Macronutrient Digestion and Absorption in the Preterm Infant

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Education Gap

Knowledge of the importance of human milk enzymes in macronutrient digestion has increased greatly over recent years, and provides a further impetus to the use of human milk, especially mother's own milk, in the nutrition of preterm infants.

Abstract

The human fetus receives oral nutrition through swallowed amniotic fluid and this makes a significant nutritional contribution to the fetus. Postnatally, macronutrient absorption and digestion appear to function well in the preterm infant. Although pancreatic function is relatively poor, the newborn infant has several mechanisms to overcome this. These include a range of digestive enzymes in human milk, novel digestive enzymes involved in fat and protein digestion that do not appear to be present in the older child or adult, and the presence of a *Bifidobacterium*-rich colonic microbiome that may "scavenge" unabsorbed macronutrients and make them available to the infant.

Objectives After completing this article, readers should be able to:

1. Understand the importance of human milk in macronutrient digestion in the preterm infant.
2. Understand the role of the colonic microbiome of human milk-fed infants in scavenging unabsorbed nutrient in the preterm infant.
3. Understand the importance of relative pancreatic exocrine insufficiency in preterm infants and the alternative digestive enzymes that serve to mitigate its effect.

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ABBREVIATIONS

BSDL	bile salt–dependent lipase
BSSL	bile salt–stimulated lipase
GLUT2	glucose transporter 2
GLUT5	glucose transporter 5
HMO	human milk oligosaccharide
IMMC	interdigestive migrating motor complex
LCPUFA	long-chain polyunsaturated fatty acid
PEPT1	peptide transporter 1
PLRP2	pancreatic lipase-related protein 2
PTL	pancreatic triglyceride lipase
SGLT1	sodium-glucose linked transporter 1

INTRODUCTION

Current nutritional recommendations in the preterm infant emphasize that postnatal growth mirrors that of the fetus of the same gestational age. Typically, this goal is not met and the growth of preterm infants, especially very low-birthweight infants (birthweight <1,500 g), is often far slower than in utero growth. (1) Although there is evidence of some improvement in recent years, (2) these nutritional deficits are associated with poorer long-term neurodevelopmental outcomes. Much of the early growth deficits of preterm infants are associated with reduced protein and energy intake, (3) and both poor growth and lower nutrient intake are associated with poorer outcomes. (2)(3)

Traditionally, neonatologists have some hesitancy when initiating and advancing enteral feedings in very preterm infants because of the perceived associated risk of necrotizing enterocolitis. However, there is good evidence that more conservative approaches to feeding, such as prolonged periods of "trophic" feedings, very slow advancement of enteral feeding volume, and delayed fortification of human milk, are not associated with lower rates of NEC. (4)(5)(6)(7)(8) Studies have found that these cautious regimens may be associated with a number of adverse outcomes such as lower nutrient intakes, (8) prolonged use of central lines, delayed establishment of full enteral feedings, (5)(6)(7) increased risk of infection, (7) poorer growth, (6) and increased length of stay. (5)

Empirically, most preterm infants seem able to adapt well to enteral feedings. Nevertheless, many neonatologists continue to remain apprehensive about feeding very preterm infants as a result of unfounded concerns about NEC and are reluctant to be the initiator of feedings. However, this misconception—that the fetus is entirely parenterally fed (via the umbilical vein) and that newborn infants (term or preterm) receive their first enteral feeding after birth—is actually incorrect.

ENTERAL NUTRITION OF THE FETUS

Fetal swallowing is first seen at 18 to 20 weeks of gestation and plays an important role in the regulation of amniotic fluid volume. The volume ingested in human fetuses is hard to assess. In fetal sheep, fluid flow along the esophagus is bidirectional, but net inward amniotic fluid flow averages 175 mL/kg per day at 75% of term gestation (30 weeks' gestation in humans) increasing to 274 mL/kg per day at 85% of term gestation (34 weeks' gestation in humans). (9) Both are far higher than the fluid intake of an adult sheep (40-60 mL/kg per day).

Ingestion of amniotic fluid by the fetus has an important *nutritional* role. There is good evidence that esophageal atresia (which prevents fetal swallowing) reduces birthweight. Birthweight Z score is lower in infants with esophageal atresia than in infants with anorectal malformations. (10) Although infants with proximal intestinal obstructions (both esophageal atresia and duodenal atresia) have associated reduced birthweight Z scores, infants with a more distal obstruction (jejunal atresia and ileal atresia) have appropriate fetal growth. Esophageal ligation in fetal rabbits leads to significant reductions in birthweight and birth length compared with sham operations, and these effects are reversed by esophageal infusions that restore fetal "swallowing." (11) Based on these studies, investigators estimated that swallowed amniotic fluid provides 10% to 14% of nutrition in the fetal rabbit. (11)

Amino Acids in Amniotic Fluid

Human amniotic fluid contains a broad range of amino acids; concentrations of alanine, lysine, and phenylalanine are high, whereas that of cysteine is low. (12) The amount of amino acids in amniotic fluid is equivalent to a protein content of approximately 0.4 g/dL (4 g/L). Although this amount is less than that found in human milk (typical estimate 1 g/dL [10 g/L]), it could be nutritionally significant if sufficient amniotic fluid is ingested.

