0
Stearic	18:0
Oleic	18:1 n-9
Mead's	20:3 n-9
Linoleic	18:2 n-6
Gamma-linolenic	18:3 n-6
Arachidonic	20:4 n-6
Docosapentaenoic	22:5 n-6
Alpha-linolenic	18:3 n-3
Stearidonic	18:4 n-3
Docosapentaenoic	22:5 n-3
Docosahexaenoic	22:6 n-3

augmentation of fatty acid *de novo* synthesis in the breast with advancing lactation. LCPUFA concentrations decline with duration of lactation and this is probably related to the decrease in fat globule membrane relative to the fat globule core (see below).

In a study of the fatty acid composition of the milk of Gambian women, Prentice and co-workers (1989) reported that breasts of lactating mothers of high parity have a markedly reduced capacity for *de novo* synthesis of fatty acids. Although the utilization of plasma lipid for milk fat was reduced, there was a more marked depression of mammary fatty acid synthesis. The mechanisms regulating substrate delivery and mammary fatty acid synthesis require further elucidation (Neville, 1989).

Phospholipids

Phospholipids consist of a glycerol molecule with two fatty acids and a phosphate group linked to an alcohol. The major classes in human milk are (wt %) phosphatidyl choline 28.4; phosphatidyl ethanolamine 19.3 and sphingomyelin 37.5 (Jensen, 1989). Smaller amounts of phosphatidyl serine (8.8%) and inositol (6.1%), along with cerebrosides and gangliosides have been identified (Bitman *et al.* 1984). Sphingomyelin contains mainly saturated fatty acids, whereas phosphatidyl choline, ethanolamine, serine and inositol have high concentrations of PUFA with 18–22 carbons and negligible amounts of medium chain fatty acids.

Phospholipids, which are important constituents of cellular membranes, particularly retinal and neural tissues (Bitman *et al.* 1984; Jensen, 1989), are synthesized *de novo* within the mammary gland and then located in the plasma membrane. During lactation the lipids coalesce into increasingly large fat droplets within the secretory cell, and migrate from the base to the apex of the secretory cell. Prior to discharge into the alveolar lumen, each droplet is enveloped in cell membrane which is rich in both phospholipid and cholesterol. Although the globules have an average diameter of 4 μm , the size of the milk fat globule changes both as a function of length of lactation and length of gestation, with colostrum having smaller globules than mature milk (Rüegg & Blanc, 1981). The more efficient packaging of triacylglycerol within the globules during the progression from colostrum to mature milk results in the need for less membrane and thus the lower levels of phospholipid and cholesterol in mature milk. It has also been suggested that the higher phospholipid and cholesterol levels in colostrum may also be related to the higher cell content of colostrum (Rüegg & Blanc, 1978).

Cholesterol

Cholesterol occurs in both free (87%) and bound (13%) form (Bitman *et al.* 1983); when bound it is usually esterified with long chain fatty acids. Cholesterol originates from *de novo* synthesis in the mammary gland and from the plasma. Most of the cholesterol is located in the milk fat globule membrane and the amount is not affected by diet (Jensen, 1989). Lammi-Keefe & Jensen (1984) detected a diurnal pattern in the cholesterol content of milk, ranging from 87.5 mg/l at 06.00 h to 112 mg/l at 22.00 h. Clark & Hundrieser (1989) found that the mean total cholesterol content of 25 milk samples was 135 mg/l, with the cholesterol ester content being 25%. In addition to being an essential component of cell membranes, cholesterol has important functions in the metabolism of bile acids, steroid hormones and calciferols.

Lipid digestion and fatty acid bioavailability

Digestion

It has been clearly demonstrated that tissue fatty acid composition is related to the fatty acid content of the diet (Witting *et al.* 1961; Mohrhauer & Holman, 1963*a,b*). In breast and formula fed infants, there are not only differences in dietary fatty acid content but also variations in digestive processes which may significantly influence fatty acid bioavailability. (Bernbäck *et al.* 1990; Hernell & Bläckberg, 1994*b*).

Lipases

Before absorption can occur, dietary triacylglycerols must be hydrolysed into absorbable products, i.e. monoacylglycerols, free fatty acids and glycerol. This process is initiated in the stomach through the action of preduodenal lipase. The origin of this lipase is uncertain. The chief cells in the mucosa of the fundus of the stomach (gastric lipase) and von Ebner's glands around the circumvillate papillae of the tongue (lingual lipase) have both been proposed as sources (Hamosh & Burns, 1977; Moreau *et al.* 1988). The lingual lipase and gastric lipase have similar molecular weight, structure and function. In contrast to the other lipases involved in fat digestion, gastric lipase has the property of being able to penetrate the milk fat globule membrane and initiate hydrolysis of the core triacylglycerol (Bernbäck *et al.* 1989). However, it is not able to hydrolyse the ester bonds of phospholipids and cholesterol esters. Gastric lipase secretion is stimulated by gastrin, does not have a known cofactor and, in contrast to pancreatic enzymes, the capacity to synthesize and secrete gastric lipase is well developed at birth (Hernell & Bläckberg, 1994*a*). Gastric lipase acts preferentially at the *sn*-3 position with a predominance of short, medium and long chain unsaturated fatty acids being released. The short and medium chain fatty acids can be directly absorbed in the stomach (Hamosh *et al.* 1989).

