Development of Lipase Activity

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Fat is essential for normal development. Before birth, long-chain fatty acids are provided by the maternal circulation and are also synthesized de novo from glucose by the fetus. After birth the newborn depends on its own digestive system for the digestion and absorption of the nutrients previously provided by the mother.

Long-chain fatty acids are essential to all mammalian tissues, both as constituents of complex lipids necessary for the structural and functional integrity of cells and as an important energy source. In the newborn, they are essential for brain development.

The diet of the newborn (breast milk or formula) provides the fatty acids in the form of triglycerides, which must be hydrolyzed to free fatty acids prior to absorption from the gut. Within the enterocyte the fatty acids are re-esterified and incorporated into chylomicrons and very low density lipoproteins prior their release into the circulation.

Chylomicrons are large particles (diameter 0.5–1.0 μm) that cannot cross the capillary endothelium. The triglyceride within these particles has therefore to be hydrolyzed at the endothelial cell surface before uptake of the fatty acids into the tissues. This process is catalyzed by lipoprotein lipase (in extrahepatic tissues) and hepatic lipase within the liver.

This chapter will address the development of several lipases that enable the infant to digest dietary fat (in breast milk and formula) and to clear the absorbed fat from the circulation. We begin with the clearing lipases because, in general, the sick infant is fed parenterally before enteral nutrition is possible.

TOTAL PARENTERAL NUTRITION

Role of Lipoprotein Lipase, Hepatic Lipase, and Lecithin Cholesterol Acyltransferase in Lipid Clearing from the Circulation

The lipid emulsions used in total parenteral nutrition (TPN) provide 1.1 calorie/ml and contain triglyceride and lecithin. The lecithin, added in the ratio of 120 mg/g triglyceride, stabilizes the triglyceride emulsion. The advantage of administration of these fat emulsions to very low birth weight (VLBW) infants or sick
infants resides in their high calorie content, as well as in the fact that they are isotonic and can be administered through peripheral veins (1). The fat particles in these emulsions are similar in size to chylomicrons and like the latter, are cleared from the circulation by lipoprotein lipase (1–3) and probably also by hepatic lipase (4–6).

Lipoprotein lipase and hepatic lipase are active at the capillary wall and under normal conditions are absent from the circulation. This specific location facilitates the uptake of the lipolytic products, free fatty acids, and monoglycerides into tissues. Whereas the role of lipoprotein lipase in the catabolism of triglyceride-rich lipoproteins has been well defined (7–9), a physiological role for hepatic lipase has been lacking until recently. Earlier studies suggested that the function of hepatic lipase is the hydrolysis of phospholipid within high-density lipoproteins. Recent in vivo studies, in which specific antibodies to hepatic lipase have been injected into rats (4) and monkeys (5), as well as evidence in humans with familial deficiency of hepatic lipase (6), show that this lipase participates with lipoprotein lipase in the hydrolysis of lipoprotein-triglyceride. It appears therefore that both enzymes function together in the clearing of circulating lipid.

Lecithin cholesterol acyltransferase (LCAT) has the dual function of initiating the catabolism of lecithin (the major phospholipid of circulating lipoproteins, as well as a major component of the lipid emulsions used in TPN) and at the same time synthesizing cholesteryl ester. This is accomplished by the transfer of one fatty acid from lecithin to cholesterol. LCAT is synthesized in the liver, is released into the circulation, and acts exclusively in plasma, where it accomplishes its function of continuous modulation of the cholesteryl ester and lecithin content of lipoproteins (10,11). Because lipoprotein lipase and hepatic lipase are tissue-bound enzymes, they can be studied only in animals. The extent of lipid clearing is directly proportional to the amount of these lipases at the endothelial surface. Both lipases are linked to the endothelium by short chains (40 nm) of heparan sulfate, and are rapidly released into the circulation after the injection of heparin (1–3). The lipolytic activity present in plasma after bolus heparin injection, called postheparin lipolytic activity (PHLA), is composed of lipoprotein lipase and hepatic lipase (about 40% and 60% of total PHLA, respectively) has a half-life of 18 to 30 min, and is a reasonably good measure of an individual's lipid-clearing ability.

**Carnitine Acyltransferase**

The final step in the catabolism of long-chain fatty acids, oxidation to H₂O and CO₂, occurs in mitochondria and depends on the presence of carnitine. Long-chain fatty acids can cross mitochondrial membranes only in the form of acylcarnitine. Carnitine is thus an essential cofactor for fatty acid oxidation, and acyl-CoA carnitine transferases are the key enzymes in fatty acid oxidation.

