CURRENT TOPIC

Fat digestion in the neonate

William G Manson, Lawrence T Weaver

Department of Child Health, Royal Hospital for Sick Children, Yorkhill Hospitals, Glasgow
W G Manson
L T Weaver

Correspondence to:
Mr William Manson, Department of Paediatric Surgery, Royal Hospital for Sick Children, Edinburgh EH9 1LF.

Fats are essential components of the diet, and have a critical role in the growth and development of the neonate. Far from being simply compact sources of energy (providing 40-50% of calorie requirements), they are also integral constituents of neural and retinal tissues.[1 2] Dietary fats come in three forms: triacylglycerols; phospholipids; and cholesterol esters, all of which contain fatty acids esterified to alcohols.

The infant consumes fats largely as triacylglycerols, which need to be broken down by enzymes in the upper gastrointestinal tract before absorption. Compared with adults, however, the newborn infant's exocrine pancreas is "immature," secreting only small amounts of lipase even in response to secretagogues.[3 4] How the neonate digests fats, and what part they play in neurodevelopment[5] is of growing importance, particularly when preterm infants of ever shorter gestation are surviving into adulthood.

Structure, nomenclature and properties of fatty acids

Fatty acids are composed of carbon–carbon (C–C) chains with a carboxylic acid group (-COOH) at one end and a methyl group ($-CH_3$) at the other. The longer the C–C chain, the more concentrated the energy source, but the more difficult the fatty acid is to metabolise. Human milk contains predominantly medium and long chain fatty acids (C:10 to C:22), but other foods contain fatty acids with longer and shorter chains.

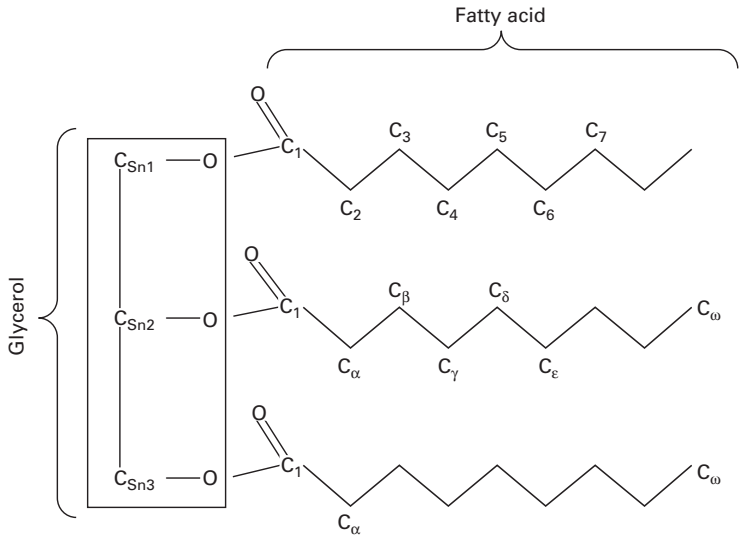

Figure 1 Triacylglycerol molecule showing the Sn positions and the numerical and alphabetical nomenclatures of fatty acids.

Fatty acids are named according to the number of carbon atoms which form the chain and the number of double bonds between them. Thus palmitic acid, which has 16 carbon atoms and no double bonds, is 16:0. Alternatively, the Greek derivation is used—hexadecanoic acid. The carbon atoms are labelled from the carboxylic acid end (fig 1). Either the carboxyl carbon is labelled C_1, followed by carbons C_2, C_3, C_4, etc. in sequence, or the first carbon after the carboxyl group is labelled "alpha" (C_α) and so on, through the Greek alphabet. The last carbon in the chain is referred to as the "omega" carbon (C_ω). The position of the double bonds is denoted either by the number of the first carbon in each bond, counting from the carboxyl end (so α-linolenic acid is 9,12,15-octadecatrienoic acid), or by reference to the number of carbon atoms from the "ω" end where the first double bond is found (so α-linolenic acid is 18:3, ω-3, sometimes written as to 18:3, n-3). The latter is becoming the more widely used notation as it is more physiologically compatible.