Methionine in amniotic fluid is of particular interest. In rodent models, maternal malnutrition leads to reduced amniotic fluid methionine (and phenylalanine) levels, and amniotic methionine is correlated with fetal birthweight. (13) Similar data are reported in humans. In a study of 625 healthy pregnancies, amniotic fluid methionine concentrations between 13 and 17 weeks' gestation were positively associated with birthweight, and amniotic fluid cysteine concentration was negatively associated with birthweight. (14)

Adaptation of Amniotic Fluid Absorption

Absorption of amniotic fluid components by the fetus seems to be adaptable. In one study, pregnant rabbits received intrauterine infusions of galactose or an inert control for 6 days. Exposure of the fetuses to galactose in the amniotic fluid led to increased galactose and glucose uptake in the proximal and distal small intestine. (15) This suggests that the fetus (and by extension, the preterm infant) may be able to adapt to new dietary components by upregulating the enzymes and transporters required to digest and absorb them.

Absorption of Macromolecules by the Fetal Gastrointestinal Tract

The fate of proteins in amniotic fluid has been studied in rhesus monkeys. (16) The authors injected an intact protein, labeled with ^{35}S-methionine, into the amniotic fluid of pregnant rhesus monkeys. The clearance of the protein was largely determined by the rate of fetal swallowing, and evidence of proteolysis of the labeled protein was observed along the length of the small intestine. Amino acids released by proteolysis of the labeled protein were incorporated into gut proteins and released into the fetal plasma as amino acids, where they equilibrated rapidly with maternal amino acids and entered the amniotic fluid amino acid pool. One day after peak ^{35}S-methionine enrichment of the amniotic amino acid pool occurred, ^{35}S-methionine-labeled amino acids were detected in the fetal plasma, indicating that amniotic fluid amino acids were being used for protein synthesis. Labeled proteins were also recovered from the fetal lung, liver, skeletal muscle, and brain. The authors estimated that amniotic fluid amino acids contributed 10% to 15% of the nitrogen requirement of the fetus. (16)

THE ROLE OF HUMAN MILK IN DIGESTION AND ABSORPTION

The ideal milk for all infants, including preterm infants, is human milk, preferably their own mother's milk, or failing that, donor human milk. The main nutritional disadvantages of human milk are the low content of energy, protein, calcium, and phosphate relative to the very high nutritional needs of preterm infants. However, it has some specific nutritional advantages over formula, such as containing many enzymes important for digestion.

Human Milk Lipase

Early evidence that human milk lipases may be important for fat digestion came from a small randomized trial in the 1980s. Preterm infants fed a preterm formula had significantly higher fecal fat excretions (11.9% ± 1.4%) than those fed a 60:40 mixture of preterm formula and fresh human milk (4.7% ± 0.5%). (17)

One enzyme that may explain these findings is bile salt–stimulated lipase (BSSL), a lipase found in human milk, and the counterpart of bile salt–dependent lipase (BSDL) secreted by the exocrine pancreas. BSSL completes the final stage of triglyceride digestion by converting monoglycerides (produced by colipase-dependent pancreatic lipase) to free fatty acids. (18) BSSL is not present in formula and is inactivated by pasteurization of human milk. (19) This may be a possible explanation for fat absorption being 17% lower in preterm infants receiving pasteurized mother's own milk compared with those receiving unpasteurized mother's own milk. (20) Human BSSL is commercially available as a recombinant protein (rhBSSL). Although one small study suggested that addition of rhBSSL to pasteurized human milk or formula improved growth and long-chain polyunsaturated fatty acid (LCPUFA) status, (21) this has not been borne out in a larger trial. (19) Although that larger study found that rhBSSL had no effect on growth of preterm infants (n=415, gestational age ≤32 weeks), significant improvements in growth were found in small-for–gestational age preterm infants in a planned subgroup analysis (n=62). (19)

Other Enzymes in Human Milk

Various other enzymes are present in human milk that may potentially play a role in the digestion of lipids, carbohydrates, and proteins (Table). (22)(23)(24) As well as containing "traditional" digestive proteins, human milk contains a number of proteases that usually perform nondigestive functions, but that are able to digest human milk proteins. (24) For example, cathepsin D is usually involved in the degradation of intracellular proteins and the inactivation of growth factors and peptide hormones, but is also able to digest at least 24 proteins found in human milk. (24)

In addition, human milk factors may make otherwise nondigestible human milk components (eg, human milk oligosaccharide [HMO]) bioavailable by modifying the microbiome of the preterm infant (to be described in more detail later in this article).