The characteristics of the enzymes participating in lipid clearing and fatty acid oxidation are listed in Table 1.
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cofactor</th>
<th>Substrate</th>
<th>Site of activity</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein lipase (LPL)</td>
<td>Apoprotein CII</td>
<td>Chylomicrons, VLDL, intravenous lipid emulsions</td>
<td>Endothelium, extrahepatic tissues</td>
<td>Hydrolysis of TG to FFA and MG</td>
</tr>
<tr>
<td>Hepatic lipase (HL)</td>
<td>—</td>
<td>HDL, VLDL</td>
<td>Endothelium, liver</td>
<td>Hydrolysis of triglycerides and phospholipids</td>
</tr>
<tr>
<td>Lecithin cholesterol acyl-transferase (LCAT)</td>
<td>Apoprotein Al</td>
<td>Lecithin, cholesterol</td>
<td>Plasma</td>
<td>FA transfer, synthesis of cholesteryl-ester</td>
</tr>
<tr>
<td>Acylcarnitine transferases (ACT)</td>
<td>Carnitine</td>
<td>Long-chain fatty acid</td>
<td>Mitochondria</td>
<td>Transfer of FA across mitochondrial membrane for oxidation</td>
</tr>
</tbody>
</table>

VLDL, very low density lipoprotein; HDL, high density lipoprotein; TG, triglycerides; FA, fatty acids; FFA, free fatty acids; MG, monoglycerides.
(From Hamosh, ref. 12.)
DEVELOPMENT OF CLEARING ENZYMES

During the last few years we have investigated the level of lipoprotein lipase, hepatic lipase, and LCAT in newborn infants. Our studies show that PHLA is lower in preterm infants under 28 weeks of gestation than in infants above this gestational age (Fig. 1) (13), that about 60% of PHLA is of hepatic origin (Fig. 2) (14), and that among VLBW infants, females have higher lipolytic activity than males (Fig. 3) (15). The response to heparin (maximal lipase release within 10 min of IV injection of heparin), half-life of lipolytic activity (18–23 min), and proportion of the two lipases in the circulation (40% lipoprotein lipase, 60% hepatic lipase) are similar in preterm infants and in adults. Thus the control mechanisms seem to be well developed even in preterm infants. It is only the total amount of

![Diagram](image-url)
FIG. 2. Total postheparin lipolytic activity (PHLA), lipoprotein lipase (LPL), and hepatic lipase (HL) activities in serum of VLBW infants maintained on total parenteral nutrition (TPN) with Intralipid. All infants received continuous on-line heparin (1 U/ml IV fluids). At the beginning of the 4-hr study period, each infant received a bolus injection of 10 U/kg heparin, for release of endothelial lipoprotein and hepatic lipase. Intralipid was infused at a rate of 0.5 g/kg/4 hr, starting at zero time. Lipase activity was measured before and after inhibition of hepatic lipase by specific antibody. (From Zaidan et al., ref. 14.)

FIG. 3. Sex difference in postheparin lipolytic activity (PHLA) in VLBW infants. (From Dhanireddy et al., ref. 15.)
enzyme that is lower, probably because of lack of adipose tissue at this age. Pre-
term and term newborns, given large amounts of heparin during exchange transfu-
sion (100 U heparin/kg body weight) have very high lipase levels (16) suggesting 
rapid transfer of enzymes from intracellular sites to the endothelium as well as 
very rapid synthesis of lipase (16).

Clearing ability, measured as PHLA, is severely impaired in malnourished in-
fants and children (17,18) and improves rapidly with proper nutrition. Sepsis, at 
all ages, is associated with low endothelial lipase levels and inefficient lipid clear-
ing (19).

Administration of lipid emulsions in excess of clearing ability leads, in addition 
to hypertriglyceridemia, to the accumulation of phospholipids and free cholesterol 
in the circulation. These lipids accumulate in a specific low density lipoprotein 
called lipoprotein-X (LP-X) within 16 to 64 hr after the infusion of 1 to 4 g of 
Intralipid/kg/day (20). Accumulation of phospholipids and free cholesterol is prob-
ably attributable to low levels of LCAT (Table 1) in preterm and term newborn 
infants (21). Our recent study shows that the cord blood of preterm infants (under 
34 weeks gestation) has less than half the LCAT activity of term infants, whereas 
the latter has less than half the activity found in the circulation of healthy adults.

**Effect of Continuous Low-Level Heparin Infusion (1 U/ml IV Fluid) on 
Triglyceride Clearing**

The use of heparin (which releases lipoprotein lipase and hepatic lipase from 
the endothelium into the circulation) for more efficient clearing of lipid in the new-
born was first suggested by Coran in 1974 (22). The concentrations of heparin 
suggested by this study (above 50 U/kg) were, however, too high to make this 
practice feasible. The use of low levels of heparin, administered continuously with 
TPN, in order to avoid clotting (a routine practice in many intensive care nurser-
ies), could affect clearing of lipid by releasing a constant low level of lipoprotein 
lipase into the circulation. We have recently tested this assumption by measuring 
the effect of continuous administration of low levels of heparin on the plasma 
PHLA, triglyceride, and free fatty acid levels in low-birth-weight neonates (23). 
These studies show that even very low levels of heparin, infused continuously, can 
affect lipid clearing (Fig. 4) (23). In contrast to infants who receive lipid infusion 
without heparin, when heparin, 1 U/ml IV fluid is present, there is a constant level 
of circulating lipases, triglyceride clearing is improved, but circulating free fatty 
acids (FFA) tend to remain high. Furthermore, when the amount of lipid infused 
is increased from 0.5 g/kg to 2 to 3 g/kg/day, lipase levels increase markedly, as 
do circulating levels of FFA, triglycerides, cholesterol, and glucose (24). The 
higher circulating levels of lipase are probably the result of continuous release of 
enzyme from the endothelium, which in turn stimulates lipase synthesis (25). The 
high FFA levels are a direct result of intravascular lipolysis (as opposed to hydrol-
ysis of fat at the endothelial wall), whereas the high triglyceride levels are prob-
FIG. 4. Effect of continuous low-level heparin infusion with total parenteral nutrition (TPN) on serum postheparin lipolytic activity (PHLA), free fatty acids (FFA), and triglyceride (TG) levels in low-birth-weight infants (gestational age 27–32 weeks) (left panel). The data are compared with those obtained from a second group of infants who did not receive heparin with TPN (right panel). Both groups received a bolus injection of 10 U of heparin/kg before the collection of blood specimens. (From Zaidan et al., ref. 23.)

