Long Chain Fatty Acids: Intake, Digestion, and Absorption in Newborn Infants

Olle Hernell and Lars Bläckberg

Department of Pediatrics, University of Umeå, S-90185 Umeå, Sweden

Triglyceride (triacylglycerol) constitutes more than 98% of the dietary fat and provides about half the energy intake for breast-fed as well as formula-fed infants. Each triglyceride molecule consists of three fatty acids esterified to one molecule of glycerol. In human milk these fatty acids are almost exclusively long chain fatty acids (LCFA), which thus become the major energy substrate during early life (1). Therefore, with only few exceptions, the milk fatty acids have been regarded merely as a source of exchangeable energy. This is evident from current guidelines on infant feeding and composition of infant formulas. Except for the amount of fat, specific recommendations on intakes are given only for the so-called essential fatty acids (2,3).

ESSENTIAL FATTY ACIDS

Because humans are devoid of enzyme systems that introduce double bonds into the n-6 and n-3 positions, linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3) must be supplied with the food to prevent deficiency symptoms. Hence these two are classified as essential fatty acids. While it is accepted that linoleic acid should account for at least 1% of the energy intake, the minimum amount of α-linolenic acid required to avoid deficiency symptoms is not known. Present recommendations for newborn infants are therefore based on the average content of human milk, i.e., 0.5% of the energy content, or 1% of the fatty acids (1,2). Generally, the concentration of 18:2 n-6 in human milk is 5–15 times higher than that of 18:3 n-3 (1,4).

Long Chain Polyunsaturated Fatty Acids

At present there are reasons to believe that, at least for preterm infants, derivatives of linoleic and α-linolenic acids should also be classified as essential nutrients. These are the parent fatty acids from which, by a series of chain elongation and desaturation
FIG. 1. Synthesis of long chain fatty acids of the n-6 and n-3 series by chain elongation and desaturation of linoleic and α-linolenic acids, respectively.

reactions, long chain polyunsaturated fatty acids (LCPUFA) of the n-6 and n-3 series, respectively, are synthesized. Although, the same enzyme systems are shared by the two series there is no interconversion between the series (Fig. 1).

As indicated (Fig. 1), certain of these LCPUFA are precursors of the biologically potent eicosanoids. Others, e.g., arachidonic acid (20:4 n-6) and docosahexaenoic acid (22:6 n-3), are important constituents of membrane phospholipids. As such they are particularly enriched in the membrane phospholipids of retina and brain grey matter, whereas these tissues contain only minor concentrations of the parent linoleic and α-linolenic acids (5,6). Thus, during the rapid phase of brain growth, i.e., the last trimester of pregnancy and early extraterine life, significant amounts of n-6 and n-3 LCPUFA are incorporated into brain lipids. The development of visual acuity in preterm infants was recently found to correlate to the n-3 LCPUFA concentration of erythrocyte membrane phospholipids (7,8). After birth this concentration is influenced by type of feeding. Compared to breast-fed infants, the erythrocyte membrane phospholipids of formula-fed infants become depleted of n-3 LCPUFA (9,10), the likely explanation being that in contrast to human milk conventional infant formulas do not contain LCPUFA (11). The conclusion has therefore been drawn that the enzyme systems required for chain elongation and desaturation of 18:2 n-6 and 18:3 n-3 (Fig. 1) are not fully developed at birth in preterm infants (12). If so, then not only the parent acids but also their LCPUFA derivatives should be considered essential nutrients, at least for a certain, as yet not defined, period of early life.

DIGESTION OF HUMAN MILK TRIGLYCERIDES

Milk lipids are secreted as fat globules composed of a core of mainly triglyceride which when secreted becomes enveloped by the apical part of the phospholipid-rich
plasma membrane of the synthesizing mammary gland epithelial cell. Although in human milk LCPUFA are also enriched in the phospholipid fraction, since this fraction accounts for less than 1% of total lipids the bulk of the LCPUFA are constituents of the triglyceride fraction (1). Hence utilization of LCPUFA is dependent on milk triglyceride digestion and subsequent product absorption (13).

**Gastric Lipolysis**

Before absorption from the aqueous portion of small intestinal contents dietary triglycerides must be hydrolyzed into absorbable products, i.e., a mixture of monoglycerides, free fatty acids and glycerol. We have recently studied the sequential steps of human milk triglyceride hydrolysis in vitro by use of purified human enzymes (14). The first step in this digestive process is accomplished by gastric lipase secreted from the chief cells of the gastric mucosa. Not only is this enzyme particularly well suited to function in the environment of gastric contents (15) but it also has the unique property that its activity is not hampered by the milk fat globule membrane (16). This first step of triglyceride digestion is important even under circumstances when the contribution of gastric lipolysis to overall triglyceride digestion is minor.