Fat digestion continues in the upper intestine through the activity of pancreatic colipase-dependent lipase. Long chain fatty acids released by the action of gastric lipase have an important role in enforcing the binding between the colipase–lipase complex and fat globules and this property enables the fat globule membrane to be penetrated by this enzyme (Bernbäck *et al.* 1989).

The enzyme bile salt stimulated lipase (BSSL) is only available to breast fed infants (Hernell *et al.* 1988). It is a product of protein synthesis in the mammary gland (Bläckberg *et al.* 1987), is secreted in breast milk, and contributes significantly to lipid hydrolysis in the breast fed infant (Hernell *et al.* 1988). Following activation by bile salts in the duodenum, BSSL acts as a non-specific lipase, supporting colipase-dependent lipase in hydrolysing triacylglycerols and diacylglycerols. A lipase, functionally very similar to BSSL, is secreted by the pancreas (Hernell *et al.* 1988). More recently this enzyme, carboxylic ester hydrolase (CEH), has been shown to have an identical primary structure to BSSL, indicating that they are almost certainly the products of a common gene (Nilsson *et al.* 1990). The contribution of CEH is relatively small compared to BSSL, with two-thirds of the combined BSSL/CEH activity in preterm infants fed human milk being provided by the milk BSSL (Fredrikzon *et al.* 1978).

Triacylglycerol configuration

The fatty acids in human milk have a highly specific positional distribution on the glycerol moiety and it has been suggested that the triacylglycerol stereospecific configuration (*sn*) may modulate the efficiency of nutrient absorption (Lien, 1994). Although human milk contains at

least 170 different triacylglycerol structures it has been reported that 30 of these represent 70% of total milk lipid. It has been demonstrated that the triacylglycerol structure most favourable for hydrolysis by the newborn infant is an unsaturated fatty acid at *sn*-1, saturated fatty acid at *sn*-2 (usually C14:0 or C16:0), and medium chain fatty acid at *sn*-3 (Winter *et al.* 1993).

Palmitic acid (16:0) is the predominant saturated fatty acid, constituting 20–25% of fatty acids in mature human milk, and 70–75% of it is esterified at the *sn*-2 position of the triacylglycerol (Breckenridge *et al.* 1969). In contrast, palmitic acid present in vegetable oils, which are most commonly used in the manufacture of infant formulas, is esterified at the *sn*-1 and *sn*-3 positions (Lien, 1994), and the *sn*-2 position is predominantly occupied by unsaturated fats (Small, 1991; Innis *et al.* 1994b). The reason for the preferential esterification of 16:0 to the 2-position of glycerol during triacylglycerol synthesis in the mammary gland is uncertain. However, it is known that pancreatic colipase-dependent lipase selectively hydrolyses the fatty acids at the *sn*-1 and *sn*-3 positions, yielding free fatty acids and 2-monoacylglycerols, and 2-monoacylpalmitin has been shown to be more efficiently absorbed than free palmitic acid which tends to form insoluble soaps with cations such as calcium and magnesium (Sammons & Wiggs, 1960; Watkins *et al.* 1974; Graham & Sackman, 1983; Quinlan *et al.* 1995).

This explanation for the predominance of palmitic on the *sn*-2 position is complicated however, by *in vitro* studies which have demonstrated that BSSL, unlike colipase-dependent pancreatic lipase, hydrolyses *sn*-2 monoacylglycerol to free fatty acid and glycerol (Hernell *et al.* 1988). This would indicate that palmitic acid could be released from monoacylglycerol and be vulnerable to insoluble soap formation. However, it appears that BSSL hydrolyses tri- and diacylglycerols at a faster rate than monoacylglycerols and therefore a significant proportion of palmitic acid will be available for absorption as 2-monoacylpalmitin (Lien, 1994).

Innis and colleagues not only provided evidence that palmitic acid is absorbed as *sn*-2 monoacylglycerol in breast fed infants, but also supported previous work by demonstrating that 70% of the fatty acids which are absorbed as *sn*-2 monoacylglycerols are conserved in that position during re-esterification to triacylglycerols in the intestinal mucosal cells (Small, 1991; Innis 1994; Innis *et al.* 1995). Studies have shown that the rate of hydrolysis and composition of remnant particles formed by lipoprotein and hepatic lipase are influenced by fatty acid composition and positional distribution (Redgrave *et al.* 1988; Small, 1991; Mortimer *et al.* 1992;). More recently, Innis and colleagues (1996) demonstrated that the lipid structure of adipose tissue reflected dietary triacylglycerol configuration. It is therefore possible that the distribution of fatty acids in human milk or formula triacylglycerols could have important metabolic effects beyond that of fat absorption.

Palmitic acid is not the only fatty acid to show a specific preference for a particular position in human milk triacylglycerols: oleic acid and stearic acid are mainly located at the *sn*-1 position whereas linoleic acid is located mainly in the *sn*-1 and *sn*-3 positions. The distribution of arachidonic acid (AA) and docosahexaenoic acid (DHA) in triacylglycerols of human milk is similar with 50% being esterified at the *sn*-2 position and 45% at the *sn*-3 position (Martin *et al.* 1993).