ably a mixture of infused lipid and newly synthesized low-density lipoprotein from the infant's liver. The accumulation of LP-X (20), i.e., high circulating levels of free cholesterol and phospholipid, is the result of infusion of lecithin in excess of the infant's clearing ability because of low levels of LCAT (21).

The high FFA levels associated with improved triglyceride hydrolysis probably reflect the limited capacity of the premature or very sick infant to oxidize fatty acids. Oxidation of fatty acids requires carnitine, which, in the form of acylcarnitine, transfers FFA into mitochondria for oxidation to H₂O and CO₂ (Table 1).
Carnitine synthesis and storage are not well developed at birth, especially in VLBW infants, who receive most of their carnitine via the feto-placental circulation. Thus, although the maternal supply of carnitine might be sufficient for adequate fatty acid oxidation during the first 48 hr after birth (26), premature infants under 34 weeks gestation, requiring TPN develop carnitine deficiency 6 to 10 days after birth (27). Carnitine is present in human milk and in cow's milk formulas, but not in currently available infusion solutions. Since the lipid and the other components of TPN do not contain carnitine, efficient lipolysis results in an increase in the level of FFA that accumulate in the circulation because their production exceeds the infant's ability to oxidize them.

High levels of FFA (which bind to plasma albumin) cause the displacement of albumin-bound bilirubin when the FFA-to-albumin molar ratio exceeds 4:1 (28). The transient increase in plasma FFA and triglyceride levels and the cumulative increase in cholesterol and glucose concentration warrant caution in the combined use of low levels of heparin and lipid emulsions in amounts exceeding 1 g/kg/day in the clinical management of low-birth-weight infants.

Further studies are necessary to investigate the ontogeny of the enzymes involved in lipid clearing as well as the ontogeny of the cofactors necessary for the efficient catabolism of lipid. A better understanding of these mechanisms will result in changes in lipid delivery during TPN and in more efficient clearing and catabolism of lipids in low birth weight and sick infants.

**FAT DIGESTION IN THE NEWBORN**

More than 95% of the fat in human milk or in infant formulas is present in the form of triglyceride-nonpolar lipids composed of three molecules of long-chain fatty acids esterfied to 1 molecule of glycerol. Fat digestion requires adequate lipase activity and bile salt levels, the former for the breakdown of triglycerides, the latter for emulsification of fat prior to and during lipolysis (29). In the newborn, and especially the premature infant, both pancreatic lipase activity (30) and bile salt concentrations (31) are low. However, reasonably good fat absorption in newborn infants (even in prematures) indicates that the newborn may depend on alternative mechanisms for the digestion of dietary fat. Indeed, recent studies in man and earlier studies in several animal species show that the newborn depends on extrapancreatic lipases for adequate fat digestion. Of special importance is intragastric lipolysis (32), where lingual (32-34) and gastric (34-36) lipases compensate for low pancreatic lipase. The products of lipolysis, fatty acids and monoglycerides, compensate for low bile salt levels by emulsifying the lipid mixture. The breast-fed newborn infant depends on an additional compensatory mechanism for fat digestion—the bile salt stimulated lipase of human milk (37-39), an enzyme found in the milk of primates. The combined activity of these two enzymes can lead to complete hydrolysis of dietary fat and thus can effectively substitute for low levels of pancreatic lipase. These compensatory mechanisms are of even
greater importance in VLBW infants (<1,500 g), whose survival has increased substantially during the past few years.