CARBOHYDRATE ABSORPTION

Carbohydrate Digestion/Absorption in Adults

In adults, large carbohydrates are digested in a 2-stage process beginning with salivary α-amylase, which is inactivated in the acid pH of the stomach, and continuing with pancreatic α-amylase in the small intestine, resulting in a combination of mono-, di-, and trisaccharide, and dextrins. These in turn are broken down by border enzymes (eg, lactase, sucrase, glucoamylase, and maltase) into monosaccharides, which are absorbed by a combination of facilitated diffusion and active transport (using transporters such as glucose transporter 2 [GLUT2], GLUT5, and sodium-glucose linked transporter 1 [SGLT1]).

In newborn infants, the situation is potentially less complex, with 3 major sources of carbohydrates:

TABLE. **Digestive Enzymes Present in Human Milk (22)(23)(24)**

MACRONUTRIENT	ENZYME	USUAL ROLE
Lipids (22)(23)	Lipoprotein lipase	Lysis of triglycerides to 2 free fatty acids and a monoglyceride
	Bile salt-stimulated lipase (BSSL)	Lysis of monoglycerides to free fatty acids and glycerol
Carbohydrate (22)(23)	α-amylase (diastase)	Hydrolysis of α-linked polysaccharides at random locations, finally producing glucose, maltose, and maltotriose
	β-amylase	Hydrolysis of 1,4-glycosidic bonds to release maltose from the nonreducing end of polysaccharides
Protein (24)	Chymotrypsin	Cleaves peptide bonds adjacent to large hydrophobic amino acids
	Pepsin	Cleaves peptide bonds adjacent to hydrophobic and aromatic amino acids
	Trypsin	Cleaves peptide bonds adjacent to lysine or arginine amino acids (unless followed by proline)
	Cathepsin D	Non-nutritional (but able to cleave α-, β-, and κ-casein)
	Plasmin	Non-nutritional (but able to cleave α-, β-, and κ-casein, and mucin-1)
	Elastase	Non-nutritional (but able to cleave α- and β-casein)
	Glutamyl endopeptidase	Non-nutritional (but able to cleave some human milk proteins)
	Proline endopeptidase	Non-nutritional (but able to cleave some human milk proteins)

1. Lactose, the dominant carbohydrate in human milk, with a concentration of 5.6 to 7.8 g/dL.
2. A diverse group of HMOs (1.2-2.1 g/dL).
3. Maltodextrins or corn syrup solids, polymers of glucose of varying length linked by 1,4-glycosidic bonds. These are found in preterm infant formulas and in human milk fortifiers.

In addition, human milk contains small amounts of free monosaccharides (typically <0.1 g/dL).

Lactose

Lactose is the predominant carbohydrate in human milk, and consists of a galactose and glucose joined by β−1,4-glycosidic bonds. It is digested by lactase bound to the brush border membrane of the small intestine.

Lactase is active in the fetus. In the lamb (human gestation equivalent ~32 weeks), intra-amniotic injection of lactose leads to a rapid rise in blood glucose suggesting that lactase and glucose transporters are present in the fetal intestine. (25) In contrast, intra-amniotic injection of sucrose or maltose does not lead to an increase in fetal blood glucose, implying that neither sucrase nor maltase is present in the fetus. (25) In the human fetus, intestinal lactase is detectable in very low levels by 12 weeks of gestation. Levels slowly increase to about 30% of the levels seen in term infants by 34 weeks' gestation and to 70% of term infant levels by 35 to 38 weeks' gestation. (26)

Postnatally, jejunal lactase activity in the piglet increases rapidly after 24 hours of colostrum or milk feedings, but not after feedings with water or after being nil per os. (27)

Lactase activity can be measured indirectly in the preterm infant from the urinary lactose-lactulose ratio. Lactase activity can be detected by 10 days after birth (the earliest time studied by the investigators) and increases at least 3-fold by 28 days after birth. (28) Lactase activity was higher in human milk–fed infants than in formula-fed infants, and lower in infants in whom initiation of feedings was more delayed. (28) Lactase activity was significantly inversely correlated with the time to reach full enteral feedings, which was lower in infants with higher lactase activity. (28) Lactase activity is also higher in infants of higher gestational age. (29)

Lactose absorption is very efficient in the preterm infant with less than 2% of lactose carbons being excreted in the feces, (30) though whether this is the result of hydrolysis by lactase is open to question. (26) The 2 carbons from lactose can be absorbed by 2 separate mechanisms (Fig). The first is lactase dependent, where lactose is digested by lactase in the brush border to galactose and glucose, which are then transported into the enterocyte by SGLT1, and across the basolateral membrane by GLUT2. The second is lactase independent, where undigested lactose undergoes bacterial fermentation in the colon, producing various short-chain fatty acids such as acetate, lactate, propionate, and butyrate, which are absorbed across the colonic mucosa. Preterm infants are known to have high breath hydrogen levels when