**Intestinal Lipolysis**

The reason behind this effect of gastric lipase is that native milk fat globules, in contrast to globules partially digested by gastric lipase, are resistant to hydrolysis by colipase-dependent pancreatic lipase. This lipase, together with its cofactor colipase, is secreted from the pancreas into the duodenal contents where it catalyzes the subsequent triglyceride digestion in the upper small intestinal contents (14). Long chain fatty acids released by gastric lipase enforce binding between the colipase-lipase complex and the globules and by this mechanism the inhibition is abolished (16).

Because it is a constituent of human milk the bile salt-stimulated lipase (BSSL) contributes to milk fat digestion in breast-fed but not in formula-fed infants. After activation by bile salts in duodenal contents, BSSL, being a non-specific lipase, will not only support colipase-dependent lipase in hydrolysis of tri- and diglyceride but it will also hydrolyze sn-2 monoglyceride. Thus, at least in vitro, BSSL causes a shift in final products of triglyceride digestion from one sn-2 monoglyceride and two free fatty acids to one glycerol and three fatty acids for each triglyceride (14,17). As discussed below, BSSL may therefore be beneficial not only to milk fat digestion but also to product absorption.

**Release of LCPUFA From Triglyceride**

Although many reports suggest that infant formulas should contain certain concentrations of LCPUFA there are virtually no studies that have focused on how well
LCPUFA are utilized from human milk or from infant formulas. There are however some indications that they may be less efficiently utilized than fatty acids containing 16–18 carbons, including the parent n-6 and n-3 precursors. For instance, when whale oil triglycerides were hydrolyzed by colipase-dependent lipase, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6, n-3) (Fig. 1) were relatively resistant to hydrolysis (18). Heimermann et al. (19) used 15 sets of synthetic triglycerides containing 12:0, 14:0, 16:0, 18:2 and one positional isomer of cis-18:1 (the position of the double bond varying from carbon 2 to carbon 16, the carboxyl carbon being number 1) to study the effect of the double bond position on hydrolysis. After a systematic exposure of these triglycerides to colipase-dependent lipase and subsequent analysis of reaction products, they concluded that ester bonds containing the isomers of 18:1 with the double bond in the second through the seventh position from the carboxyl group were relatively resistant to hydrolysis, the most hindered being the isomer with the double bond between carbons 5 and 6, while no discrimination could be seen beyond carbon 7. In this context it should be noted that position-5 coincides with the position of the nearest double bond in EPA and arachidonic acid, while it is 4 for DHA, 9 for linoleic acid and α-linolenic acid.

Relevance of these in vitro studies may gain support from a recent observation of LCPUFA absorption in adults. Relative to α-linolenic acid (free acid or linseed oil), when given as fish oil triglycerides EPA and DHA were absorbed to only 68% and 57%, respectively, as compared to greater than 95% when given as free acids. After stereospecific analysis it was concluded that the results obtained were best explained by EPA and DHA being relatively resistant to hydrolysis by colipase-dependent lipase, which seemed to be independent of the $sn$ position of the fatty acid (20).

**Does BSSL Augment Utilization of Milk Triglyceride LCPUFA?**

We have recently initiated studies addressing the question whether or not also human milk triglyceride LCPUFA are less well utilized than their precursor fatty acids. In a first series of in vitro experiments we used double labeled rat chylomicron triglyceride as substrate. These chylomicrons were incubated with purified human colipase-dependent lipase alone, with purified BSSL alone, or with the two lipases operating simultaneously to resemble the situation in breast-fed infants (21). When colipase-dependent lipase was incubated with 18:2 n-6 and 20:4 n-6 double labeled chylomicrons the release of 20:4 was clearly retarded relative to 18:2 (Fig. 2). After 60 min incubation the mol% 20:4 released was only 60% that of 18:2. When the two lipases were operating together this difference was almost abolished (Fig. 2A, left panel), the mol% released as free fatty acid being about 70 for both fatty acids (Fig. 2A, middle panel). As expected, the explanation was that BSSL, in contrast to colipase-dependent lipase, does not discriminate between the two fatty acids (Fig. 2A, right panel).