LINGUAL AND GASTRIC LIPASES

Lingual lipase is secreted from lingual serous glands (von Ebner), a group of tubuloalveolar glands located beneath the entire region of the circumvallate papillae in man (Fig. 5) (33,40). Gastric lipase is also present in man and is secreted from isolated gastric glands, in response to specific secretagogues (41). The exact contribution of these two lipases to fat digestion in the newborn is not yet known. These two lipases have very similar characteristics, since both act primarily in the stomach (Table 2). It seems, however, that lingual lipase could continue to act in the intestine, because it is less inhibited by bile salts than gastric lipase. Our studies have shown that high levels of lipase are present in gastric aspirates of newborn infants (Fig. 6) (34), and that the lipase is not inactivated at pH >2.0 (42). Thus it can accumulate in the stomach between meals, so that there is extensive hydrolysis of formula fat in the stomach of preterm infants (43). Furthermore, because

![Diagram of the human tongue](image-url)
TABLE 2. Characteristics of the lipolytic activity in gastric aspirates of newborn infants

1. Origin: Oral (lingual serous glands), gastric
2. Absent from secretions of major salivary glands
3. Development: Present from 24 weeks of gestation
4. Site of action: Stomach
5. Substrate: TG, DG, MG
6. Enzyme characteristics:
   a. pH optimum: 3.5–6.0
   b. Rate of hydrolysis: MCT > LCT; TG containing unsaturated FA > saturated FA; specific for primary esters
   c. Products (FFA) inhibitory
   d. Bile salt effects on lipolysis: lingual lipase stimulated (17 mM taurodeoxycholate); gastric lipase inhibited
   e. Reaction products: DG, MG, glycerol, FFA
   f. Molecular weight: 46,000–48,000
7. Function: Hydrolysis of dietary fat in the stomach

TG, triglyceride; DG, diglyceride; MG, monoglyceride; FA, fatty acids; FFA, free fatty acids; MCT, medium-chain triglycerides; LCT, long-chain triglycerides.

of the hydrophobic nature of lingual and gastric lipases, they can penetrate into the milk fat globules of human milk and can initiate the hydrolysis of milk triglycerides within the core of these globules (44). Initial hydrolysis of the fat within the core of the milk fat globule by lingual and gastric lipase probably facilitates the subsequent action of the bile salt stimulated lipase (BSSL) of human milk. The latter (37–39) hydrolyzes milk fat at pH 7.5 to 8.0 in the presence of bile salts, thus acting in the intestine to complete the digestive process initiated in the stomach by lingual and gastric lipases.

Hydrolysis of milk fat by lingual lipase produces relatively large amounts of monolauroglycerol (45), a substance with antibacterial, antiviral, and antifungal activity (46). On the basis of earlier studies in children with complete absence of pancreatic lipase, which show absorption of 60% to 70% of dietary fat (47), as well as in light of our recent studies in cystic fibrosis patients (48), one may assume that lingual and gastric lipases are of major importance in physiological and pathological conditions associated with pancreatic insufficiency (low levels of pancreatic lipase and low duodenal pH) such as prematurity (29), congenital absence of pancreatic lipase (47), or cystic fibrosis (48).

BILE SALT STIMULATED LIPASE OF HUMAN MILK

The possible compensatory role of a second lipase, present in human milk, became apparent when we (49) and others (50) observed better fat absorption in premature infants fed fresh (not heated) milk than in infants fed formula only (49) or heated breast milk (50). Although the fat in human milk is absorbed to a greater extent than that in bovine milk or in certain formulas, because of the specific configuration of human milk triglycerides, the marked improvement of fat absorption after feeding fresh human milk probably resides in its specific BSSL.
Studies on this lipase in term human milk (Table 3) (37,38,40), have shown that it is a glycoprotein with a molecular weight of 90,000 to 125,000 daltons and that it accounts for as much as 1% of the total protein in human milk (51). The enzyme is heat labile and is rapidly inactivated at temperatures above 55°C. Bile salts are necessary for both catalytic activity and protection from proteolytic enzymes (51).

Unlike pancreatic lipase, human milk lipase has a low substrate specificity: It hydrolyzes long-chain triglycerides as well as naturally occurring esters such as retinyl palmitate, the main component of vitamin A. The enzyme is stable for 1 hr or longer at pH 3.5, suggesting that it will not be inactivated when milk enters an empty stomach. The BSSL of term human milk has been shown to be active in vitro under conditions simulating those found in the neonatal intestine. Calcula-
TABLE 3. Characteristics of human milk bile salt stimulated lipase

1. Origin: Mammary gland of primates (human, gorilla) and carnivores
2. Absent from milk of several other species
3. Present in milk of women who deliver prematurely (25–36 weeks) or at term
4. Substrate: LCT, MCT, water-soluble esters
5. Mechanism of triglyceride hydrolysis:
   a. pH optimum: 7.5–9.0
   b. Bile salts (0.5–5 mM) obligatory
   c. TG hydrolyzed to FFA + glycerol: low substrate specificity
   d. Fat particle size strongly affects lipolysis
6. Molecular weight: 90,000
7. Amounts to 1% of total milk protein (10 mg/dl)
8. Site of action: Intestine of the newborn
9. Acid resistant (1 hr, pH 3.5), maintains activity in the intestine for 1–2 hr
10. Function: Hydrolysis of milk fat in the intestine

LCT, long-chain triglycerides; MCT, medium-chain triglycerides; TG, triglycerides; FFA, free fatty acids.