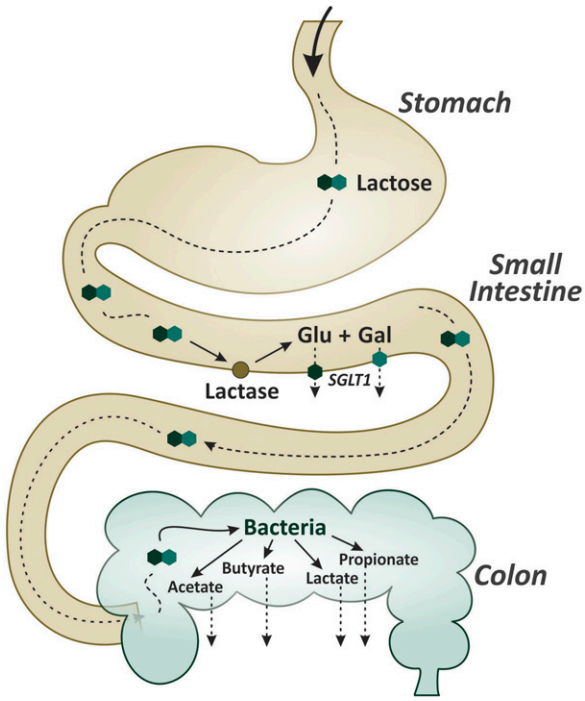

Figure. Schematic representation of lactose absorption. Lactose can be digested in the small intestine by brush border–bound lactase into glucose and galactose, which are then absorbed via the transporter sodium-glucose linked transporter 1 (SGLT1). Unabsorbed lactose can be fermented in the colon by bacteria into acetate, butyrate, lactate, and propionate which can be absorbed across the colonic mucosa. Less than 2% of lactose carbons are excreted in the stool in preterm infants.

fed lactose, which would be consistent with significant levels of colonic fermentation. It is also possible that unabsorbed glucose and galactose may undergo fermentation in the small bowel. Human colonic bacteria (with or without lactobacilli) have been shown to be able to ferment lactose to acetate, lactate, propionate, and butyrate, (31) and stable isotope studies in preterm infants have demonstrated that they have a high capacity to absorb acetate, which could be sufficient to explain the absorption of a significant proportion of lactose carbon. (32)

Regardless of the balance between the 2 mechanisms, lactose is very well-tolerated by preterm infants without evidence of malabsorption (such as diarrhea or poor growth).

Oligosaccharides

The concentration of HMOs is high in early lactation but falls as lactation proceeds. (33) Human milk contains more than 100 HMOs. All begin with the lactose linkage of glucose-β−1,4-glucose, but a variety of sugars (including galactose, N-acetylglucosamine, fucose, and N-acetylneuraminic acid) are bound to the glucose residue. (33) Humans lack the required enzymes to digest HMOs, so they are resistant to digestion in the small intestine. (33) Small amounts of intact HMOs are absorbed and may be subsequently excreted in the urine, but the majority of HMOs enter the colon intact. (33)

In the colon, bacteria digest the HMOs and this is a significant contribution to breath hydrogen. (34) Not all bacteria, however, are able to digest HMOs. *Bifidobacterium* (35) and *Bacteroides* species are especially well-suited to digest HMOs. (36) Some biovars of *Bifidobacterium longum* are able to grow well in vitro with HMOs as the sole carbon source. (37) In contrast, other "pathogenic" bacterium such as *Enterococcus*, *Streptococcus*, *Clostridium*, and *Escherichia coli* strains are less able to digest HMOs. (36) It has been suggested that humans and *Bifidobacterium* have coevolved, with humans producing milk that is especially well-suited to support *Bifidobacterium* growth in the colon, in return for the beneficial results of a *Bifidobacterium*-rich colonic microbiota. (37)

The products of bacterial fermentation of HMO–acetate, butyrate, lactate, and propionate–are readily absorbed in the colon, and serve to largely offset the energetic costs of maternal synthesis of HMOs.

Glucose Polymers

Glucose polymers, often described as maltodextrins or corn syrup solids, are present in infant formulas and in human milk fortifier. They compose a collection of polymers of different length joined by 1,4-glycosidic bonds. These bonds are susceptible to digestion by amylases. There are 4 main sources of amylase in the preterm infant: 1) α-amylase in human milk; 2) β-amylase in human milk; 3) salivary α-amylase; 4) pancreatic α-amylase.

α-amylase randomly hydrolyzes 1,4-glycosidic bonds along the length of the polymer to produce a mixture of glucose, maltose, and maltotriose, whereas β-amylase removes a maltose from the nonreducing end of the polymer.

Both α-amylase and β-amylase are present in the milk of mothers delivering preterm infants. Levels of α-amylase are similar in mothers delivering infants at 25 to 30 weeks, 31 to 35 weeks, and 36 to 40 weeks of gestation. Levels are highest in colostrum and then decline with increasing duration of lactation. (38)

Salivary α-amylase levels are variable in preterm infants. Enzyme levels tend to be higher with increasing gestational age, but can be detected in preterm infants as young as 26 weeks' gestation (the youngest age studied by the investigators). (39)

However, pancreatic α-amylase levels are typically low in preterm infants. In one study, pancreatic α-amylase was undetectable at birth, and remained so after 30 days of

feeding. (40) However, other studies have noted different findings. In neonatal piglets (an excellent model for human nutrition), pancreatic amylase is low in preterm compared with term piglets, but did increase after the start of enteral feedings (but not if the piglet was parenterally nourished). (41) It has been suggested that salivary α-amylase may be able to partially compensate for low levels of pancreatic α-amylase levels in preterm infants. (42)