The reason why 20:4 was released at a slower rate by colipase-dependent lipase was that this acid, but not 18:2, accumulated in the diglyceride fraction; more than
LCFA ABSORPTION IN NEWBORNS

FIG. 2. Mesenteric lymph duct cannulated rats were fed, via a gastric fistula, $^{14}$C-labeled linoleic acid (18:2 n-6) and $^3$H-labeled arachidonic acid (20:4 n-6) dispersed in a parenteral fat emulsion. Chyle was collected and chylomicrons were isolated by ultracentrifugation. The chylomicrons were used as lipase substrate in vitro as previously described (22). Final bile salt concentration was 10.5 mM (sodium taurocholate:sodium taurodeoxycholate, 4.5:6). Incubations were done with purified human colipase-dependent lipase and colipase (PL, left panels) alone, with purified bile salt-stimulated lipase (BSSL, right panels) alone, or with the two in combination (COMB, middle panels). During the 60 min of incubation, aliquots were withdrawn at various time intervals, the lipids were extracted, lipid fractions were separated by thin-layer chromatography, and the different lipid fractions were transferred to counting vials and the radioactivity determined (22). The amount of the respective labeled fatty acid present in triglycerides (TG) and free fatty acids (FFA) is shown in the upper panels (A) and amount present in diglyceride (DG) is shown in the lower panels (B). Data are presented as percentage of the total lipid radioactivity ($^{14}$C and $^3$H, respectively) present in each lipid class.
25 mol% as compared to less than 10 mol% after 60 min (Fig. 2B, left panel). This is in accord with previous observations (17,18,21), and with the resistance to hydrolysis being dependent on the fatty acid itself rather than on its sn position on the acylglycerol. No such accumulation was seen with BSSL alone or with the two lipases in combination (Fig. 2B, right and middle panels). This further illustrates the nonspecific nature of BSSL as a lipase; its activity has previously been shown to be relatively independent of the physical state as well as of the chemical structure of the lipid substrate (23). This may well be explained by the fact that BSSL is structurally clearly different from colipase-dependent lipase. In fact, the N-terminal half of BSSL shows striking homology to acetylcholine esterase, while the C-terminal part is unique (24).

To compare LCPUFA from the n-6 and n-3 series we carried out identical experiments, but with chylomicrons labeled with 20:4 n-6 and 20:5 n-3. There was no obvious difference in rate of release between the two when incubated with colipase-dependent lipase alone, with BSSL alone, or with the two lipases in combination. However, when hydrolysis by colipase-dependent lipase on one hand and BSSL on the other were compared, both fatty acids were released slower by the former lipase. Thus, when the two lipases acted simultaneously, in this case the release of both fatty acids was augmented. It is interesting to note that for both 20:4 n-6 and 20:5 n-3 the double bond nearest to the carboxyl group is positioned between carbons 5 and 6, which coincides with the most unfavorable position for hydrolysis by colipase-dependent lipase (19).

**PHYSICAL-CHEMICAL BEHAVIOR AND ABSORPTION OF LIPOLYSIS PRODUCTS**

Efficient utilization of dietary triglycerides depends on sufficient capacity for gastrointestinal lipolysis, and on subsequent efficient solubilization of the water-insoluble lipolysis products in the aqueous portion of upper small intestinal contents. Such solubilization is achieved by bile salt micelles (25), and, after transport as mixed micelles to the mucosal surface, absorption of the products occurs, presumably in monomolecular state. Not only is the newborn infant’s endogenous capacity for fat digestion limited because of low intraluminal concentrations of colipase-dependent lipase (26), but due to low intraluminal bile salt concentrations the capacity for micellar solubilization and transport is also comparatively low (27). The compensatory role of BSSL makes fat digestion an efficient process in breast-fed infants. The relatively unimpaired fat absorption in breast-fed infants, together with the observation in adult patients with bile fistulas (28) that even in the almost complete absence of bile salts in the intestinal contents more than 50% of dietary triglyceride is digested and absorbed, suggests that a micellar phase is not indispensable for substantial fat absorption.

The Physical-Chemical Phases of Lipids in Intestinal Contents

We have recently studied the physical-chemical state of lipolysis products during established fat digestion and absorption, first by the use of model experiments in
vitro (29) and then by chemical and physical-chemical ex vivo analyses of aspirated duodenal contents after triglyceride-rich meals given to healthy human adults (30). Equilibrium phase diagrams corresponding to the aqueous lipid compositions of upper small intestinal contents were developed. We identified (Fig. 3) two one-phase zones composed of mixed micelles (A1) and lamellar liquid crystals (A2), respectively, and two two-phase zones, one composed of cholesterol crystals and cholesterol-saturated micelles (B1) and the other of physiologically relevant (B2) composed of coexisting cholesterol- and mixed lipids (ML)-saturated mixed micelles and unilamellar vesicles, as judged by size and freeze-fracture technique. A single large three-phase zone in the system (C), was composed of cholesterol-saturated micelles, cholesterol crystals, and liquid crystals (29).