Fusions based on in vitro studies, in which fresh whole milk was used as the source of both enzyme and substrate, suggest that as much as 40% of the triglyceride in milk can be hydrolyzed within 2 hr (52), the estimated intestinal transit time. Indeed, feeding a mixture of low-birth-weight formula and the mother’s fresh milk markedly improves fat absorption in small premature infants (body weight 660–1,700 g) (Table 4) (49).

We have recently shown that BSSL is present in the milk of women who deliver prematurely at similar concentrations as that in the milk of term mothers (39). Furthermore, the enzyme is stable in milk stored at −70°C for years, and is present in equal amounts in milk collected by manual expression, by pump, or by drip—

TABLE 4. Effect of addition of fresh human milk to low-birth-weight formula on fat excretion in VLBW infants (mean ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Human milk* + (40%)</th>
<th>Formula* + (60%)</th>
<th>Formula* (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infants</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1,344 ± 124</td>
<td>1,227 ± 127</td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>30.8 ± 0.85</td>
<td>30.1 ± 0.75</td>
<td></td>
</tr>
<tr>
<td>During study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (days)</td>
<td>16.6 ± 5.3</td>
<td>23.6 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>1,432 ± 117</td>
<td>1,264 ± 99</td>
<td></td>
</tr>
<tr>
<td>Fat intake (g/kg/day)</td>
<td>5.85 ± 0.25</td>
<td>6.16 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>Fat excretion (% of intake)</td>
<td>4.68 ± 0.50</td>
<td>11.89 ± 1.37</td>
<td></td>
</tr>
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</table>

*Fresh (not heated) milk provided by the infant’s mother.

bShort- and medium-chain triglycerides: 65.3% of the total fat.

(From Alemi et al. ref. 49.)
DEVELOPMENT OF LIPASE ACTIVITY

Thus banked human milk (which in many countries is drip-milk) may be stored fresh-frozen for long time periods without any loss in its fat digestive potential.

An additional function of the BSSL of human milk may be the hydrolysis of microbial lipids. A recent report that human milk is giardiacidal suggests that the latter is the result of hydrolysis of protozoal lipids by the BSSL of human milk (53).

The combined action of lingual and gastric lipases, which leads to hydrolysis of 50% to 70% of dietary fat, followed by that of BSSL, which accounts for the hydrolysis of 30% to 40% of milk fat, can achieve the complete hydrolysis of dietary lipid in the absence of pancreatic lipase (Fig. 7). These two compensatory mecha-

![Diagram](image)

**FIG. 7.** Compensatory mechanisms for fat digestion in the newborn. (1) Lingual and (5) gastric lipases—intragastric lipolysis: pH optimum: 3.5–6.0; reaction products: TG→DG, MG, FFA; reaction products are amphiphilic lipids that emulsify fat. This process can hydrolyze 50–70% of dietary fat. (2) Human milk lipase—intestinal hydrolysis: pH optimum: 7.5–8.0; bile salts (0.5–5.0 mM) obligatory; reaction products: TG→FFA, glycerol. This process can hydrolyze 30–40% of milk fat. (3) Pancreatic lipase—intestinal lipolysis: pH optimum: 7.0–8.0; bile salts and colipase obligatory; reaction products: TG→MG, FFA; activity very low in newborn infants. (4) Bile salt synthesis and concentration very low in newborn infants. (Modified from Hamosh, ref. 40).
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nisms, the first initiating fat digestion in the stomach, the second completing the hydrolysis in the intestine, enable the premature infant to overcome the lack of adequate amounts of pancreatic lipase and bile acids.

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REFERENCES


**DISCUSSION**

*Dr. Vileisis:* You mentioned that postheparin lipolytic activity is decreased with extreme malnutrition and with sepsis. What about the effect of hypoxia? What about the effect of liver disease, let us say, secondary to total parenteral nutrition?

*Dr. Hamosh:* We do not have any data available on these aspects.

*Dr. Rubaltelli:* Disappearance of serum triglycerides in parenterally fed newborns, either preterm or full-term, receiving intravenous fat emulsions is not a good index of FFA oxidation. When we give glucose and other calories, the disappearance of FFA is more a matter of storage than a matter of oxidation. Glucose and insulin have a very strong effect on the re-esterification of FFA. Do not you think that we therefore need to be very careful in interpreting these data?

*Dr. Hamosh:* I agree with you that quantitation of serum triglyceride levels alone is not a good indicator of what is happening in the baby, mainly because there seems to be a very high rate of re-esterification in the liver. Serum triglycerides during infusion of fat emulsions are really a mixture of the infused exogenous lipid and newly synthesized, endogenous very low density lipoprotein (VLDL). In the future it is important to analyze this mixture and to differentiate between VLDL triglyceride and the infused at the same time.

*Dr. Rubaltelli:* Could you comment on the role of BSSL in the jaundice of breast-fed infants?