The products of α-amylase need to be converted to monosaccharides before absorption. This appears to be likely as the levels of sucrase, maltase, and isomaltase are usually comparable to the term infant. (43)

In practice, glucose polymers seem to be well absorbed in preterm infants. Absorption of glucose polymers has been assessed directly in infants (gestational age 28–42 weeks) using a gastrointestinal perfusion model. (44) This absorption increased with postnatal age, and with length of time on full enteral feedings. (44) In fact, absorption of glucose polymers was greater than that of lactose or of a combination of lactose and glucose polymers. (44)

Monosaccharide Transport

The final steps of carbohydrate absorption in preterm infants is transport of glucose and galactose across the apical membrane of the enterocyte (via SGLT1) and then across the basolateral membrane into the circulation (via GLUT5). (45) Glucose transport across the gut appears to be intact in the fetal lamb (32 weeks' equivalent gestation) because intra-amniotic infusion of glucose leads to a rapid increase in fetal blood glucose, while no such increase is found for maltose or sucrose infusion. (25)

In a postnatal rat model, increased dietary carbohydrate increases glucose and galactose transport, as does increased enteral feeding. (45) Starvation and prolonged parenteral nutrition reduce glucose transport and it seems that "*luminal nutrition may be required to maintain glucose transporters.*"(45) Similar results are seen in preterm infants, with glucose absorption increasing as the proportion of milk feedings increases, and as feedings are infused over shorter periods. (46)

FAT AND LIPID ABSORPTION

Lipid Digestion and Absorption

Dietary lipids are important not only as a critical source of energy, but also as a substrate for bioactive compounds such as essential fatty acids, structural components of cell membrane, and regulators of gene expression.

Fat Digestion in Adults

In adults, fat digestion begins in the mouth with lingual lipase that is secreted from the serous glands, and continues in the stomach with gastric lipase that is secreted by the chief cells in the fundus. Both enzymes have a preference for fatty acids on the sn-3 position of the triacylglycerol. Together they account for 10% to 30% of fat hydrolysis.

Chyme entering the duodenum stimulates the release of pancreatic lipases and colipase, which act synergistically in the digestion of emulsified fat. Pancreatic triglyceride lipase (PTL) seems to account for the majority of lipase activity in vitro. It binds to the oil/water interface of the triglyceride oil droplet, and preferentially hydrolyzes the triglyceride at the sn-1 and sn-3 positions. Many constituents of duodenal contents inhibit PTL, but colipase restores its activity by forming a complex that anchors PTL to the substrate. Phospholipase A catalyzes the hydrolysis of the fatty acid ester linkage at carbon 2 of phosphatidylcholine, leading to release of free fatty acid and lysophosphatidylcholine.

BSDL, also known as *carboxyl ester lipase*, has activity against various lipid substrates, but no human studies have suggested a function for BSDL in adults.

Bile, a mixture of mainly bile salts, phospholipids, cholesterol, and bicarbonate, is secreted by the liver and temporarily stored in the gallbladder. It is highly surface active, and acts as an emulsifier to ensure a large accessible surface area for the lipases. The absorption of many lipid-soluble substances, including cholesterol, vitamin D, vitamin K, and carotene, are almost completely dependent on the presence of bile. The products of triacylglycerol digestion, along with the bile salts, phospholipids, cholesterol, and other fat-soluble substances, form micelles in the small intestine.

Fat Absorption in Adults

Despite its complexity, lipid digestion is very efficient and adults absorbed about 95% of dietary fat, regardless of fat intakes. Fatty acids with 12 or more carbon atoms are absorbed into the lymphatic system as chylomicrons, while those with 10 or fewer carbon atoms (short- and medium-chain fatty acids) are absorbed directly into the portal circulation.

Up to 50% of fat can be absorbed in the absence of bile, as free fatty acids, predominantly through direct portal absorption.

Lipid Nutrition in Neonates

At birth, the human fetus switches from a predominantly glucose energy supply to a lipid-dominating source. Milk

fat provides 40% to 60% of the energy requirement in neonates. The content and composition of human milk fat varies with the mother's diet and stage of lactation. Human milk total lipid content increases during lactation from 2 g/dL in colostrum to 4.9 g/dL in mature milk. The ratio of saturated to unsaturated fat in human milk is similar to that in adipose tissue, and is appropriate for cell membrane function. Human milk fat is secreted as milk fat globules, which consist of a hydrophobic triacylglycerol-rich core enveloped by a triple layer membrane. This membrane contains amphipathic compounds such as phospholipids, proteins including enzymes, and cholesterol. It also contains membrane proteins and glycoproteins, and is rich in bioactive components.

The LCPUFAs, arachidonic acid and docosahexaenoic acid, are important functional components in human milk. These LCPUFAs are necessary for normal brain development as well as for immune function.

