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REVIEW ARTICLE

Development of Gastrointestinal Function: Risk Factors for Necrotizing Enterocolitis

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The intestinal tract of the fetus matures rapidly in the third trimester of the pregnancy. The premature infant has decreased intestinal motility, limited digestion, absorption and excretion, and poor intestinal barrier defense. These limitations place the infant at high risk for acute intestinal injury, necrotizing enterocolitis. This article reviews the development of the gastrointestinal tract in the fetus, the barriers to feeding the high risk, premature infant, and the most serious intestinal disease, necrotizing enterocolitis.

KEYWORDS: gastrointestinal tract, necrotizing enterocolitis, prematurity

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INTRODUCTION

The fetus must develop four major intestinal functions to allow adequate nutrition and growth after birth. These are motility, digestion and absorption, excretion, and defense against noxious chemicals and microorganisms.[1-4] Most of these functions are relatively mature once the fetus has reached 34 weeks gestation. Prior to 34 weeks, motility, digestion, and defense are poorer in smaller and more immature babies. The immature intestine is susceptible to injury especially after the initiation of feedings. Necrotizing enterocolitis (NEC) is the most devastating enteric illness, especially for the very small premature infant. This manuscript provides an overview of gastrointestinal tract development and maturation. The risk factors for NEC, an overview of the varied pathophysiology, treatment of NEC and possible preventive measures are discussed.

GASTROINTESTINAL DEVELOPMENT

Motility

Gastrointestinal motility is defined in multiple stages including sucking, swallowing, gastric emptying, intestinal transit, and defecation.[2] The swallowing reflex first appears at 16 weeks gestation and the sucking reflex appears at 24 weeks gestation. However, the suck-to-swallow coordination of those two reflexes is not mature until approximately 34 weeks gestational age. Therefore, virtually all preterm infants require feedings by tubes inserted into the stomach until they can nipple.[5,6]

ABBREVIATIONS: CCK, Cholecystokinin; NEC, necrotizing enterocolitis; PAF, platelet activating factor

Gastric emptying begins at 20 weeks gestation and by 28 weeks gestation the antrum can exert a pressure of 25% that of a full-term infant. There is a thin stomach muscular layer before 34 weeks gestation. Therefore, there is low esophageal sphincter pressure and a significant tendency in preterm infants to exhibit gastroesophageal reflux.[7] Gastric emptying is delayed in the first 12 hours in both term and preterm infants. Larger babies, in general, have faster emptying rates. Body position, especially right side down, appears to have little effect on gastric emptying.

The composition of the feeding affects gastric emptying. Specifically, increased caloric density delays gastric emptying.[8] Although monosaccharides (e.g., glucose) empty from the stomach readily, the disaccharides, especially lactose, de-

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lay gastric emptying. Protein digestion is initiated in the stomach and the casein fraction of milk, be it breast milk or the protein in cow's milk based formula, prolongs gastric emptying.[7,9] Complex fats, especially triglycerides with long chain fatty acids, also delay gastric emptying.

In the small intestine, peristalsis can first be detected at 16 weeks gestation.[10] However, a mature motor pattern of peristalsis is not found until 34 weeks gestation.[11] Intestinal transit time from mouth to colon is approximately 9 hours in a 32 week gestation infant, whereas a 40 week gestation infant requires only 4 hours for food to reach the colon. The time from the mouth to the anus at 40 weeks gestation is approximately 13 hours, an additional 9 hours beyond the time it requires for food to reach the colon.

Prolonged transit time in the small intestine of preterm infants is due to multiple factors: an immature muscular layer, an immature enteric nervous system, poor coordination of peristaltic waves and increased antiperistaltic waves.[12] In addition, the decreased secretion of hormones, especially vasoactive intestinal polypeptide, CCK, and gastrin also delay intestinal motility.[13]

Intestinal motility appears to be improved in preterm infants whose mothers have received antenatal corticosteroids.[14] Although intended for accelerating lung maturation, this positive effect has been observed within the intestine.[15] Early hypocaloric feedings maintain developing intestine motility.[16-18] Prokinetic agents, such as cisapride and erythromycin, are ineffective in improving the intestinal motility of preterm infants due to the immaturity of the enteric nervous system and the muscularis layer. In the term infant and in the majority of preterm infants, theophylline, caffeine and prenatal magnesium given to the mother for hypertension or to inhibit labor, all delay gastrointestinal motility.[11]

Digestion and Absorption

All the vitamins and minerals are reasonably well-absorbed from the intestinal tract of both term and preterm infants. The absorption of other major nutrients, carbohydrates, proteins and fats, has various developmental sequences.[19-21] In the typical feedings of a full-term infant, approximately 50% of the calories are derived from fat, 15% from protein, and 35% from carbohydrate.[21]