Micellar phase boundaries (A1) for typical physiological conditions were expanded by increase in total lipid concentration (0.25–5 g/dl), pH (5.5–7.5), and FA-to-MG molar ratio (5–20:1), resulting in reduction of the size of the physiologically two-phase (B2) zone (Fig. 3). Mean particle size (hydrodynamic radii, Rh), measured by quasielastic light scattering (QLS), demonstrated an abrupt increase from micellar (<40 Å) to micelle plus vesicle sizes (400–700 Å) as the B2 zone was entered. By phase separation and analysis, tie lines for the constituent phases of this zone demonstrated that the mixed micelles were saturated with mixed lipids and cholesterol, whereas the coexisting vesicles were saturated with bile salts but not with cholesterol (Fig. 3).

Analyses ex vivo of aspirated duodenal contents, in which lipolysis was immediately inhibited (30), confirmed the principal observations made in the model experiments. Relative lipid compositions of the so-called micellar phase, collected after ultracentrifugation, generally fell within the physiologically two-phase (B2) zone of the condensed ternary phase diagram (Fig. 3). As judged by QLS, ex vivo micellar sizes were similar (Rh < 40 Å), whereas unilamellar vesicle sizes (Rh = 200–600 Å) were smaller. When followed as functions of time, vesicles frequently dissolved
spontaneously into mixed micelles, indicating that under the nonequilibrium in vivo conditions the constituent micellar phase was often unsaturated with lipids.

These two studies (29,30) support a model of intestinal fat digestion and absorption by which biliary lipids, mainly BS, phospholipid, and cholesterol, first mix with colipase-dependent lipase/colipase complex, and together adsorb to the crude tri- and diglyceride emulsion surfaces resulting from gastric lipolysis, entering from the stomach. The emulsion particles are thus further stabilized and inhibition of colipase-dependent lipase relieved, initially by FA released by gastric lipase and then continuously as lipolysis products are formed. Any surplus of biliary lipids will remain as a coexisting micellar phase in aqueous duodenal contents. During lipolysis, products formed will locate mainly at the emulsion surface, presumably as multilamellar, liquid-crystalline bilayers (31). As the core of the emulsion droplet shrinks, parts of the surface coat pinch off as large liquid-crystalline structures. Provided sufficient bile salts are present, they will catalyze formation of small unilamellar vesicles from these multilamellar liposomes, and, in healthy adults via the continued presence of bile, a two-phase system of mixed micelles and unilamellar vesicles (zone B2, Fig. 3) will form.

Provided that dissolution of vesicles into unsaturated micelles is faster than mucosal product absorption the micellar phase will become more and more saturated with products of lipolysis. Most of the products will be carried by micelles prior to absorption. However, absorption could also take place from unilamellar, and perhaps also multilamellar, vesicles. Under conditions of low intraluminal bile salt concentrations, the normal state for newborn infants, the abundance of multi- and unilamellar vesicles could explain the relatively unimpaired fat absorption. If so, one would expect hydrolysis of MG to glycerol and FA by BSSL in breast-fed infants to improve overall fat absorption for two reasons. The first is that the micellar phase is expanded by increase in the FA:MG molar ratio (29), and the second is that it is likely, even in the complete absence of bile salts, that spontaneous vesiculation will occur in the presence of high dilution, physiological ionic strength, and multilayers of partially ionized fatty acids.

**DIRECTIONS FOR FUTURE RESEARCH**

Research by many groups in the last 25 years has provided much information concerning the lipases involved in gastrointestinal fat digestion and absorption. The structure of gastric lipase, colipase-dependent lipase, and bile salt-stimulated milk lipase has been revealed from amino acid sequencing or cloning and sequencing of isolated cDNAs, and many of the physiologically relevant properties and other characteristics of these enzymes are known from studies in vitro. With accessibility to relevant probes it will now be possible to study how the levels and activities of these lipases are regulated on a molecular level and what effects dietary components, hormones, and gut peptides have on these enzymes.

During the last decade we have gained further insight into the physical-chemical
behavior of dietary lipids during established fat digestion. It now seems that lipolysis products are dissolved in the aqueous portion of duodenal contents not only by the previously recognized mixed micelles. Moreover, absorption may occur directly from these other particles, e.g., unilamellar vesicles. Better definition of the occurrence and function of such "new" product phases in health and disease may have considerable impact on our understanding of intestinal absorption of lipids as well as of other nutrients, antibiotics, carcinogens, etc.