Long chain polyunsaturated fatty acids (LCPUFA)

The rate of release of LCPUFA from triacylglycerol by pancreatic colipase-dependent lipase at the *sn*-3 (and *sn*-1) positions is dependent upon the positions of double bonds, with a relative resistance to the release of AA (C20:4 n-6) and eicosapentaenoic acid (EPA) (C20:5 n-3) from triacylglycerols compared to the more saturated fatty acids, e.g. linoleic acid (C18:2 n-6) and oleic acid (C18:1 n-9). However, BSSL efficiently hydrolyses the ester bonds of DHA and AA at all positions, even at the low levels of bile salt concentration which are present during the

newborn period. These data indicate that an important function of BSSL in breast fed infants is to enable optimal release and utilization of LCPUFA from human milk (Chen *et al.* 1989, 1994; Hernell *et al.* 1993).

There is evidence that the *sn*-position of DHA may determine the metabolic fate and tissue distribution of the fatty acid. In a study of newborn rats who were fed oils with DHA located in the *sn*-2 position or DHA randomly distributed, the specifically structured triacylglycerol resulted in a higher level of DHA in the brain compared with the randomized oil which had higher levels of DHA in the liver (Christensen & Høy, 1997). Other recent data from this group indicate that these metabolic differences may influence sensory and cognitive function (Christensen *et al.* 1996). Further studies are required to investigate the hypothesis that optimal utilization of LCPUFA is dependent on appropriate stereospecific configuration of the triacylglycerols and the availability of BSSL.

Other lipolytic enzymes

Additional lipolytic enzymes secreted by the pancreas are phospholipase A₂, which releases the phosphate group from *sn*-3 position of phospholipids, and cholesterol ester hydrolase which cleaves cholesterol esters into free fatty acids and cholesterol.

Lipid composition of formulas—What are the issues?

Total lipid content?

It was previously recommended that the total lipid content of infant formula should have a lower limit of 3.3 g/100 kcal and an upper limit of 6.0 or 6.5 g/100 kcal (Food and Drug Administration, 1985). More recently, however, it has been suggested that such a low fat content is not desirable because a significant proportion of the caloric content would have to come from a high protein or carbohydrate intake and this would increase the osmolarity of the milk and also increase the renal solute load. Therefore, ESPGAN (1991) favour a minimum fat content of 4.4 g/100 kcal (40% of energy intake, which is similar to the lower range of human milk fat values).

Triacylglycerol structure adaptation?

The positional specificity of fatty acids on the glycerol moiety has prompted the development of 'structured lipids'. Adaptation of the triacylglycerol structure of infant formula lipids to approximate more closely to that of human milk seems a logical step in the further development of infant formulas. The triacylglycerol structure of infant formula lipids can be modified by the process of 1,3-enzymic interesterification (Lien *et al.* 1993). The fatty acids are released from their natural positions by 1,3 selective lipases and redistributed equally to all three positions on the glycerol molecule. Long chain saturated fatty acid absorption has been shown to be significantly improved in rats fed a vegetable oil blend containing a high proportion of palmitic acid in the *sn*-2 position (Betapol) compared with rats who were fed a mixture of vegetable oils with the palmitic acid predominantly in the *sn*-1,3 positions (Carnielli *et al.* 1995a). More recently Carnielli *et al.* (1995b) conducted fat and mineral balances on preterm infants receiving a Betapol-containing formula using a randomized crossover design. Although the

overall fat absorption from the two formulas was not significantly different the absorptions of palmitic and myristic acids were significantly higher and calcium retention greater in the Betapol formula. A more recent randomized trial of synthetic triacylglycerol formula in preterm infants reported similar results (Lucas *et al.* 1997). In an early study of term infants, a formula based on natural lard which contains palmitic acid mainly in the *sn*-2 position was compared with a formula based on randomized lard. The absorption of all fatty acids was improved in the infants receiving natural lard, especially palmitic and stearic acids (Filer *et al.* 1969). This important finding needs to be supported by further long term studies to define more clearly the nutritional, growth and developmental effects of structured lipids in term infants.

Saturated v. unsaturated fatty acids?

The polyunsaturated to saturated fatty acid ratio (P/S) in human milk is in the region of 0.3 and differs from bovine milk which has a P/S ratio of 0.04 (Lawrence, 1994). Most manufacturers have adapted their formulas to achieve a P/S ratio of 0.2–0.5 (Koletzko & Bremer, 1989). With the widely accepted view that dietary saturated fatty acids are associated with an elevation of plasma cholesterol, the saturated fatty acid component of formulas is of considerable relevance. Studies have demonstrated that saturated fatty acids suppress receptor-mediated clearance of low density lipoproteins (LDL) (Shepherd *et al.* 1980; Spady & Dietschy, 1985) and this may be due to saturates potentiating the action of cholesterol to suppress gene expression for LDL receptors in liver cells (Goldstein & Brown, 1977).

Whether each of the dietary saturated fatty acids exerts a similar cholesterolaemic effect has been studied in primates and the evidence suggests that lauric (12:0) and myristic acids (14:0) are most cholesterolaemic (and therefore atherogenic) with 16:0 having a modest effect and 18:0 possibly being neutral (Hayes *et al.* 1991). On this basis it has been recommended that lauric and myristic acids should not exceed 15% of fat in infant formulas (Commission of the European Communities, 1991; ESPGAN, 1991).