Monosaccharide absorption can first be detected at 12 to 14 weeks gestation. Disaccharidases can be detected as early as 10 weeks gestation but the enzyme lactase is in relatively small quantity until 34 weeks of gestational age.[8] Therefore, the vast majority of preterm infants have some degree of lactose malabsorption. Lactose has not been removed from the formulas for preterm infants because it is important for absorption of calcium. Eighty-five to ninety percent of the calcium in the full-term infant accumulates during the last 12 weeks of gestation.[21]

Any carbohydrate that is not digested and absorbed becomes available to the bacteria colonizing the colon.[22] These bacteria will then readily ferment the carbohydrate yielding several gases, hydrogen and carbon dioxide and a series of short chain fatty acids to include lactic, acetic, butyric, propionic, and formic acids depending on the specific organism. Consequently the preterm infant develops gaseous abdominal distention and, due to the irritation of the short chain fatty acids, some loose watery stools.[22]

Protein digestion and absorption can first be detected with proteolytic enzymes developed between 20 and 26 weeks of gestation.[8,23] The amniotic fluid proteins swallowed by the preterm infant are very similar to the whey proteins found in human milk contributing to the evolution of these enzymes. Amino acid transport and macromolecule absorption can be detected as early as 20 weeks of gestation.[10]

Lipid digestion and absorption is most problematic for the preterm infant. Preterm infants have great difficulty digesting fats, not only due to decreased pancreatic lipase and colipase, but also to the low bile acid concentration found in the duodenum and jejunum.[3,24] There is decreased secretory response and once absorbed, the lymphatic system is immature and the fats are poorly handled. Table 1 illustrates the various lipases that are involved in lipid digestion. Even full-term infants have some compromised ability to digest fats. To meet the needs of preterm infants, medium chain triglycerides are added to formula. These triglycerides can be digested by lingual and gastric lipase. They are active at a low pH and do not require the action of bile salts.

Defense

Intestinal defense includes defense via mechanical, chemical, immune and microorganisms.[24,25] The best mechanical defense is motility. Any substance that may irritate the intestine can be readily

Table 1. Characterization of Human Lipases

Lipase	Gestational Age*	Activity at Term Gestation†
Lingual	30	> 100
Gastric	25	> 100
Pancreatic	20	10
Colipase (Pancreatic)	Unknown	Unknown
Bile Acids	22	50

**weeks at which activity first detected*
†% of adult activity

pushed through. The direct barrier defense includes the production of mucous and the glycoproteins that can trap the offending agent.[26] A tight epithelial barrier junction is the ultimate mechanical defense. With sufficient inflammation the epithelium can desquamate and the entire mucosal surface can be replaced over the course of three to four days.

Defense against microorganisms includes a chemical defense beginning with gastric acidity.[7] The low pH in the stomach not only kills bacteria, but also inhibits the growth of many organisms. In the small intestine, bile salts inhibit the growth of many Gram-negative rods. The enzymes for digestion of proteins also adversely affect bacterial survival.

Beyond the mechanical and chemical defenses within the intestinal tract, there are a series of cellular elements that can respond to microorganisms.[24,27] Resident mast cells have the most potent neutrophil and eosinophil chemotactic factors found anywhere in the body. Neutrophils and eosinophils are readily recruited along with lymphocytes and macrophages to handle invading organisms. In addition, there is some redundancy in the portal system and the reticuloendothelial system of the liver that captures organisms that have penetrated the intestinal barrier.

At birth, the intestinal content is sterile. Within the intestine, bacteria compete for the same resources. Selective colonization of the intestine by organisms that produce less toxic substances and are less likely to invade is a selective advantage.[28] For example, breast milk promotes the growth of lactobacilli and bifidobacteria. Although there are many immune factors in breast milk that assist in maintaining colonization, lactoferrin, an iron binding protein, both improves the absorption of iron as well as sequesters it from organisms such as *Klebsiella* and *Escherichia coli* that are more pathogenic.

Table 2 provides an overview of the advantages and disadvantages of both enteral and parenteral nutrition.[29] Although the goal of nutrition is to match intrauterine growth, due to high metabolic activity and limited ability of the liver, kidneys and lungs of the preterm infant to excrete waste products, this is rarely achieved. It is difficult to match ideal body composition, in that the placenta preferentially provides nutrients and clears waste products for maximal fetal growth.