Most fatty acids are bound as esters to a glycerol molecule, to form triacylglycerols (fig 1), more commonly but less correctly called triglycerides. In this form they are hydrophobic: they do not dissolve in, or mix well with, water, and therefore they have an important role by binding to, and thus aiding, the transport of fat soluble vitamins. However, this hydrophobia means that they provide a concentrated energy source compared with carbohydrates (9 kcal/g *vs* 4 kcal/g) which can bind up to 2 g of water for each gram of carbohydrate. Fatty acids are also bound as esters to cholesterol (a precursor of steroid hormones and bile salts), and to phosphate containing alcohols as phospholipids. These are "ambiphilic," with one end hydrophobic and the other hydrophilic, making them ideally suited to form membranes at the interface between aqueous and fat layers (fig 2). Unsaturated fatty acids are usually long chain (>C:16). One or more of the C–C bonds is a double bond and the molecule is therefore not "saturated" with hydrogen. They are more rigid and require an extra metabolic step to break the double bond and to "saturate" the

Table 1 Differences in fatty acid composition of human and cow's milk

Fatty acid	Mature human milk (%)	Unmodified cow's milk (%)
10:0	1.4	3.5
12:0	5.4	4.1
14:0	7.3	12.0
16:0	26.5	31.3
16:1	4.0	1.3
18:0	9.5	9.2
18:1	35.5	21.7
18:2	7.2	1.6
18:3	0.8	0.4
20:0	0.2	0.2
20:4	0.3	0.1
22:6	1.1	0.1
Total fat (g/l)	42	38

Adapted from reference 11.

molecule before oxidation. Such fatty acids tend to be used other than for energy: they are essential constituents of the growing brain and retina and precursors of the prostaglandins.[1 2 6]

To be assimilated, the hydrophobia of dietary fatty acids must be masked so that they can mix with water. In milk they are found in fat globules which contain triacylglycerols surrounded by a membrane formed of ambiphilic phospholipids and cholesterol esters, with their lipophilic ends pointing inwards and their hydrophilic ends outwards (fig 2). These globules can mix with water to form an emulsion. However, if left to stand, being less dense than water, they rise to form a fat layer above an aqueous layer.

Triacylglycerols, cholesterol esters, and phospholipids have an important role in the nutrition of the neonate. In this review we will discuss the assimilation of triacylglycerols and fatty acids in early life, with particular reference to how they are digested in the gastrointestinal tract of the infant.

Table 2 Lipid composition of human milk on different days of lactation

	Lacation day				
	3	7	21	42	84
Total fat (g/dl)	2.04	2.98	3.45	3.19	4.87
Lipid class					
Triacylglycerol (%)	97.6	98.5	98.7	98.9	99.0
Cholesterol (%)	1.3	0.7	0.5	0.5	0.4
Phospholipid (%)	1.1	0.8	0.8	0.6	0.6

Adapted from reference 10.

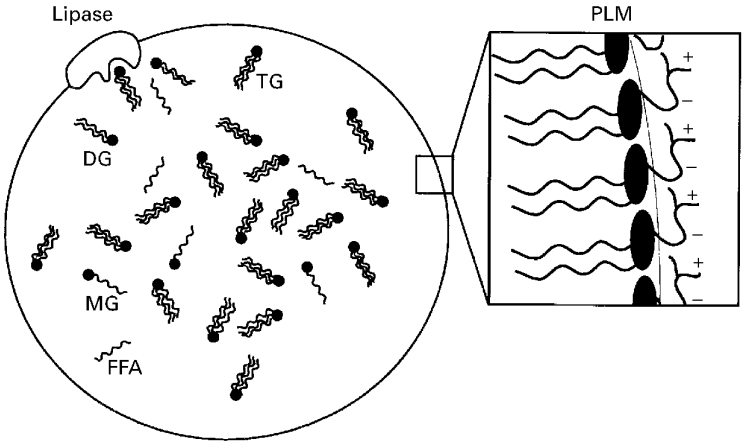

Figure 2 Fat globule with a core of triacylglycerol (TG), diacylglycerol (DG), monoacylglycerol (MG) and free fatty acids (FFA). A section of ambiphilic phospholipid membrane (PLM) is shown enlarged. A preduodenal lipase (Lipase) is attached to the membrane, acting on a triacylglycerol molecule.