Of particular interest to this workshop have been the LCPUFA of the n-6 and n-3 series. Triglycerides rich in such fatty acids are presently fed to many patients for various purposes. For instance, there are indications that LCPUFA should be added to infant formulas in a concentration approximating that of human milk. Data presented at this meeting suggest that some LCPUFAs are more resistant than others to hydrolysis from triglycerides by colipase-dependent pancreatic lipase. However, such differences are abolished when bile salt-stimulated lipase acts together with colipase-dependent lipase. These data may favor a view that LCPUFAs are more efficiently utilized in breast-fed than in formula-fed infants. If so, this must be an important factor to consider when decisions are made about which LCPUFAs, and in what proportions, should be added to formulas. This, and the general mechanisms of LCPUFA utilization, are important questions to address within the near future.

ACKNOWLEDGMENTS

Financial support from the Swedish Medical Research Council (19X-05708) and the Medical Faculty, University of Umeå is gratefully acknowledged.

REFERENCES


**DISCUSSION**

*Dr. Small:* In the two-phase region, even if you are very close to single phase on the right hand side (which is actually a mixed, probably multilamellar, phase at that concentration) you still have micelles in equilibrium with vesicles. Over a long stretch of the intestine those micelles might be adequate to carry all the lipid molecules for absorption.
Dr. Hernell: I agree, but it is very difficult to establish that you are not completely devoid of micelles. In our *in vitro* system we could separate vesicles from micelles by centrifugation, but this is not possible with intestinal contents. Thus it is difficult to give a definite answer to your question.

Dr. Small: Since the pH precludes the formation of micelles by fatty acids, you can rule out the presence of micelles by measuring bile salt concentration and showing that it is clearly well below the mixed micelle concentration.

Dr. Bazan: Your work indicates the future directions in nutrition. The cDNA cloning that you have described for colipase-dependent lipase and the BSSL opens up the opportunity to develop probes to see how dietary intake would modify messenger abundance for these lipases. I wonder whether you have done any such studies.

Dr. Hernell: The data that I presented was on the milk lipase, on the BSSL, but the colipase-dependent lipase has also been cloned. No, we have not yet done such experiments but we certainly intend to do so.

Dr. Spector: How much modification is there in the dietary fat in the intestinal mucosa? In other words is the mucosa an active site for elongation and desaturation of different fatty acids?

Dr. Hernell: I don’t know but I believe Dr. Clandinin may be able to answer.

Dr. Clandinin: We have done some recent work on this with Alan Thompson’s group. The enterocyte does contain Δ-9 and Δ-6 desaturase activities. Both respond fairly quickly to a number of physiological changes such as overnight fasting and change in fatty acid intake.

Dr. Spector: What about the phospholipids in the chylomicrons? It would seem that the phospholipid fatty acid composition would have to be somewhat more regulated than would be the case if it simply reflected whatever is available in the diet.

Dr. Clandinin: The composition of the chylomicrons reflects the triglyceride composition of the fat fed. The aspect that has been of interest to us is how the membrane in the enterocyte is altered by diet. This involves both the absorptive membrane and the membrane that is coated on the chylomicrons.

Dr. Crawford: When considering these phosphoglycerides one is not just thinking about the coating of the chylomicrons but also about the synthesis of lipoprotein by the small intestine, which involves picking up a lot of phosphoglyceride en route; this phosphoglyceride would go preferentially down the portal system. The early experiments of Borgstrom show that significant amounts of the phosphoglyceride fatty acids were indeed going via that route rather than via the lymphatics. What is known about what happens to these phosphoglycerides? Do they provide the lipoprotein in human milk?

Dr. Hernell: To my knowledge no one has looked at the milk phospholipids and the fate of the phospholipid fraction.

Dr. Crawford: It is relevant that there is a small proportion of the fatty-acid-rich pool which is dominated by the phosphoglyceride pattern. This is destined for some different purpose than being dumped in adipose tissue for energy, and this may have a quite disproportionately interesting biological significance because its destination would be for cell membrane growth, differentiation, and regulatory processes.

Dr. Hernell: Dr. Åke Nilsson has pursued this problem by studying the hydrolysis of similarly labeled chylomicrons by lipoprotein lipase—that is, what happens when the chylomicrons enter the blood stream. It appears that lipoprotein lipase discriminates between fatty acids. Those with the first double bond close to the carboxyl carbon are not so readily hydrolyzed. Thus such fatty acids remain in the diglycerides, and perhaps this is a way to channel these fatty acids to the liver as chylomicron remnants. This would fit a hypothesis of a specific route to spare certain fatty acids for a particular purpose.