Monounsaturated fatty acids are mainly represented by oleic acid (18:1 n-9) which alone accounts for approximately one third of the lipids in breast milk. It is a major source of energy and from a structural viewpoint is an important component of myelin which is formed during the first two years of life. The early adult work of Hegsted and colleagues (1965) and Keys and colleagues (1965) indicated that monounsaturated fatty acids, especially oleic acid, lower the concentration of total cholesterol when exchanged for saturated fatty acids and it was later demonstrated that the change was due to a decrease in LDL cholesterol levels with high density lipoprotein (HDL) cholesterol concentrations being unchanged (Mattson & Grundy, 1985).

How these animal and adult human studies relate to infant nutrition remains uncertain. However, a recent study of formula fed term infants showed that enrichment of the diet with oleic acid during weaning enabled the lipoprotein profile to more closely resemble that present during breast feeding (Uauy *et al.* 1990). Further studies relating formula P/S ratios to later plasma lipid profiles and to cardiovascular risk factors are required.

Trans-fatty acids?

Trans-isomeric unsaturated fatty acids are formed during industrial and biological hydrogenation of unsaturated fats. C18 *trans*-monounsaturated fatty acids are the major *trans*-

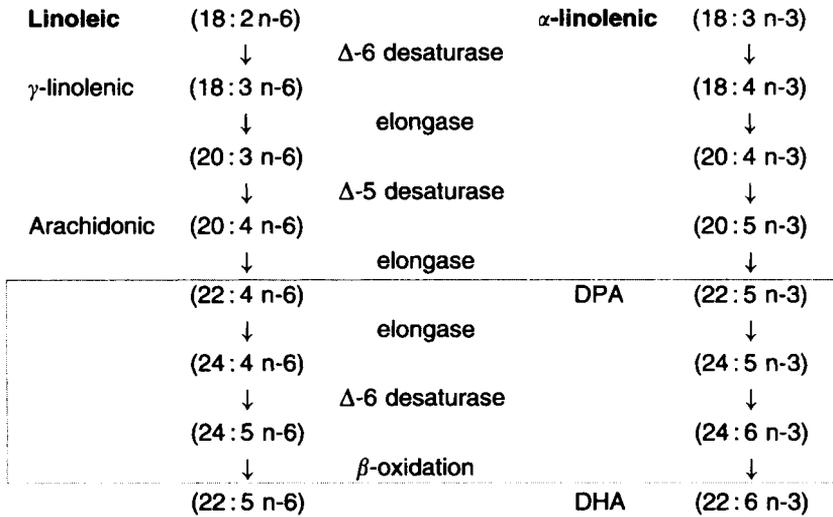
isomers found in partly hydrogenated vegetable oil and animal fats (Sanders, 1988). This is reflected in the *trans*-fatty acid composition of breast milk with the major proportion of *trans*-fatty acids being 18:1t (Koletzko *et al.* 1988). In that study *trans*-fatty acids accounted for 4.4% of fatty acids in breast milk. The *trans*-fatty acid content of human milk reflects the *trans*-fatty acid content of the previous day's diet (Emken, 1995). In studies of Canadian women human milk fat was found to contain 6% fatty acids when a 35% *trans*-fatty acid diet was fed and 2.5% for a 13% *trans*-fatty acid diet (Chappell *et al.* 1985). *Trans*-fatty acids are mainly incorporated into the triacylglycerol fraction but can also be located in phospholipids and cholesterol. Their metabolism and utilization for energy production is similar to saturated and *cis*-monounsaturated fatty acids. *Trans*-fatty acids have been implicated in the development of cardiovascular and thrombotic disease and cancer in adults; however, this has been questioned (Kritchevsky, 1982; Hunter *et al.* 1985). The effects of *trans*-fatty acid intake in infancy are uncertain, but there is some evidence that they may impair biosynthesis of LCPUFA and compromise growth in preterm infants (Koletzko, 1992b). Further research is required and in the meantime it has been recommended that the *trans*-fatty acid content of infant formulas should not exceed 4% (Commission of the European Communities, 1991).

Ratio of linoleic acid to α -linolenic acid?

The essential fatty acids, linoleic acid and α -linolenic acid, are substrates for lengthening and desaturation reactions which take place mainly in the liver endoplasmic reticulum, and which lead to the synthesis of LCPUFA with 20–22 carbons and 2–6 double bonds (Fig. 1). Desaturation enzymes are mixed function oxygenases that are named according to the site at which the double bond is inserted. The position of desaturation is indicated by Δ and occurs at carbon atoms 9, 6, 5, and 4 from the carboxyl group of the fatty acid. Recent studies show that Δ -4 desaturation involves three enzymic steps, rather than a single step by a Δ -4 desaturation enzyme. This reaction proceeds by initial chain elongation, followed by Δ -6 desaturation and then chain shortening by β -oxidation retroconversion in the peroxisomes (Voss *et al.* 1991).