Lipid Digestion in Neonates

Lipase accumulates in the proximal pouch of infants with esophageal atresia, consistent with a lingual site of production. (47) It is active at low pH and in the absence of bile salts.

Gastric lipase can be found in samples from fetuses as early as 18 weeks of gestation, attains significant levels of activity by 27 weeks, and reaches normal adult levels after the first few months of age. (48) Lipolytic activity is present in gastric aspirates as early as 26 weeks of gestational age in human infants. (47)

Preduodenal (lingual and gastric) lipases are essential for the digestion of human milk fat because, contrary to pancreatic lipases, they can penetrate into the milk fat globule and initiate the digestive process. (49)

Even though some authors proposed that higher rates of gastric lipase activity may exist to compensate for the low pancreatic lipase activity, the pre- and postprandial gastric lipase concentration in infants is similar (50) or lower (51) than that in adults, and gastric lipolysis in premature infants is similar to that found in adults. (50)

Neonates have a relative exocrine pancreatic insufficiency, despite their high dietary fat intake. The capacity to digest fat is suboptimal at birth due to low pancreatic enzyme levels and low intraluminal bile salt concentrations. At birth, the expression of PTL and phospholipase A2 is very low or undetectable (52) and only reaches adult activity levels in the duodenum by 1 to 2 years of age. (53)

The 2 pancreatic enzymes predominantly involved in fat digestion early in life are BSDL and pancreatic lipase-related protein 2 (PLRP2). PLRP2 messenger RNA is present by 16 weeks in the pancreas of human fetuses. (54) PLRP2 seems to have a minor digestive role, if any, in adults, but seems to play a significant role in the digestion of long-chain triglycerides when in the presence of colipase in infants. (55) Furthermore, in an in vitro model of human lipases and digestion of human milk and formula, PLRP2 has been shown to act synergistically with gastric lipase and BSDL from human milk in the digestion of human milk, especially in the presence of colipase. Predigestion with gastric lipase increased the activity of both enzymes in formula by 11-fold. (56)

BSSL, an enzyme with close similarity to the pancreatic BSDL, is present in human milk at all stages of lactation, even in mothers who deliver prematurely. (57) BSSL is inactive in the milk, but is activated by bile in the small intestine. BSSL has an optimal pH between 7.0 and 8.0 and requires bile salts for its lipolytic activity. Based on in vitro studies, BSSL activity is sufficient to completely hydrolyze milk triglycerides within 30 minutes in the small intestine. (58) However, BSSL is inactivated by heat (eg, pasteurization), (59) resulting in decreased fat absorption. (60)

Early in life, the reabsorption of bile salts is confined to the distal ileus and enterohepatic recycling is inefficient. In rats, active bile salt–transporting capacity increases with age, which may be related to a change in microvillus membrane lipid composition with increase in cholesterol resulting in a decrease in membrane fluidity. To what extent dietary effects contribute to these changes remains to be elucidated. (61) The postnatal development of the enterohepatic circulation results in an increase in the bile salt pool that leads to efficient absorption, as lipolysis and solubilization are better facilitated. Breastfed infants show a larger bile salt pool and higher intraluminal bile salt concentration than formula-fed infants, both at 11 and 35 days of age. (62)

Lipid absorption is lower in newborns than in adults. Term infants excrete approximately 10% of consumed lipids, whereas preterm infants excrete 10% to 30%. (63) The amount of unabsorbed fat appears to depend on gestational and postnatal age and the type of fat. (64) The absorption of saturated and long-chain fatty acids may be particularly impaired in formula-fed infants, especially for docosahexaenoic acid. (65)

Unabsorbed lipids reach the colon where they may be used by the colonic microbiota. However, the presence of prebiotic oligosaccharides may mask the effect of unabsorbed long-chain fatty acids on the colonic microbiome.

PROTEIN, AMINO ACID, AND POLYPEPTIDE ABSORPTION

Protein Digestion in Adults

Protein digestion is complex and involves a wide variety of enzymes. It occurs in 4 phases: gastric, luminal, mucosal/brush border, and intracellular.

Gastric Phase. Gastrin chief cells secrete 2 proenzymes, pepsinogen I and II, which are cleaved in the acid pH of the stomach into pepsin. Pepsin functions best at low pH and cleaves internal bonds, especially those involving phenylalanine, tyrosine, and leucine. Pepsin produces polypeptides and smaller oligopeptides. (66)

Luminal Phase. The pancreas secretes 5 main proteases; 3 are endoproteases (trypsin, chymotrypsin, and elastase) that cleave internal amino bonds, and 2 are exopeptidases (carboxypeptidase A and B) that cleave the terminal amino acid from the peptide, releasing a free amino acid. All are secreted as inactive zymogens. The most important is trypsin because it can activate all 5 zymogens to release the active protease. Trypsin itself is released from trypsinogen by brush border enterokinase or by trypsin (autolysis).

The different enzymes have different specificities for the various amino-amino bonds of proteins, and in concert, produce a mixture of oligopeptides, dipeptides, tripeptides, and free amino acids. (66)

Mucosal/Brush Border Phase. The brush border membrane contains a range of exopeptidases (including aminopeptidase N, aminopeptidase A, dipeptidylcarboxypeptidase, and dipeptidylaminopeptidase IV), endopeptidases, and dipeptidases to further digest peptides.