Table 2. Advantages and Disadvantages of Enteral and Parenteral nutrition

	Advantages	Disadvantages
Enteral	Mucosal growth	GE reflux
	Motility	Apnea
	Intestinal Hormone Secretion	Aspiration
	Peptide release	Antigens/Allergens
	Disaccharidase activity	Malabsorption
Parenteral	Intake known	Intestinal atrophy (decreased DNA, Protein)
	Fluids	Cholestasis
	Calories	Catheter Sepsis
	Nutrients	Necrotizing Enterocolitis
		IV Infiltration
		Expense

NECROTIZING ENTEROCOLITIS

Although many difficulties are associated with feeding the preterm infant, the most serious of these is necrotizing enterocolitis (NEC). NEC is the most common cause of death of premature babies who survive early onset respiratory distress.[30,31] The incidence is 5% to 30% of infants born weighing less than 1500 grams.[32,33]

Historically, it was believed that NEC was caused by intestinal ischemia followed by reperfusion injury.[34] This is no longer believed to be a sufficient explanation. Well over 90% of preterm infants with NEC do not have an apparent hypoxic or ischemic insult.[35] These infants are resuscitated well. In the neonatal intensive care nursery, the heart rate, respiratory effort, blood pressure and oxygenation of premature infants are monitored frequently. Preterm infants less than 34 weeks gestation are at greatest risk for NEC with highest risk at lower gestational age and birth weight.[36,37] The majority of the preterm infants who develop NEC have been enterally fed, something not pos-

sible in infants whose intestines have suffered an ischemic insult.[38] However, one quarter of all cases of NEC occur beyond one month after birth, primarily in extremely low birth weight babies whose corrected age is less than 34 weeks postconception.[33]

Currently there are three logical categories for the etiology of NEC: 1) infection, 2) ischemic reperfusion injury, and 3) intestinal inflammation. Of these, inflammation of the bowel is by far the most common, accounting for 85% to 90% of all cases.[22,27] There are several clinical risk factors that have been identified. These include decreased stools following increased feedings. There is often evidence of carbohydrate intolerance with reducing sugars found in the stool and positive breath hydrogen, a derivative of intestinal fermentation of undigested carbohydrate.[27] Eosinophilia (greater than 5% eosinophils) suggests involvement of the intestinal mast cells, which attract eosinophils to help defend against bacterial invasion.

Signs and Symptoms of NEC

Clinical signs specific to the intestinal tract include abdominal distension, gastric retention or vomiting, blood and hematochezia.[37] Many other non-specific findings imply systemic disease. These include temperature instability, lethargy, apnea, metabolic acidosis, thrombocytopenia, neutropenia, and coagulopathy. A decline in stool frequency as feedings are increased is a common finding.[37]

Radiographic criteria for NEC includes pneumatosis intestinalis, gas within the bowel wall.[27,39] Several studies have shown these gases to be a mixture of carbon dioxide and hydrogen, both derived from bacterial fermentation of carbohydrate. In neonates with early onset of disease in the first several days after birth, pneumatosis is seldom found primarily as the infants have not been fed and the intestinal tract is not yet colonized. In the preterm infant with NEC, hepatic portal venous gas may be seen. Bowel wall edema, indicating inflammation, is common. Free intraperitoneal air or an ileus (sentinel loop), are indications for surgical intervention.

Acute Treatment

The treatment of the infant with NEC is symptomatic and supportive. If feedings have been initiated, they are stopped and the gastrointestinal tract is decompressed using an oral or nasal gastric tube. Once cultures are obtained, an empiric course of parenteral antibiotics is initiated. The enteric flora are varied and the choice of antibiotics must consider aerobes (Gram negative and Gram positive bacteria) as well as anaerobes. If an organism is isolated from the blood culture, the antibiotic regimen may be revised. Serial abdominal x-rays, complete blood counts, platelet counts and blood gases are necessary to monitor progression of intestinal disease. Severe disease includes intestinal perforation, bleeding and metabolic acidosis. Aggressive supportive care includes compensation for respiratory insufficiency caused by abdominal distension, which limits diaphragm function. Acidosis is corrected and hypotension due to volume loss is addressed by administration of fluids. Surgical intervention is necessary if there is an intestinal perforation or deterioration with aggressive medical support.

During the recovery phase, common complications include strictures and adhesions of the intestine. These occur primarily in the distal ileum and the proximal colon. Pericolonic abscesses and enterocolic fistulae have been reported. Some of the infants require surgical resection of the intestine leading to malabsorption and malnutrition and postsurgical short bowel syndrome.[40] These infants receive parenteral nutrition which has attendant complications of infection and direct metabolic effects on hepatic function.

Infection

Upon examining the various etiologies of necrotizing enterocolitis, interesting patterns develop. No consistent pathogen has been associated with necrotizing enterocolitis.[28,41] Toxins are rarely found and organisms commonly need a cofactor to directly affect the intestine.[42] However, a series of bacteria and viruses have been reported including *E coli, Klebsiella*, Coronavirus and Rotavirus.[43] Each of these organisms has the potential to initiate serious disease in children and adults and may on occasion be associated as a direct infectious cause of intestinal damage in preterm infants.