The rate limiting step in fatty acid metabolism is desaturation, especially Δ -6 desaturation. Fatty acids of the n-3, n-6, and n-9 series compete as substrates for the same desaturation enzymes (Fig. 1). The Δ -6 desaturation enzyme shows a preference for fatty acids in the order 18:3 n-3 > 18:2 n-6 > 18:1 n-9 (Brenner, 1981). Desaturation of oleic acid (18:1 n-9) is more marked when 18:2 n-6 and 18:3 n-3 availability is low. An elevated concentration of Mead acid (20:3 n-9) may therefore be an indicator of essential fatty acid deficiency (British Nutrition Foundation, 1992).

The competitiveness between metabolic pathways for n-6 and n-3 fatty acid elongation and desaturation has important implications for recommendations on LCPUFA requirements for infants. In human milk the ratio of linoleic acid to α -linolenic acid (LA/ALA) is generally between 5 and 10. Ratios on either side of this range produced fatty acid profiles markedly different from that of breast fed infants (Clark *et al.* 1992), and extremes of LA/ALA ratio altered 22-carbon fatty acid concentration in the brain (Dyer & Greenwood, 1991). Previous recommendations on levels of LA and ALA in formulas have referred to the amounts of linoleic acid and α -linolenic acid in human milk and thus manufacturers have opted for an LA/ALA ratio of approximately 10:1. This does not allow for the preformed LCPUFA which are present in human milk (Gibson *et al.* 1994). The optimum LA/ALA ratio can therefore only be considered in parallel with the discussion on whether formulas should be supplemented with



3 Enzyme reactions involved in Δ -6 desaturation

Figure 1. Desaturation and elongation reactions of essential fatty acids linoleic and α -linolenic acids

LCPUFA. It has been recommended, however, that the LA/ALA ratio should be not less than 5 and not greater than 15 (Commission of the European Communities, 1991).

LCPUFA supplementation for term infants?

Biochemical evidence

Breast fed infants receive a dietary LCPUFA supply which is considered to meet their requirements for tissue accretion, even if they are born prematurely (Clandinin *et al.* 1989). In contrast to human milk, commercial infant formulas until recently did not contain LCPUFA (Koletzko & Bremer, 1989). Term and particularly preterm infants, fed formulas that contain linoleic acid and α -linolenic acid but are devoid of LCPUFA, develop LCPUFA depletion of structural lipids, as measured by plasma and erythrocyte membrane concentrations (Putnam *et al.* 1982; Koletzko *et al.* 1989; Makrides *et al.* 1994; Decsi *et al.* 1995). Studies assessing the relationship between brain fatty acids and diet in infancy have demonstrated that breast fed infants have higher concentrations of DHA in their cerebral cortex compared with infants fed formula milk (Farquharson *et al.* 1992, 1995; Makrides *et al.* 1994). Furthermore, the accumulation of DHA in the cerebral cortex is dependent on the length of breast feeding (Makrides *et al.* 1994). In contrast there were no differences in the level of cortical AA between breast and formula fed infants, suggesting that either AA is rigorously conserved in brain tissue or the amount of AA produced from the precursor in formula is sufficient to meet the AA requirements of the rapidly growing brain.

Higher levels of AA and DHA have been achieved in infants fed formulas containing preformed LCPUFA compared with infants fed formulas with high concentrations of the precursor essential fatty acids. The most important factor determining the plasma and ery-

throcyte content of AA and DHA in young infants is therefore the dietary intake of preformed LCPUFA rather than the dietary parent essential fatty acids, and this supports the view that elongation and desaturation pathways may be impaired in preterm and term infants during the first months of life.

Clinical evidence

As the accretion of polyunsaturated fatty acids is greatest in the phospholipids of cell membranes in the central nervous system, there has been intense investigation of the effects of LCPUFA on brain development and cortical and visual function in the infant.

Several studies have focused on the maturation of the visual pathway because photoreceptor outer segment membranes in the retina contain uniquely high concentrations of DHA, their presence being important for normal photochemical activity of the visual pigment rhodopsin (Wiedmann *et al.* 1988). Animal studies have demonstrated that LCPUFA may have important biochemical and physiological effects on retinal function (Weisinger *et al.* 1996; Neuringer *et al.* 1986).

Several human infant studies have indicated that n-3 LCPUFA supplementation may enhance visual acuity in preterm infants (Birch *et al.* 1992*a,b*; Carlson *et al.* 1993), but the evidence for term infants is not consistent. Four nonrandomized studies have compared LCPUFA status with assessments of visual acuity in breast and formula fed infants. Three studies reported better visual function indices and higher DHA levels in breast fed infants (Birch *et al.* 1993; Makrides *et al.* 1993; Jorgensen *et al.* 1996) while the fourth demonstrated differences in erythrocyte DHA levels but visual acuity was not significantly different between breast and formula fed infants (Innis *et al.* 1994*b*).

Randomized studies comparing infant diets with and without DHA have been undertaken in order to define more clearly whether there is a causative link between dietary DHA and neural development. Three randomized controlled studies of the effects of formula feeding with and without LCPUFA on visual function have been reported and several studies are in progress (Makrides *et al.* 1995; Carlson *et al.* 1996*a,b*; Auestad *et al.* 1995). The methods of assessing visual function differ and the results are equivocal (Table 2). In the Makrides study (1995) supplemented infants received a fish oil containing EPA (20:5 n-3) and DHA, and primrose oil containing γ -linolenic acid (GLA; 18:3 n-6) as a precursor of AA. The infants were studied using visual evoked potentials at 16 and 30 weeks and the supplemented group were shown to have enhanced visual acuity. Carlson and colleagues (1996*a,b*) randomized term infants to a formula containing DHA and AA and measured visual function using acuity cards. Differences in acuity were noted at 2 months but not at 4, 6, 9 and 12 months. The Auestad study (1995) involved 135 subjects who were randomized to a standard formula without LCPUFA supplementation, or a formula with DHA derived from fish oil with low concentration of EPA, or a formula containing DHA and AA derived from egg phospholipid. Visual function was measured by visual evoked potential, acuity cards and electroretinogram. No differences in visual function were detected between feeding groups during the first year of life.