Di- and tripeptides can be transported into the cell by peptide transporter 1 while free amino acids are taken up by various amino acid transporters of different specificities. (67)(68)

Intracellular Phase. Intracellular proteases further digest the absorbed di- and tripeptides to free amino acids, which are transported into the basolateral cell membrane into the circulation using the same amino acid transporters found in the brush border. (66)

Most protein digestion and absorption occurs in the duodenum and jejunum. (66) But amino acid transporters are expressed in the colon and it is theoretically possible that unabsorbed amino acids might also be absorbed in the colon. (69) The functional contribution of amino acid transporters in the colon is unclear.

Protein Digestion in Neonates

Proteases in Human Milk. A wide variety of proteases are present in human milk including anionic trypsin, anionic elastase, plasmin, and both tissue-type and urokinase-type plasminogen activators cathepsin D and kallikrein. (70)

Most milk protease and antiprotease concentrations do not change with gestational age or postnatal age. However, this is not true of all human milk proteases. The concentration and activity of kallikrein, the most abundant and active protease in preterm milk, increases over time in milk from mothers of very premature infants, but remains more stable in the milk of mothers who deliver during mid- and late gestational age. (70)(71)

The action of carboxypeptidase B2 is higher in preterm milk than in term milk, and may explain the higher level of α-1-casein–derived peptides found in preterm human milk compared with term human milk. (71) Plasmin activity is also higher, and endogenous human milk peptides are more abundant in preterm human milk than term human milk, especially in early lactation. (71) The higher protein degradation by endogenous proteases in preterm milk may contribute to improved net digestion in the immature digestive system of the premature infant. (71)

Protein Degradation in the Stomach. Pepsin is present in the stomach of fetuses as early as 16 weeks of gestation (72) and is produced at birth by both term and preterm infants, though at levels far lower than that of adults. (73) Recent studies on the peptidome in human milk suggest the presence of an enzyme with pepsinlike activity that may be seen at higher pH than pepsin. (74) Whether this is a novel enzyme not yet described in human milk remains to be elucidated.

Gastric aspirates of newborn infants also contain a protease with electrophoretic mobility and immunoreactivity similar to that of calf chymosin, a protease that cleaves κ-casein. This protease is unique in that it disappears from gastric fluid in the postpartum period and is not found in adult gastric fluid. (75)

Gastric proteolysis depends on the proteases present as well as the gastric pH, because pH influences enzyme activity. Typically, highly acidic pH causes protein denaturation, rendering the molecule more susceptible to protease cleavage. Because of their low acid production and the milk's buffering capacity, newborns maintain their postprandial gastric contents at near neutral pH, preventing protein denaturing. (76) Premature infants have a gastric pH of 5 to 7 for up to 1 hour after feeding, decreasing 3 hours after a feeding to a pH of 3 to 3.5. (50) The only human milk proteases with the potential to hydrolyze milk proteins at that pH are cathepsin D and plasmin. (77) These proteases seem to contribute very little to gastric protein digestion.

Duodenal Protein Digestion. Proteins present in human milk, human milk fortifiers, preterm formulas, and whey

protein concentrates are digested in vitro by duodenal juice from healthy preterm infants. (78) Casein is degraded most rapidly, and whey proteins more slowly. (78) Bovine whey proteins in human milk fortifiers and in preterm formulas are relatively slowly digested in vitro by normal duodenal juice. (78)

Luminal Proteases. Key luminal proteases involved in adult intestinal proteolysis (trypsin, chymotrypsin, elastase, enterokinase, and carboxypeptidase B) are present in both term and premature infants, but their concentrations and activities are much lower than those in adults. (79)

Enterokinase (which is responsible for activation of trypsin) has been detected in the duodenal mucosa of infants at 24 to 26 weeks of gestation (80) and it is present and active at birth in both term and premature infants. However, enterokinase activity was only 6% and 20% of that of older children in premature infants and term infants, respectively, (80) and trypsin concentration in the duodenum of premature infants is lower than in term infants. (81) At birth, both groups had lower trypsin activities than did adults, (40) but reached normal levels by 1 month of age. (40)(53)

Chymotrypsin and carboxypeptidase B are present in similar concentration and activity in duodenal fluids of both term and preterm infants at birth and at 30 days of age but lower than those found in older children and adults. (53)

In summary, even though major luminal proteases are present at birth and have similar activity in premature and term infants, particularly by 30 days after birth, lower enterokinase activity in the first several weeks may limit protein digestion in premature infants. (79)

Brush Border Peptidases. Once peptide fragments reach the brush border of the intestinal lining, a large variety of brush border peptidases such as di- and tripeptidases continue their breakdown. (82) Substantial quantities of brush border proteases, including γ-glutamyltranspeptidase, oligoaminopeptidase, dipeptidylaminopeptidase IV and carboxypeptidase, are present by 22 weeks of gestation, and some as early as 10 weeks of gestation. (82) Some enzymes have concentrations similar to those seen in older children and adults. The role of brush border peptidases early in fetal life is unclear but may contribute to extraction of amino acids from swallowed amniotic fluid, known to provide about 15% of fetal protein accretion. (83)