*Dr. Hamosh:* There exists a lot of controversy over this question. Jaundice develops in a small number of babies; the milks that are fed to these babies do not have higher lipase activity, but seem to have slightly higher levels of FFA. In general, when human milk is kept in the refrigerator for a day or two, the levels of FFA rise above the levels in fresh milk. Lipoprotein lipase in milk is extremely low, it is about one to two orders of magnitude lower than BSSL. It is therefore hard to imagine that lipoprotein lipase alone could be the enzyme that leads to the accumulation of FFA, which in turn play a role in the pathogenesis of breast milk jaundice. However, BSSL is not active at all on milk fat unless bile salts, or other hydrophobic agents, are present that can act at the active site of the enzyme;
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it could very well be that a certain amount, even a very small amount of FFA (which are hydrophobic enough to act instead of the bile salts), facilitates the interaction of the human milk BSSL with the milk fat globule (1). As it now stands, both enzymes probably have a role in breast milk jaundice. One may also postulate differences in the composition of the milk fat globule membrane (a barrier between the interaction of lipases and the triglyceride contained within the globule core) that might facilitate the interaction between enzymes and substrate. This has not yet been explored, but we are planning to look into it.

Dr. Stern: Both of you seem to agree that the breast milk jaundice is lipid mediated and not, as had been supposed for about 20 years by Arias and Gartner and a few others, the result of an aberrant pregnandiol in the breast milk, either 3α, 20 α or 3 α, 20 β-diol. This may well be the case because the evidence for pregnane 3 α, 20 β-diol is poor: It is based on in vitro inhibition studies, but feeding 3α, 20 β-pregnandiol has given very different results; for instance, we used the same material as Arias and we did not see any difference. Our attempts to isolate the pregnandiol from the breast milk by thin-layer chromatography produced an abnormal substance that was not 3 α, 20 β or 3 α, 20 α. If however there is a lipid involved, then you may need to answer the question: Is this an aberrant type of FFA or is it a quantitative phenomenon of FFA? If this is the case, I would urge you not to study the system on the basis of jaundice in the newborn because the variability of glucuronidation activity in normal 5-day-old newborns can be an 8 times factor. The substance should be studied in a tissue culture system to show that it actually inhibits glucuronidation. Whether you produce jaundice from it is really a function not simply of the inhibitor but of the maturation of the liver, and this is too variable a phenomenon to be able to measure as clinical jaundice.

Dr. Hamosh: It is possible that a small change in the fatty acid composition of breast milk might well be associated with this phenomenon; for example, in one of the membrane long-chain fatty acids. Ninety-eight percent of the 3% to 4% fat that is contained in human milk is found in triglycerides (2). Phospholipids amount to no more than 0.5% of total fat, and contain mostly the long-chain polyunsaturated fatty acids of the omega-3 and omega-6 variety (2). You would therefore need a very small quantitative change to have a different membrane with different permeability characteristics. Indeed, one should look at this question in cell cultures.

Dr. Stern: There is a very good and elegant system for that; the Lathe and Walker technique (3). It uses a fairly simple substrate, acetaminophen, glucose as a driver, and rat liver microsomes as a source of glucuronidase. It allows you to measure the glucuronidated products of acetaminophen after adding various inhibitors.

Dr. Chouraqui: When you give heparin, do you add it to the lipid infusion or do you administer it by another way?

Dr. Hamosh: The heparin is administered separately.

Dr. Chouraqui: Is the poor bile salts production in very preterm babies sufficient to stimulate milk lipase?

Dr. Hamosh: The bile salt concentrations present in very preterm babies are unable, in vitro, to stimulate the milk lipase, because in vitro lipolysis occurs only at and above 6 to 12 millimolar bile salt. However, when the feed has been predigested in the stomach it contains a mixture of tri-, di-, and monoglycerides as well as FFA. Partial glycerides and emulsified lipid are, however, hydrolyzed at much lower bile salt concentrations. Once the mixture of partial glycerides and FFA enters the duodenum, BSSL is able to act at the physiological bile salt levels of the newborn infant.

Dr. Chouraqui: What happens with lipase in pasteurized pooled human milk?
Dr. Hamosh: Both lipoprotein lipase and BSSL are completely inactivated at 63.5°C. They are, however, completely stable in frozen milk. We have just completed a study (4) during which we stored milk at −20°C and −70°C and also carried out experiments with quick freezing and thawing. The enzymes retained the initial activity. However, in order to really feed the baby milk closely similar to breast milk, one should store the milk at −70°C because at −20°C there is a continuous low-grade hydrolysis of the fat so that after 2 months or so there is an accumulation of 3% to 5% FFA (4). Breast milk jaundice has already been shown at 2% FFA. It would therefore be a little risky to feed this milk. I would advocate freezing at −70°C and not pasteurizing the milk if one wants to keep the lipase active.

Dr. Vidailhet: Which technique did you use to measure plasmatic clearance of the infused fat emulsion? And did you check the effect of carnitine on the FFA levels, which are very high in your studies?

Dr. Hamosh: We do quantitate the triglycerides during the infusion by enzymatic triglyceride analysis (5). We did not yet study the effect of carnitine on the clearing of intravenous lipid emulsions.