Human milk

Human milk is a complex mixture of nutrients and non-nutritional factors which provide nourishment and aid the growth and development of the baby. Milk is the sole food for most newborn mammals and it must, therefore, contain a complete and sufficient supply of fluid and nutrients. Milk supplies energy (fat and carbohydrate), protein, vitamins, minerals, immunoproteins, trophic factors and other bioactive substances which play a part in helping the newborn adapt to extrauterine life.[7]

The fatty acids in human milk have single, unbranched chains with an even number of carbon atoms and varying numbers of double bonds. Small amounts of branched and cyclic fatty acids, and fatty acids with odd numbers of carbon atoms, are also found: these are thought to derive from maternal dietary intake of such fats and do not seem to be of nutritional importance to the infant.[8–10] Chain length varies largely between 10 to 22, but fatty acids of 8 and 24 carbon atoms have been found. Fatty acids occur in different ratios which meet the various nutritional requirements of the neonate for them. Table1 shows the relative concentrations of some fatty acids in mature human milk and, for comparison, in unmodified cow's milk.[11]

Ninety nine per cent of fatty acids in milk are in the form of triacylglycerols.[8–10 12–15] A very small proportion (<0.1%) occurs as diacylglycerols and free fatty acids, but this may be an artefact from processing the milk for assay. The other 1% occurs as cholesterol esters (10–15 mg/dl) and phospholipids (15–20 mg/dl).

The fat content of human milk changes during early lactation. It increases from 2.0 g/dl in colostrum to 4.9 g/dl in mature milk, reflecting the increasing energy requirement of the growing infant. However, the fat content of milk also varies during feeds, from 3.0 g/dl in midday foremilk to 4.0 g/dl in midday hindmilk, and during the day, from 3.0 g/dl in early morning milk to 4.5 g/dl in evening milk.[8 9]

During the transition from colostrum to mature milk (table 2),[10] the proportions of cholesterol and phospholipid relative to total fat content fall (1.3% down to 0.4%, and 1.1% to 0.6%, respectively). However, this is almost entirely due to an increase in concentration of triacylglycerols rather than to a decrease in concentration of the other two lipids: phospholipids actually increase in concentration from 22.4 to 29.2 mg/dl.

Humans can elongate fatty acids to extend chain lengths and, in some circumstances, can desaturate the chain to make double bonds. However, double bonds cannot be inserted beyond the C_9 carbon and so a supply of ω-3 and ω-6 fatty acids (such as linoleic (18:2, ω-6) and α-linolenic (18:3, ω-3) acids) is required to synthesise arachidonic (20:4, ω-6; AA) and docosahexaenoic (22:6, ω-3; DHA) acids. These are essential structural components of neural tissue, and also precursors of the

Table 3 Relative proportions of fatty acids at Sn positions of triacylglycerol molecule

	Sn position		
Fatty acid (mol%)	*1*	*2*	*3*
10:0	0.2	0.2	1.8
12:0	1.3	2.1	6.1
14:0	3.2	7.3	7.1
16:0	16.1	58.2	6.2
16:1	3.6	4.7	7.3
18:0	15.0	3.3	2.0
18:1	46.1	12.7	49.7
18:2	11.0	7.3	2.0
18:3	0.4	0.6	1.6
20:1	1.5	0.7	0.5

Adapted from reference 16.

eicosanoids.[5] In the neonate these enzyme systems (elongases and desaturases) are not fully developed. Therefore, although in adults linoleic and α-linolenic are regarded as the only essential fatty acids, the newborn infant also has a dietary requirement for AA and DHA.

Triacylglycerols are stereo-specific and the three ester bonds are not equally susceptible to hydrolysis by lipase enzymes. Fatty acids are not randomly distributed among the three stereo-specific numbering (*Sn*) positions, but are found selectively placed to provide the ideal mixture of fatty acids and monoacylglycerols for the neonate (table 3)[16]: for example, a relative abundance of 16:0 (palmitic acid) at the *Sn2* position provides the monoacylglycerol 2-palmitoyl-glycerol which is a potent antimicrobial, and with palmitate in this position, the absorption of other fatty acids may increase.[17 18]

Lipases

The lipases which act in the infant gut can be categorised into preduodenal, pancreatic, and breast milk lipases.

PREDUODENAL LIPASE

There is uncertainty about the nature and origin of preduodenal lipases. The fundus of the stomach and von Ebner's glands around the circumvillate papillae of the tongue have both been proposed as sources.[19–21] The evidence for a "gastric lipase" is that, when incubated with triacylglycerol, samples of gastric fundus release free fatty acids. The samples used have included gastric biopsy specimens from all ages, and pieces of stomach obtained from babies dying of cot death, stillborn babies, and aborted fetuses. Gastric lipase can be found in samples from fetuses as early as 18 weeks of gestation, attain significant levels of activity by 27 weeks,[19] but do not reach normal adult levels until the first few months of age. However, this gastric lipase activity may well derive from lipase secreted by the tongue which has been adsorbed on to the gastric mucosa and has not been washed off specimens during preparation.[22]