Bacterial Proteases. The bacteria of the intestinal microbiota also produce proteases and contribute to the digestion of dietary proteins. The resulting amino acids seem to be metabolized rapidly by the bacteria. Various human intestinal bacteria can break down protein, including *Bacteroides* species, *Propionibacterium* species, and some members of *Streptococcus*, *Clostridium*, *Bacillus*, and *Staphylococcus*. (84) These proteins are first broken into peptides and then into volatile fatty acids, ammonia, dicarboxylic acids, and various phenolic compounds. (85) A wide variety of anaerobes can ferment amino acids.

GASTROINTESTINAL MOTILITY

In addition to having the appropriate digestive and absorption machinery, preterm infants have other requirements to be able to successfully tolerate enteral nutrition including an adequate absorptive surface area and adequate gastrointestinal motility. Unless nutrients are able to pass from the stomach to more distal areas in the small intestine where they are absorbed, feeding will be unsuccessful. Anecdotally, most clinicians are able to recall very preterm infants (especially those of gestational age <24 weeks) whose feeding advancements were delayed by large gastric residuals (if they were being assessed), frequent emesis, or infrequent stools and abdominal distention.

The topic of gastrointestinal motility has been reviewed previously and will not be repeated in depth here. (86) Briefly, the intestinal motor pattern of the adult undergoes 5 phases after enteral feedings are given. This is known as the interdigestive migrating motor complex (IMMC).

Phase I: The motor activity of the gut is suppressed and little motor activity occurs.

Phase II: Single or multiple contractions develop at various levels of the gut, with limited coordination, and limited propagation along the gastrointestinal tract.

Phase III: Sustained contractions lasting up to 10 minutes develop and are propagated down the gastrointestinal tract in a coordinated manner.

Phase IV: Contractions once again become more random, and then quiescent.

During feedings, there are widespread uncoordinated contractions of the gut intended to mix the ingested food with digestive secretions and enzymes. (86)

The development of the IMMC is controlled by the cells of Cajal, stimulated in part by motilin. The cells of Cajal are typically present by 20 to 22 weeks' gestation and preterm infants can show evidence of gastrointestinal motility as early as 24 weeks' gestation, but a mature pattern of IMMC is rarely seen before 34 to 36 weeks. (86) The proportion of infants with a mature IMMC gradually increases with

gestational age, but up to 10% of term infants fail to show a mature IMMC at birth. (86)

Motilin levels in preterm infants are similar to those seen in adults, but they fail to cycle in the way those of adults do. (86) This may, in part, be responsible for the delayed onset of mature IMMC in preterm infants. Enteral feedings have been shown to increase the rate of maturity of gastrointestinal motor function, (86) which is another reason why enteral feedings should be encouraged.

Many of the anatomic requirements for gastrointestinal motor activity are present before 20 weeks of gestation, including the circular and longitudinal muscle layers, the myenteric plexus, the submucosal plexus, and mature neuroblasts.

SUMMARY AND CONCLUSIONS

Macronutrient absorption by the preterm infant is generally good, though fat absorption is less than that seen in the adult. Many digestive enzymes and transport systems are present in the preterm infant or can be induced by exposure to substrates.

Exocrine pancreatic function is generally poor in preterm infants. Levels of pancreatic α-amylase and pancreatic lipase, for example, are low in preterm infants. However, secondary mechanisms largely compensate for this deficiency.

The preterm infant has several important additional absorptive resources. The first source is the wide range of digestive enzymes present in human milk that may counterbalance poor pancreatic function. Examples include BSSL (lipid digestion) and proteases (protein digestion). The second source is the microbiome of the colon (including *Bifidobacterium*) that may assist in digestion of nutrients. The best example of this is the fermentation of HMOs into short-chain fatty acids, but it is also possible that colonic bacteria have a role in protein, amino acid, and fat digestion and absorption as well. Finally, the preterm infant may also have digestive enzymes that are not present in the adult (eg, BSSL, PLRP2, novel gastric proteases).

The preterm infant seems well-suited to continuing enteral feedings after birth, and the literature provides no reason for preferring parenteral over enteral feedings, and many reasons for preferring enteral feedings.

Difficulties with establishing enteral feedings are far more likely due to delayed gut motility rather than problems with nutrient digestion and absorption. Therefore, the primary route of nutrition for preterm infants should be the enteral route, unless clearly contraindicated.

American Board of Pediatrics Neonatal-Perinatal Content Specifications

- Know the physiology of protein/amino acid digestion (absorption and metabolism) in newborn infants.
- Know the physiology of fat digestion, absorption, and metabolism in newborn infants.
- Know the physiology of carbohydrate digestion, absorption, and metabolism in newborn infants.
- Know the advantages and disadvantages of the use of donor human milk.

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