Studies investigating the relationship of LCPUFA supplementation to cognitive function are few (Table 3). Agostoni and colleagues (1994, 1995*a,b*, 1997), investigating a cohort of 56 infants supplemented with DHA and AA derived from egg phospholipid, assessed infant development using the Brunet-Lezine psychomotor development test, and found higher development quotient scores in supplemented infants at 4 months but no difference at age 1 and 2 years. In Auestad's study (1995) infant development was assessed using Bayley Scales at 12 months and scores were not significantly different between groups. An important finding of this group was that vocabulary scores using the MacArthur's Communicative Developmental

Table 2. Randomized clinical studies of the effect of LCPUFA supplementation on visual function in term infants

Reference	n	Milk	Supplement	Assessment	Age	Results
Makrides <i>et al.</i> (1995)	26	HM/Suppl F/St F	DHA, EPA, GLA	VEP	4 months 7 months	HM/Suppl F > St F
Carlson <i>et al.</i> (1996a)	39	HM/Suppl F/St F	DHA, AA	Acuity Cards	2 months 4,6,9,12 months	HM/Suppl F > St F No differences
Auestad <i>et al.</i> (1995)	135	HM/St F/Suppl F1 Suppl F2	DHA, AA DHA, EPA	Sweep VEP Acuity Cards, ERG	2,4,6,9,12 months 2,4,6,9,12 months	No differences No differences

HM: human milk; Suppl F: supplemented formula; St F: standard formula

VEP: visual evoked potential; ERG: electroretinogram

Table 3. Randomized clinical studies of the effect of LCPUFA supplementation on cognitive function in term infants

Reference	n	Milk	Supplement	Assessment	Age	Results
Agostoni <i>et al.</i> (1995)	56	HM/Suppl F/St F	DHA, AA	Brunet-Lezine DQ	4 months 12 months 24 months	HM/Suppl F > St F No differences No differences
Auestad <i>et al.</i> (1995)	135	HM/St F/Suppl F1 Suppl F2	DHA, AA DHA, EPA	Bayley Scales McArthur CDI	12 months 14 months	No difference Negative correlation between red blood cell DHA & CDI
Forsyth <i>et al.</i> (1996)	56	HM/Suppl F/St F	DHA, AA	Infant Habituation	4 months	Late peak fixators
Willatts <i>et al.</i> (1998)	44	HM/Suppl F/St F	DHA, AA	Problem Solving	10 months	HM/Suppl F > St F Suppl F > St F

HM: human milk; Suppl F: supplemented formula; St F: standard formula
 MacArthur's Communicative Developmental Inventories (CDI)

Inventories at 14 months showed reduced scores in the supplemented group and there was a negative correlation between the Communicative Developmental Inventories and red blood cell DHA levels. Forsyth & Willatts (1996), assessed infants by the process of infant habituation, which is described as a decrease in the attention a baby pays to a stimulus over repeated presentations, and has become a useful tool for studying perception, memory and learning in infancy. Measures of habituation are thought to reflect the infant's capacity for processing and learning information, and visual fixation times were found to be shorter in LCPUFA supplemented growth retarded term infants compared with similarly growth retarded infants who were not supplemented. Duration of visual fixation times have previously been shown to be inversely correlated with childhood IQ (Bornstein & Sigman, 1986; Rose *et al.* 1986). More recently Willatts *et al.* (1998) reported that term infants randomized to a formula supplemented with AA and DHA achieved higher scores for problem solving tasks at the age of 10 months than infants who were randomized to an unsupplemented formula.

Comparison of these trials is difficult because of small samples studied, variations in quantity and quality of LCPUFA supplementation and the different measures of visual and cognitive assessments employed. There is a need for larger randomized studies in which the LCPUFA supplementation and the assessments are appropriate and standardized.

LCPUFA Supplement

EC Directives have recently indicated that long chain (C20 and C22) fatty acids may be added to infant formulas for term infants despite the current paucity of information on clinical benefit (Commission of the European Communities, 1996). Moreover, there are currently no accepted specifications on quality and quantity of an optimum LCPUFA supplement. The safety of LCPUFA supplementation has not been properly evaluated. There are published studies which have reported potentially hazardous complications of LCPUFA supplementation including increased risk of infection and necrotizing enterocolitis (Carlson *et al.* 1996a,b), increased bleeding time (Uauy *et al.* 1993) and impaired postnatal growth (Carlson *et al.* 1992).