Lipase activity has also been detected in the upper oesophageal pouches of babies with congenital oesophageal atresia.[23] It is found in the tongue of the rat fetus at 20 days,[24] and there is evidence for its presence in the glands of von Ebner in humans.[20] However, it has been argued that lipase found in oesophageal pouches represents reflux of gastric secretions through the tracheo-oesophageal fistula, present in many of the babies studied. Moreau *et al*[21] found no lypolytic activity in biopsy specimens of tongue, pharynx, and oesophagus, taken at endoscopy and from transplant donors, suggesting that preduodenal lipase originates from the stomach alone. Both lingual and gastric lipases may exist, but the extent to which each contributes to preduodenal lipolysis remains unclear. These two moieties of lipase, lingual and gastric, seem to have similar molecular weights, structures, and conditions for action[13 15 25] and hereafter they will be referred to collectively as "preduodenal" lipase.

Preduodenal lipase consists of a polypeptide chain of 379 amino acid residues with a molecular weight of around 43000 Daltons.[25] It embeds itself in the phospholipid surface layer of the milk fat globule and digests the lipids within(fig 2). It acts preferentially at the *Sn3* position, hydrolysing only very small amounts of fatty acids at the *Sn1* and *Sn2* positions. When Hamosh *et al*[26] measured the free fatty acids released in the neonatal stomach by preduodenal lipolysis, they found a predominance of medium chain saturated and long chain unsaturated fatty acids, and concluded that preduodenal lipase preferentially hydrolysed these fatty acids. However, another study[27] has questioned this conclusion, suggesting that an abundance of such fatty acids at the *Sn3* position and preference of preduodenal lipase to hydrolyse fatty acids at this position would explain to the findings described by Hamosh *et al*.[26]

Preduodenal lipase has a low optimal pH (2.5–6.5), and is resistant to the acid conditions of the stomach and to gastric proteases. It does not require cofactors or bile salts and is rapidly inactivated by pancreatic trypsin and therefore ceases to be active when the milk bolus passes into the duodenum.[25] However, in cystic fibrosis, where pancreatic function and hence trypsin concentrations are low, its action may continue in the duodenum and compensate to some extent for depressed pancreatic lipase activities.[28]

Preduodenal lipase has an important role in the initiation of lipolysis in the stomach, with the liberation of short and medium chain and ω-3 and ω-6 fatty acids, and the preparation of the milk emulsion for further lipolysis by pancreatic and breast milk lipase.[13 26 29]

PANCREATIC LIPASE

Lipase is secreted by the pancreas from approximately 30 weeks of gestation onwards.[30] However, in both term and preterm infants it is present at very low concentrations until well into the first year of life.[3] It is a polypeptide of 449 amino acid residues, has a molecular weight of approximately 50 000 Daltons,[25] an optimal pH of 6.5–8.0 and an absolute requirement for colipase and bile salts. It has little action on soluble esters, preferring a lipid/water interface,[31] and hydrolyses triacylglycerols at the *Sn1* and *Sn3* positions, liberating 2-monoacylglycerols and free fatty acids.

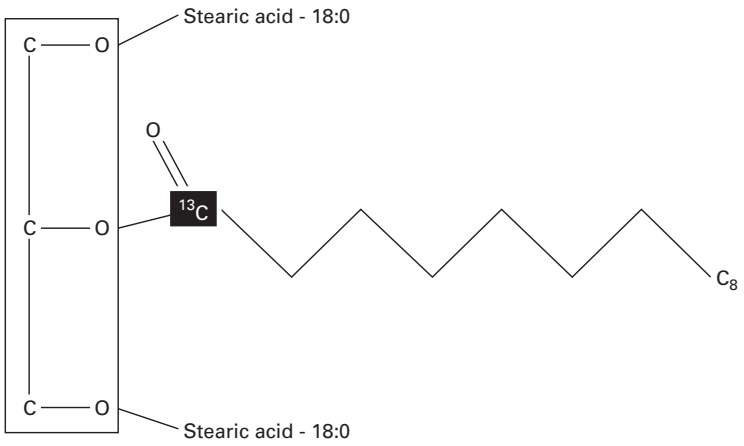

Figure 3 The isotope labelled mixed triacylglycerol 1,3-distearic 2-^{13}C-octanoic acid.