Ischemia-Reperfusion

Although ischemic and reperfusion injury were thought to be the primary pathogenesis of NEC, current evidence would relegate it to be a cause of relatively few cases.[27,34] These are associated with perinatal asphyxia, severe respiratory distress and

Table 3. Clinical Correlates of Necrotizing Enterocolitis and Feeding

Clinical Variables	Unfed Infant	Fed Infant
Gestational Age	Preterm to term	< 34 wks, < 1500 gm
Perinatal Asphyxia	Common	Less likely
Bacterial Colonization	Little	Well established
Onset of NEC	< 7 days after birth	7 days–3 mo (25% > 1 mo)
Location of Necrosis	Small bowel; single site	Ileum, colon; Multiple sites common
Organ Systems involved	Multiple (brain, kidneys, heart, liver)	Intestine first then systemic

hypoxia, hypotension due to severe volume loss and polycythemia. A double volume exchange transfusion performed due to severe hyperbilirubinemia has the potential to decrease intestinal blood flow. Patent ductus arteriosis and umbilical artery catheterization could interfere with aortic flow. These may account for some cases of NEC within the first few days after birth.

Intestinal Inflammation

Table 3 compares the clinical correlates of NEC of the infant who has not been fed versus the infant who has been fed.[44,45] In a fed infant, perinatal asphyxia as the cause of NEC is unlikely. Bacterial colonization is well established by four to five days. The onset of NEC is from seven days to three months. The first location of necrosis is in the ileum and colon. This is where the undigested carbohydrate in a preterm infant first reaches significant numbers of bacteria. In these children, the intestine is the first organ affected and then other organs are affected as the disease progresses. In contrast, in the infant who has not been fed, multiple organs are affected including the brain and kidneys. If the intestine is affected, it is usually a single, discreet perforation site in the small intestine.

Multiple agents can disrupt the mucosal barrier of the intestine. The most important of these are the short chain organic acids, derived in the process of fermentation of undigested dietary carbohydrate.[46] These irritants initiate an inflammatory process at the level of the mucosal surface by disrupting epithelial cells and stimulating the underlying mast cells into action. Systemic neutrophils, eosinophils and macrophages are recruited. A series of mediators are released by these cells. These include the pro-inflammatory leukotrienes, thromboxane, platelet activating factor, nitric oxide, complement, endothelin-1, interleukin-6, oxygen radicals, tumor necrosis factor and many others.[47,48] The results are a rapidly evolving inflammatory process which in the preterm infant results in compromised microcirculation and intestinal damage.[49,50]

Platelet activating factor (PAF) may play a central role in this process.[47,50] The evidence in humans is limited. Animal models initiating PAF release commonly induce intestinal damage after a severe hypothermic and hypoxic stress to a term gestation newborn animal. Cold stress and hypoxia are vigorously addressed and minimized in the care of preterm infants.

Therapeutic approaches, which appear to be ineffective both in animals and to a limited extent in humans, include vasodilators, neutrophil antagonists, and mast cell stabilizers. Antagonists of PAF minimize damage in rodent models of NEC. There is no PAF antagonist available for use in human newborns.

More current concepts looking at the prevention of NEC suggest that clinicians need to modify feeding practices to include continuous slow volume "gut priming" feeding even during an acute illness. Feedings should be increased cautiously looking for an appropriate pattern of intestinal motility and defecation.[27,51,52]

In addition, improving the digestibility and absorption of formulas made for premature infants would provide less substrate to the organisms of the lower intestinal tract. Breast milk factors which may be of some benefit and not currently available in formula include lactoferrin (an iron binder), immunoglobulins, and prostaglandins which promote mucosal integrity.[53] The mucosal barrier may be enhanced by antenatal steroids. However, postnatal administration of steroids, especially in pharmacologic doses to improve pulmonary function, impacts the intestinal mucosa adversely. Epidermal growth factors, insulin-like growth factors, and prostaglandin E analogues all given orally, could enhance the mucosal barrier.

Although improving motility would appear to be desirable, due to the immaturity of the enteric nervous system, it is highly unlikely the preterm infant would be responsive to any agent effective in older children or adults.[11,54] Antagonists of in-

flammatory mediators are difficult to use in adults and have shown significant toxicity. They could not be used in preterm infants in whom suppressive inflammation could result in overwhelming infection, especially bacterial infection.[27] There is some hope that free radical scavengers such as super oxide dismutase could be used to limit the extent of intestinal necrosis once inflammation of the intestine has been identified.

CONCLUSION

In summary, the preterm infant is uniquely susceptible to adverse events within the developing intestine. The combination of poor intestinal motility, underdeveloped intestinal motility, and poor mucosal barrier function combine to increase the risk of severe intestinal inflammation and necrosis.

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