Attempts have been made to define the LCPUFA requirement of the term infant by estimating the intake of a breast fed infant. The essential fatty acid intake of infants fed human milk has been calculated from the average composition of mature milk of European mothers (Koletzko *et al.* 1992a) assuming an average fat content of 35 g/l and a daily intake of 175 ml/kg. The average daily supply of total LCPUFA is 100 mg/kg (an amount that clearly exceeds intrauterine LCP deposition in brain of approximately 6.5 to 16.5 mg/d (Clandinin *et al.* 1989; Martinez, 1991). The average daily supply of linoleic acid was 660 mg/kg, α -linolenic acid 50 mg/kg, n-6 LCPUFA 70 mg/kg and n-3 LCPUFA 33 mg/kg. In human milk the n-6/n-3 LCPUFA ratio is kept relatively constant at about 2.0 (Koletzko *et al.* 1988) and brain lipids of newborn infants contain about twice as much n-6 LCPUFA as n-3 LCPUFA (Svennerholm 1968; Clandinin *et al.* 1989). A recommendation has therefore been made that n-6 LCPUFA content should not exceed 2% of total fat content and n-3 LCPUFA content should not exceed 1% (Statutory Instrument, 1995).

The LCPUFA supplement needs to be safe and commercially viable. AA and DHA are not present in currently used vegetable oils and alternative sources have therefore been explored. An early source of DHA was a fish oil which contained higher levels of EPA than human milk. In a study using this source, poorer growth was reported in the supplemented preterm infants (Carlson *et al.* 1992). More recent studies have tried to avoid the addition of EPA by using fish oils high in DHA but low in EPA, such as tuna oil (Auestad *et al.* 1995). Borage and evening primrose oil contain GLA, a metabolic precursor of AA which by-passes the rate limiting step of Δ -6 desaturase enzyme and this has been evaluated as a source of AA. However, data from

two studies indicate that GLA is not able to prevent a decline in AA levels in formula fed infants (Makrides *et al.* 1995; Ghebremeskel *et al.* 1995).

European studies have used egg phospholipids as a source of DHA and AA (Agostoni *et al.* 1995a,b; Forsyth & Willatts, 1996). Egg lipids have a high content of phosphatidylcholine and cholesterol (Agostoni *et al.* 1994) and since large quantities are required to achieve the LCPUFA levels in human milk, the amounts of lecithin and cholesterol could exceed that allowed as an additive (Ministry of Agriculture, Fisheries & Food, 1992). In egg lipids the LCPUFA are predominantly in phospholipids whereas in human milk they are principally located in the triacylglycerol component.

The production of LCPUFA by microorganisms in large scale controlled conditions is a promising source (Kyle, 1996). The two microorganisms presently used for DHA and AA production are a common marine microalga *Cryptocodinium cohnii* and the soil fungus *Mortierella alpina* respectively (Boswell *et al.* 1996). Since the sources of single-cell LCPUFA have not previously been used as food for man, the oils are considered to be novel and consequently their safety and nutritional aspects are currently being assessed by the Advisory Committee on Novel Foods and Processes (Wells, 1996).

Cholesterol supplementation?

Whether infant formulas should be supplemented with cholesterol has been the subject of speculation and debate for several decades. Central to this debate is the functional significance of the cholesterol present in human milk.

Biochemical evidence

The human infant needs cholesterol for growth and development including cognitive, visual and neurological functions (Fomon, 1974). However, cholesterol is not an essential dietary nutrient, the human fetus being able to synthesize it endogenously from the 11th week of gestation (Pitkin *et al.* 1972; Lin *et al.* 1977). It has been estimated that the total daily cholesterol required by a 4 month old (6 kg) infant for growth and metabolism is in the region of 103 mg or 267 mol (15–20 mg/kg; 45–50 mol/kg) (Hachey *et al.* 1996). Human milk contains 115–150 mg/l and, assuming that 50% of cholesterol intake is absorbed (Zilversmit & Hughes, 1974), the breast fed infant will receive only half of its requirement for growth and metabolism by diet.

The brain contains relatively high levels of LDL receptors and CEH activity (Pitkänen *et al.* 1986), both of which are necessary to deliver cholesterol to the tissues. Interestingly, an isotope tracer study has suggested that most cholesterol in the brain is derived from synthesis *in situ*, with little or none obtained from circulating lipoproteins (Edmond *et al.* 1991).

Clinical Issues

The cholesterol content of a typical commercially available infant formula is usually less than one-third that of human milk. Several studies comparing breast fed infants with infants fed a standard formula have demonstrated that breast fed infants develop higher total plasma cholesterol levels, and plasma LDL cholesterol concentrations and a higher LDL : HDL ratio than infants fed low cholesterol formulas (Jooste *et al.* 1991; Hayes *et al.* 1992; Kallio *et al.* 1992). However, these differences tend to disappear by the age of one year.

Only a few cholesterol supplementation studies of infant formulas in term infants have been reported. Growth and development was not influenced by cholesterol supplementation.