Pancreatic lipase by itself is not very effective at hydrolysing triacylglycerols found in milk in vitro. However, if milk is predigested with preduodenal lipase, there is a 20-fold increase in the release of free fatty acids compared with that from milk digested with pancreatic lipase alone.[13] It has been suggested that as much as 25% of free fatty acids are hydrolysed by preduodenal lipase.[29]

BREAST MILK LIPASE

It has been known since the turn of the century that human milk has the capacity to hydrolyse esters.[32] A breast milk lipase was first described in 1953[33] and since then, human milk lipoprotein lipase has been detected and characterised.

Because of its absolute requirement for bile salts, breast milk lipase is more commonly referred to as bile salt stimulated lipase (BSSL). BSSL is present in term and preterm milk and is found in the highest concentrations in the colostrum of mothers of preterm infants. Although the amount of lipase secreted by different women varies, each mother produces relatively constant concentrations of BSSL until weaning.[34]

BSSL has 722 amino acid residues and a molecular weight of around 90 000 Daltons. Differences in reported molecular weights may be explained by differences in glycosylation of the enzyme. BSSL shares little homology with other human lipases, but sequences are similar to those in esterases such as acetyl choline esterase. This may explain, in part, the non-specific action of BSSL compared with other lipases.[25]

BSSL is present in the aqueous fraction of the milk emulsion and does not hydrolyse triacylglycerol, which is held inside the milk fat globule, until the milk reaches the duodenum. BSSL is activated by primary bile salts (cholate and chenodeoxycholate) in two ways: firstly, the size of the globules is reduced by the action of bile, increasing the surface area of the globules on which BSSL can act; secondly, the bile salts bind with BSSL in such a way as to facilitate hydrolysis of triacylglycerols. BSSL is non-specific in its action on the triacylglycerol molecule: it hydrolyses fatty acids at all three positions (*Sn1, 2,* and *3*) to release glycerol and free fatty acids.

With growing evidence that long chain polyunsaturated fatty acids have an important role in neonatal development, it is possible that, in the presence of low concentrations of pancreatic lipase, the action of BSSL may be fundamental to the optimal nutrition and neurofunction of neonates. It is important to note that pasteurising or boiling donor expressed breast milk reduces fat absorption to 73% and 64%, respectively, compared with raw human milk.[35]

Gastrointestinal digestion and absorption of milk lipids

The digestion and absorption of fat in the gastrointestinal tract occurs in several stages. After ingestion milk is further emulsified in the stomach: gastric motility and acidity act on the milk to decrease the size of the fat globules. This promotes the action of preduodenal lipase, resulting in partial digestion of lipids. The milk then enters the duodenum as a coarse chyme and is mixed with bile, which further reduces the size of the milk globules and promotes hydrolysis. The smaller fat globules present a larger surface area relative to volume for the action of pancreatic lipase and BSSL. At a critical concentration bile salts aggregate to form micelles, which have a highly polar surface and a non-polar, hydrophobic core. They solubilise the products of hydrolysis (glycerol, monoacylglycerols, and the hydrophobic free fatty acids) to form mixed micelles. The hydrophobic core attracts other non-polar molecules such as fat soluble vitamins. The resultant small globule, with its polar, hydrophilic surface, then undergoes absorption. The mixed micelle comes into contact with the brush border of the small intestine and free fatty acids, and acylglycerols diffuse into the mucosal cell. In the endoplasmic reticulum fatty acids bind with fatty acid binding protein and triacylglycerols are resynthesised. These are released into the circulation as chylomicrons and pass via the portal system to the liver where they are metabolised. The short and medium chain fatty acids are used for energy, either being oxidised immediately to carbon dioxide and water, or being transferred to fat stores. Most longer chain unsaturated fatty acids are used, either as they are or after further desaturation and/or elongation, for the synthesis of cell membranes and bioactive molecules, such as prostaglandins.

Conclusions

Most of our understanding of the digestion of milk fat by the newborn infant is based on extrapolation from adult studies, experiments performed on neonatal mammals, or measurements of the lipolytic activity of secretions from human fetuses and infants in vitro. Fat balance studies have provided some measure of the efficiency of lipid digestion and absorption in the newborn, but there are few published studies of the functional capacity of the neonate to digest fat.

Stable isotopes provide a means of advancing our knowledge and understanding in this area. The labelling of dietary fats with the non-

radioactive isotope of carbon (^{13}C) offers ways in which the fate of ingested lipids can be studied safely and non-invasively.