Dr. Senterre: It has often been said that one of the advantages of human milk fat is the particular structure of its triglyceride, with palmitic acid in beta position. On the other hand, unlike pancreatic lipase, human milk BSSL has very low specificity for hydrolysis. What do you think is more important in human milk: the beta position of palmitic acid or the BSSL that hydrolyzes palmitic-2-monoglyceride?

Dr. Hamosh: I have never had much faith in the importance of the beta position, mainly because studies show that some babies, who do not absorb fat well, have a problem with formula as well as with human milk. The beta position was considered of the utmost importance as long as we knew little about the milk lipases. I definitely believe that the lipase is a much more important factor for the hydrolysis of human milk (6,7). Another important observation is the much higher concentration of medium-chain fatty acids in milk produced by mothers of premature infants who deliver at 25 to 36 weeks, than in term human milk (2). This means that the baby receives a fat that is much more easily solubilized and hydrolyzed because all the lipases hydrolyze medium-chain triglycerides (MCT) better than long-chain triglycerides (LCT). Yet another factor is that colostrum and transitional milk after a premature delivery (during the early period of milk secretion) contain much higher levels of long-chain polyunsaturated fatty acids, which are extremely important for the brain and for membrane synthesis (2).

Dr. Rubaltelli: You mentioned that preterm and mature human milk differ in their MCT content. I would like to mention that the same applies for carnitine and short-chain acylcarnitine, which is very rapidly utilized as an energy source. Preterm colostrum and preterm milk contain more carnitine than term colostrum and mature human milk; furthermore, carnitine levels are also higher in mature colostrum than in mature milk. The levels of short-chain acylcarnitine are also high. This may be a physiological adaptation during the transition from fetal life, during which glucose and amino acids are used as an energy source, to postnatal life, during which fat is used in much larger amounts.

Dr. Hamosh: This is fascinating, although I do not really believe that the mammary gland knows that at the other end of it there is a preterm baby. I am firmly convinced that these differences in the concentration of various components of human milk result from the metabolism of a slightly immature exocrine gland in which the junctions are not tight yet and in which paracellular transport will occur. It is very interesting that whatever the immature human breast does is to the advantage of the baby. This immature period is a very short one because the preterm milk catches up very fast; after the transitional period, it is
already very similar to the milk produced by the term mother; the sole exceptions are the medium-chain fatty acids, which continue to be present in higher concentrations for almost the entire first 3 months of lactation (2). With regard to the long-chain polyunsaturated ones, the proteins, and many other components, lactating function seems to mature very fast. Indeed, as we heard in Dr. Lebenthal's presentation, it seems as if the exteriorized fetus, the premature baby in its incubator, developed much faster than it would have done in utero. The hormonal changes following the preterm delivery contribute to a much faster maturation of the mammary gland. I therefore agree with you that there is a very nice adjustment of the mother to the baby and the baby to the mother so that the dyad can progress together.

Dr. Stern: Does this maturation that appears to be linked to extraterine life of the infant depend on whether the baby actually nurses?

Dr. Hamosh: With regard to fat digestion, our observations are based mostly on formula-fed babies.

Dr. Stern: So you would suggest it is a hormonal event, postpartum?

Dr. Hamosh: I think so. The faster maturation of the newborn is due to the stressful situation that goes with extraterine life.

Dr. Stern: What about the maturation of the breast?

Dr. Hamosh: I think that is because of the very abrupt early parturition and the complete change in the hormonal milieu.

Dr. Stern: Would sucking stimulate those changes?

Dr. Hamosh: It will be a while before we shall be able to answer this question because the preterm mother is pumping, very often and very thoroughly, so the volumes of milk increase very fast. I do not know if pumping might not, in certain cases, be more efficient than the sucking newborn.

Dr. Heim: Prolactin determines the composition of preterm milk, and the prolactin secretion, as you said, is very much dependent on the manipulation with the nipple. If you apply an electric or a mechanical pump, you can increase the prolactin level tremendously and that influences the high protein and high sodium concentrations of the milk. So I think this is an artificial effect.

Dr. Hamosh: Yes, this could very well be.

Dr. Marini: When we look at preterm milk, we look always at concentrations, and very seldom at daily amounts. Is this important?

Dr. Hamosh: There are data that show that the preterm mammary gland adjusts quite fast. In the beginning the volumes are indeed low for the first few days, but then they increase. After 2 weeks or so, the same volumes are produced as by the mature mammary gland (8,9).

Dr. Heim: Dr. Hamosh, you emphasize that one should distinguish two components in the intragastric lipolysis, the one owing to lipase secreted by the lingual serous gland, and the other to parietal cell secreted lipase. How did you differentiate between the two secretion pathways and what is the quantitative relation of the supragastric lipase and the gastric mucosal lipase?