Infants fed supplemented formulas had higher plasma total cholesterol and plasma LDL cholesterol concentrations and a higher LDL:HDL cholesterol ratio than standard formula fed infants (Rassin *et al.* 1990; Wong *et al.* 1993; Cruz *et al.* 1994). This occurs despite evidence that a high intake of cholesterol down-regulates endogenous cholesterol synthesis and increases faecal cholesterol excretion (Wong *et al.* 1993; Boehm *et al.* 1995). Van Biervliet *et al.* (1992) reported that addition of cholesterol to formula affected maturation of the HDL particles and postulated that exogenous cholesterol may enhance the delivery of cholesterol and AA to the developing human brain. Most recently Katoku and colleagues (1996) demonstrated that the plasma cholesterol concentration and fatty acid pattern of the red cell membranes in infants fed a cholesterol-fortified formula were much closer to those of breast fed infants than infants fed a cholesterol unfortified formula. In particular, there was a decrease in n-3 LCPUFA, especially DHA and EPA, in the red blood cells of the unfortified group at age 6 months. There was an accompanying increase in the n-6 LCPUFA and therefore the n-6 to n-3 ratio was increased. It has been suggested that cholesterol preferentially associates with certain membrane bound enzyme activities which may influence fatty acid desaturase activity in the liver microsome membrane (Garda & Brenner, 1985).

The conjecture that cholesterol in milk may endow the nursing infant with an enhanced ability to metabolize dietary cholesterol later in life was originally hypothesized by Reiser and colleagues (Reiser & Sidelman, 1972; Reiser *et al.* 1979). In the 1980s Mott and coworkers tested the theory again in baboons who are considered to have lipid metabolism which is similar to that of the human. Adult baboons that were breast fed during infancy have lower HDL cholesterol concentrations, higher LDL + VLDL:HDL cholesterol ratios, lower cholesterol production and more extensive arterial lesions than those fed formula (Mott *et al.* 1982, 1985, 1995).

There are very few studies which have examined the relationship of modified cholesterol intakes in infancy to lipid profiles in later childhood. The earliest studies in this area were those of Glueck *et al.* (1972) and Friedman & Goldberg (1975), neither of which showed long term associations. A study by Hodgson *et al.* (1976) found that infants fed a low cholesterol commercial formula had lower serum cholesterol levels at age 7–12 years than those fed either human milk or cow's milk. However, perhaps the most definitive study in this area was by Fomon *et al.* (1984) who studied 469 children up to the age of 8 years. The children had received breast milk or a low cholesterol formula as infants and although a transient rise in serum cholesterol was observed during the neonatal period in both males and females fed human milk, the difference in cholesterol levels was not significant in any group at 8 years of age. This conclusion has been supported by later studies (Wagner & von Stockhausen, 1988).

Fall *et al.* (1992) showed that adult men born during 1911–30 who were exclusively breast fed during the first year of life or exclusively bottle fed from birth had higher mortality rates from ischaemic heart disease and higher serum total cholesterol and LDL cholesterol concentrations than men who were either breast and bottle fed or breast fed but weaned before one year. No information was available on the fat composition of the bottle feeds, but it is assumed that these formulas contained a high percentage of cholesterol-rich milk fat. On the other hand, as these individuals were on exclusive milk feeds for at least one year, the higher mortality from ischaemic heart disease may have been related to a degree of undernutrition during the first year of life. Pathological data have shown that lipid deposition in the arterial intima can begin in the first years of life (Stary, 1989; Strong *et al.* 1992) and high serum total cholesterol and LDL cholesterol have been reported to correlate positively with the extent of atherosclerotic lesions in the aorta and coronary arteries of children and young adults (Newman *et al.* 1986, 1991).

Conclusions

Despite potential concerns there is currently no clear evidence to indicate whether cholesterol intake during infancy is beneficial or harmful to long term health. A recent review concluded that dietary cholesterol in human milk offered no long term benefit to the majority of infants nor did it increase the risk of cardiovascular disease (Hachey *et al.* 1996). Further well designed long term studies are required to resolve this debate.

Recommendations

Compositional recommendations

	Minimum	Maximum
Lipid content	1.05 g/100 kJ (4.4 g/100 kcal)	1.5 g/100 kJ (6.5 g/100 kcal)
Lauric acid	–	15% of total fat content
Myristic acid	–	15% of total fat
Linoleic acid	70 mg/100 kJ (300 mg/100 kcal)	285 mg/100 kJ (1200 mg/100 kcal)
α -Linolenic acid	Not less than 12 mg/100 kJ	(50 mg/100 kcal)

Linoleic/ α -linolenic acid ratio not less than 5 and not greater than 15

Trans fatty acid content not greater than 4% of the total fat content

Long chain (20C and 22C) polyunsaturated fatty acids may be added to formulas as follows:

n-3 LCPUFA 1% of total fat content
 n-6 LCPUFA 2% of total fat content
 (arachidonic acid 1% of total fat content)

Eicosapentaenoic acid (20:5 n-3) content should not exceed the content of docosahexaenoic acid (22:6 n-3).

(Statutory Instrument, 1995, 1997)

Research recommendations

Further research is required to define more clearly the long term nutritional, growth and developmental effects of structured lipids in formulas for term infants.

More information is required on the differential handling of LCPUFA and other fatty acids at the organ and cellular level.

There is a need for large (multi-centre) randomized studies to determine the short and long term functional effects of LCPUFA supplementation.

Further research and development is required to determine a commercial source of LCPUFA which is safe, effective and economic.

Further information is required on the short and long term effects of cholesterol intake during infancy, and in particular its relationship to LCPUFA metabolism.

Long term studies should be initiated to determine the relationship of infant diet (especially saturated fatty acid and cholesterol intake) to the development of cardiovascular disease.

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