Fats labelled with ^{13}C have been used to assess pancreatic function in adult health and disease,[36] and in children to assess fat digestion in cystic fibrosis.[37] The substrate used in these studies was a "mixed triacylglycerol" (MTG) with ^{13}C labelled octanoic acid in the *Sn-2* position (fig 3). The stearic acids on the *Sn-1* and *Sn-3* positions are hydolysed by lipases, releasing labelled monoglyceride which is absorbed and oxidised, releasing $^{13}CO_2$. The percentage dose of ^{13}C recovered after 6 hours (PDR) is calculated and used as an expression of functional fat digestion.[38] The choice of octanoic acid (which is rapidly absorbed and oxidised[39–43]) as the fatty acid in the *Sn2* position ensures that the rate limiting step is the digestion of the two long chain fatty acids in the *Sn1* and *Sn3* positions. As human milk contains very little, if any, octanoic acid,[9] the labelled tracer is not significantly diluted by unlabelled substrate.

Fat digestion in infancy has also been studied using this technique. Hoshi *et al*[44] studied five term neonates at 3 days of age and five "growing preterm infants," using ^{13}C-trioctanoyl-glycerol (trioctanoin) as a substrate. They reported mean PDRs of 53% in term neonates and 46% in the preterm group, values significantly higher than those obtained in older children by McClean *et al*,[45] who reported a mean PDR of 24%. More recently, MTG (1,3-distearyl, 2-^{13}C-octanoyl glycerol) has gained popularity over trioctanoin because the combination of fatty acids on the triacylglycerol is specific for pancreatic lipase. Using the MTG breath test to measure lipase activity in preterm infants of 27 to 35 weeks gestational age and aged 14 to 55 days, van Aalst[46] reported a mean PDR of 25%. When we performed MTG breath tests on neonates aged 1 to 3 days, we recorded a mean PDR of 16% (range of 0–32%). By 7–21 days this had increased to 23% and by late infancy to 29%.[47]

Together, these studies suggest that the capacity of the term newborn to digest fat during early neonatal life varies widely, ranging from nil to near normal adult levels. Thereafter, it increases in both term and preterm infants. In some children with cystic fibrosis and depressed pancreatic function PDR can be zero,[37] suggesting that the MTG is an appropriate substrate with which to measure pancreatic lipase acitivity.

"Designer lipids," triacylglycerols with labelled fatty acids in different combinations and at different sites, offer a means of studying the changing capacity of infants to digest fats in early life. They can be tailor-made to measure the activity of specific enzymes in the gastrointestinal tract so that the relative contributions of preduodenal, pancreatic, and breast milk lipases to overall fat digestion can be determined.

For example, ^{13}C-labelled cholesterol ester could be used to measure the activity of BSSL alone, as it is hydrolysed by this enzyme but not by preduodenal or pancreatic lipases.[40] A triacylglycerol with a labelled fatty acid at the *Sn1* position could distinguish pancreatic from preduodenal lipolysis, as the former acts at the *Sn1* position of the triacylglycerol molecule, whereas the latter does not. To distinguish differences in digestion (as opposed to absorption or oxidation), a shorter chain fatty acid (such as octanoate) is best used as the labelled moiety, as it is rapidly absorbed and oxidised.[39–43]

The use of ^{13}C-labelled lipids to study fat digestion heralds a renewed interest in the assimilation of lipids by the newborn, and an advance from cross-sectional studies of lipolysis using invasive or indirect methods, to dynamic measures of the functional capacity of the growing infant to make use of a major component of milk.

The significant difference, not only in the relative proportions of fatty acids in human and cow's milk, but also in their distribution on the triacylglycerol molecule, has major implications for the neurodevelopment of the newborn.[5] Milk is the only source of essential fatty acids for the growing infant, and it is now recognised that feeding babies on formulas based on cow's milk may be associated with deficient neurodevelopment and retinal function in infancy[48] and, in preterm infants, reduced intelligence quotient in later childhood.[49] It is therefore vital that we understand more fully the fate of ingested lipids, what regulates their digestion, and as a result, which fatty acids are available for absorption and deposition in neural tissues.