Dr. Hamosh: We do not know yet if it is the parietal cell that secretes the gastric lipase. How do we differentiate them? The whole aspect of gastric lipase started when we studied infants with esophageal atresia; in these infants there was no connection between the oral cavity and the stomach and yet we found quite high lipase activity in the esophageal pouch as well as in the stomach (10). This led to the gastric lipase studies, mainly because there were some old observations of lipase activity in the stomach that I attributed (after I discovered lingual lipase) to lingual lipase that was adsorbed to the gastric mucosa. Indeed this
is true in the rat and the mouse, in which no gastric lipase is to be found, but which have high lingual lipase. The rabbit, on the contrary, has no lingual lipase but has a very potent gastric lipase. We use a preparation of isolated gastric glands that are maintained in short-term organ culture and are responsive to secretagogues; gastric lipase is released in response to CCK and to carbachol; we have shown also that these secretagogues act via two different sets of receptors that can be inhibited by different antagonists (11). In the human, we have also succeeded in preparing isolated gastric glands (obtained from gastric specimens taken during gastroplasty for severe obesity) that secrete lipase (12). This lipase has very similar characteristics to that in the rabbit’s stomach. Indeed, it is very difficult to differentiate between lingual and gastric lipase. Both lipases have very similar characteristics.

**Dr. Tsang:** Is there any differential effect for your lingual lipase or gastric lipase if you feed human milk versus cow’s milk?

**Dr. Hamosh:** We have not yet studied this aspect. We are now starting to look at whether there are differences in the hydrolysis of some of the formulas compared to human milk. So far, we see no major differences, but these are very preliminary studies.

**Dr. Heim:** Could you explain why these supragastric lipases act more vigorously on MCT and unsaturated fatty acids? My understanding is that all these enzymes basically attack the ester bound between the glycerol and fatty acids. What really makes them more sensitive to MCT and unsaturated fatty acids?

**Dr. Hamosh:** It is mostly a question of solubility. As the fatty acids get shorter or more polyunsaturated, they are more miscible with water so the solubility of the mixed molecules differs and the lipase will just have more access to them. Lingual lipase and gastric and bile salt stimulated lipase are low specificity enzymes, yet all will work much better on MCT than on LCT. Pancreatic lipase activity rate is also higher with MCT than LCT. This really has no bearing on what happens in vivo: We have fed babies, the same baby acting as its own control, MCT and LCT formulas and we found exactly the same extent of fat absorption. Babies can be divided into good absorbers and bad absorbers. In the good absorbers about 90% of fat is absorbed with either formula. Poor absorbers will absorb about 60% of fat from the LCT formula and will not do much better on the MCT formula, absorbing maybe 70% of the fat. It will depend more on the developmental stage of the gastrointestinal tract of each baby than on the different formulas. The difference between this study and the previous ones, in which the investigators compared one group of infants on one formula to a second group on a second formula, is that we used each baby as its own control. Thus, we could distinguish between good and bad absorbers.

**Dr. Phienvit:** The fatty acid pattern of milk is influenced by the quantity of fat and the fatty acid pattern of the mother’s diet: Could the mother’s dietary fat influence the digestion or the hydrolysis or the absorption of fat? Could it have an influence on the production of BSSL and could it also influence the absorption and digestion of the mother’s milk?

**Dr. Hamosh:** We really have no answers to your questions. Yes, indeed if the woman consumes mostly carbohydrates, that will result in palmitic acid rich milk: palmitic acid is mostly synthesized from glucose; the milk will contain slightly higher amounts of myristic acid and higher C12, C10, and C8. In general, the milk seems to maintain its composition quite consistently, unless we go to extreme situations, which we very seldom see in real life. If the woman consumes only polyunsaturated fats, and very large amounts of them, then, the milk will be very rich in polyunsaturated fat. We see an increase in the intake of polyunsaturated fats in the American diet; 20 years ago human milk contained about 8% linoleic acid, now it is up to 12% to 14%. As far as I know, nobody has yet looked at the level of BSSL as a function of maternal diet.
Another thing is that maternal diets are notoriously difficult to study: It is a burden on the mother and on the investigator. Unless well-defined populations who consume certain types of diet can be studied in a multicountry study, it is very hard to obtain the data.

Dr. Phienvit: In Western countries, diets are more or less evenly distributed, but this does not necessarily apply for developing countries. For example, in Bangkok, about 30% of the calories ingested come from fat, but in the northeast of Thailand, most of the calories come from carbohydrates, and fat contributes only to 4% to 8% of total calories ingested.

Dr. Hamosh: You are right, if one goes back to Ahrens’ studies in 1958 to 1959, which are still the classics in the field, one sees that these were very drastic studies in which the women were maintained in the metabolic ward of the hospital and were fed exclusively one type of diet, to which, of course, the mammary gland responded by producing milk with very different fat composition. It would be very interesting to conduct a study between the northeast and the south and see what happens.

Dr. Rubaltelli: A study was recently conducted to see if different diets given to mothers in the first week after delivery could influence the occurrence of jaundice in the newborns. The mothers were given two diets differing in their lipid composition: one was a mediterranean diet containing mostly unsaturated fatty acids like olive oil; the other was the classic diet in use in Finland containing large amounts of saturated fatty acids. The milk contained the same proportion of saturated and unsaturated fatty acids as in the mother’s diet, so the diet of the mother influenced the composition of the lipid fraction of the milk. There were no detectable differences in growth in the infants of both groups. There were also no differences in the appearance of jaundice.

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