1 Svennerholm L, Vanier M. The distribution of lipids in the human nervous system. III. Fatty acid composition of phosphoglycerides of human foetal and infant brain. *Brain Res* 1973;**50**:341-51.
2 Svennerholm L, Vanier M. The distribution of lipids in the human nervous system. II. Lipid composition of human fetal and infant brain. *Brain Res* 1972;**47**:457-68.
3 Zoppi G, Andreotti G, Pajno-Ferrara F, Njai DM, Garburro D. Exocrine pancreas function in premature and full term neonates. *Pediatr Res* 1972;**6**:880-6.
4 Lebenthal E, Lee PC. Development of functional response in human exocrine pancreas. *Pediatrics* 1980;**66**:556-60.
5 Cockburn F. Neonatal brain and dietary lipids. *Arch Dis Child* 1994;**70**:F1-F2.
6 Stryer L. Hormone action. In: Stryer L, ed. *Biochemistry*. New York: WH Freeman & Co, 1988:975-1004.
7 Weaver LT. Breast and gut: the relationship between lactating mammary function and neonatal gastrointestinal function. *Proc Nutr Soc* 1992;**51**:155-63.
8 Jensen RG, Hagerty MM, McMahon KE. Lipids of human milk and infant formulas: a review. *Am J Clin Nutr* 1978;**31**:990-1016.
9 Jensen RG, Clark RM, Ferris AM. Composition of the lipids in human milk: a review. *Lipids* 1980;**15**:345-55.
10 Hamosh M. Lipids and nutrition. In: Kelley VC, ed. *Practice of Pediatrics*. Philadelphia: Harper and Row, 1987:1-13.
11 Williams AF. Lactation and infant feeding. In: McLaren DS, Burman D, Belton NR, Williams AF, eds. *Textbook of paediatric nutrition*. Edinburgh: Churhill Livingstone, 1991:21-45.
12 Hamosh M. Lipid metabolism. In: Hay WW, ed. *Neonatal nutrition and metabolism*. St Louis: CV Mosby, 1991:122-42.
13 Hamosh M, Iverson SJ, Mehta NR, Spear ML, Bitman J. Lipids in human milk and their digestion by the newborn infant. In: Ghisolfi J, Putet G, eds. *Essential fatty acids and infant nutrition*. Paris: John Libbey Eurotext, 1992:119-37.
14 Innis SM. Human milk and formula fatty acids. *J Pediatr* 1992;**120**:S56-S61.
15 Hamosh M, Iverson SJ, Kirk CL, Hamosh P. Milk lipids and neonatal fat digestion: relationship between fatty acid composition, endogenous and exogenous digestive enzymes and digestion of milk fat. 1994;**75**:86-91.
16 Jensen RG. Lipids in human milk - composition and fat soluble vitamins. In: Lebenthal E, ed. *Textbook of gastroenterology and nutrition in infancy*. New York: Raven Press, 1989.
17 Tomarelli RM, Meyer BJ, Weaber JR, Bernhart FW. Effect of positional distribution on the absorption of the fatty acids of human milk and infant formulas. *J Nutrition* 1968;**95**:583-90.

18 Carnielli VP, Luijendijk IHT, van Goudoever JB, *et al*. Feeding premature newborn infants palmitic acid in amounts and stereoisomeric position similar to that of human milk: effects on fat and mineral balance. *Am J Clin Nutr* 1995;**61**:1037-42.

19 Sarles J, Moreau H, Verger R. Human gastric lipase: ontogeny and variations in children. *Acta Paediatr* 1992;**81**:511-13.

20 Hamosh M, Burns BW. Lipolytic activity of human lingual glands (Ebner). *Lab Invest* 1977;**37**:603-8.

21 Moreau H, Laugier R, Gargouri Y, Ferrato F, Verger R. Human preduodenal lipase is entirely of gastric fundic origin. *Gastroenterol* 1988;**95**:1221-6.

22 Hamosh M, Scanlon JW, Ganot D, Likel M, Scanlon KB, Hamosh P. Fat digestion in the newborn. *J Clin Invest* 1981;**67**:838-46.

23 Salzman-Mann C, Hamosh M, Sivasubramanian KN, et al. Congenital esophageal atresia. Lipase activity is present in esophageal pouch and stomach. *Dig Dis Sci* 1982;**27**:124-8.

24 Hamosh M. Lingual and breast milk lipases. *Adv Pediatr* 1982;**29**:33-67.

25 Hernell O, Blackberg L. Molecular aspects of fat digestion in the newborn. *Acta Paediatr* 1994;**Suppl 405**:65-9.

26 Hamosh M, Bitman J, Liao TH, *et al*. Gastric lipolysis and fat absorption in preterm infants: effect of medium-chain triglyceride or long-chain triglyceride-containing formulas. *Pediatr* 1989;**83**:86-92.

27 Gargouri Y, Pieroni G, Riviere C, *et al*. Kinetic assay of human gastric lipase on short- and long-chain triacylglycerol emulsions. *Gastroenterol* 1986;**91**:919-25.

28 Abrams CK, Hamosh M, Hubbard VS, Dutta SK, Hamosh P. Lingual lipase in cystic fibrosis. Quantitation of enzyme activity in the upper small intestine of patients with exocrine pancreatic insufficiency. *J Clin Invest* 1984;**73**:374-82.

29 Carriere F, Barrowman JA, Verger R, Laugier R. Secretion and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans. *Gastroenterol* 1993;**105**:876-88.

30 Keene MFL, Hewer EE. Digestive enzymes of the human foetus. *Lancet* 1929;**i**:767-9.

31 Sarda L, Desnuelle P. Action de la lipase pancréatique sur les esters en emulsion. *Biochim Biophys Acta* 1958;**30**:513-21.

32 Marfan AB. Allaitment naturel et allaitment artificiel. *Presse Med* 1901;**9**:13-17.

33 Freudenberg E. Die frauenmilch lipase. *Bibl Paediatr* 1953;**54**.

34 Mehta NR, Jones JB, Hamosh M. Lipases in preterm human milk: ontogeny and physiologic significance. *J Pediatr Gastroenterol Nutr* 1982;**i**:317-26.

35 Williamson S, Finucane E, Ellis H, Gamsu HR. Effect of heat treatment of human milk on absorption of nitrogen, fat, sodium, calcium, and phosphorous by preterm infants. *Arch Dis Child* 1978;**53**:555-63.

36 Vantrappen GR, Rutgeerts PJ, Ghoos YF, Hiele MI. Mixed triglyceride breath test: a noninvasive test of pancreatic lipase activity in the duodenum. *Gastroenterol* 1989;**96**:1126-34.

37 Amarri S, Harding M, Coward WA, Evans TJ, Paton JY, Weaver LT. ^{13}C-mixed triglyceride breath test: a non invasive measure of exocrine pancreatic function in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1995;**120**:457.

38 Amarri S, Weaver LT. ^{13}C-breath tests to measure fat and carbohydrate digestion in clinical practice. *Clin Nutr* 1995;**14**:149-54.

39 Metges CC, Wolfram G. Medium- and long-chain triglycerides labelled with ^{13}C: a comparison of oxidation after oral or parenteral administration in humans. *J Nutrition* 1991;**121**:31-6.

40 Greenberger NJ, Rodgers JB, Isselbacher KJ. Absorption of medium and long chain triglycerides: factors influencing their hydrolysis and transport. *J Clin Invest* 1966;**45**:217-27.

41 Paust H, Keles T, Park W, Knoblach G. Fatty acid metabolism in infants. In: Chapman TE, Berger R, Reyngoud DJ, Okken A, eds. *Stable isotopes in paediatric nutritional and metabolic research*. Andover: Intercept Ltd, 1991:1-22.

42 Sulkers EJ, Lafeber HN, Sauer PJJ. Quantitation of oxidation of medium-chain triglycerides in preterm infants. *Pediatr Res* 1989;**26**:294-7.

43 Schwabe AD, Bennett LR, Bowman LP. Octanoic acid absorption and oxidation in humans. *J Appl Physiol* 1964;**19**:335-7.

44 Hoshi J, Nishida H, Yasui M, Ohishi M, Takahashi M. [13C] breath test of medium-chain triglycerides and oligosaccharides in neonates. *Acta Paediatr Jpn* 1992;**34**:674-7.

45 McClean P, Harding M, Coward WA, Green MR, Weaver LT. Measurement of fat digestion in early life using a stable isotope breath test. *Arch Dis Child* 1993;**69**:366-70.

46 van Aalst K, Veerman-Wauters G, vd Schoor S, Ghoos YF, Devlieger H, Eggremont E. The ^{13}C mixed triglyceride breath test for assessment of lipase activity in preterm infants. *J Pediatr Gastroenterol Nutr* 1995;**20**:459.

47 Manson WG, Dale E, Weaver LT. Measuring fat digestion in early life using ^{13}C-labelled lipids. *J Pediatr Gastroenterol Nutr* 1996;**22**:427.

48 Agostoni C, Trojan S, Bellu R, Riva E, Giovannini M. Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: the role of long-chain polyunsaturated fatty acids. *Pediatr Res* 1995;**38**:262-6.

49 Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. *Lancet* 1994;**339**:261-4.