MINIREVIEW

Gut Responses to Enteral Nutrition in Preterm Infants and Animals

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Preterm birth is associated with immature digestive function that may require the use of total parenteral nutrition and special oral feeding regimens. Little is known about the responses to oral food in the preterm neonate and how enteral nutrients affect the immature gastrointestinal tract (GIT). *In vivo* studies are difficult to perform in laboratory rodents because of their small body size and that of immature organs at birth, and this makes the large farm animals (e.g., pigs, cattle, sheep) more attractive models in this field. In these species, preterm delivery at 88%–95% gestation is associated clinical complications and degrees of GIT immaturity similar to those in infants born at 70%–90% gestation. Studies in both animals and infants indicate that the immature GIT responds to the first enteral food with rapid increases in gut mass and surface area, blood flow, motility, digestive capacity, and nutrient absorption. To a large extent, the enteral food responses are birth independent, and can be elicited also *in utero*, at least during late gestation. Nevertheless, preterm neonates show compromised GIT structure, function, and immunology, particularly when delivered by caesarean section and fed diets other than mother's milk. Formula-fed preterm infants are thus at increased risk of developing diseases such as necrotizing enterocolitis, unless special care is taken to avoid excessive nutrient fermentation and bacterial overgrowth. The extent to which results obtained in preterm animals (most notably the pig) can be used to reflect similar conditions in preterm infants is discussed. Exp Biol Med 231:1695–1711, 2006

Key words: animal model; gastrointestinal; fetus; newborn; milk; necrotizing enterocolitis

Introduction

Birth requires that the body rapidly adapts to meet a major change in the mode of nutrition. Before birth, the fetus receives a constant flow of maternal elemental nutrients *via* the placenta. After birth, the neonate must adjust to a variable uptake of nutrients from milk, which are available only after specialized digestive processes have occurred in the gastrointestinal tract (GIT). The final maturation of the GIT for enteral nutrient intake occurs shortly before or after term. Thus, preterm birth is associated with immature digestion and absorption, which, coupled with other complications (respiratory, circulatory, excretory, metabolic), may lead to problems in accomplishing a smooth transition from parenteral to enteral nutrition in the perinatal period.

Preterm delivery in man, defined as birth before 90% gestation, is a significant global health problem, with considerable variation within and across populations, ranging from 5%–10% of births in industrialized countries to as high as 25% of births in areas of Asia and Africa (1–6). In industrialized countries, preterm birth is probably the most important risk factor for neonatal survival and health. Epidemiologic studies indicate that preterm birth affects development and health mainly in the short term, whereas another related and partly overlapping complication, intrauterine growth retardation, tends to have life-long health consequences (5). Much research in this area has been done to investigate lung and brain maturation, and the respiratory and cerebral defects associated with preterm birth. Less is known about gut maturation and the GIT responses to the first enteral food, despite the fact that the clinical aspects of feeding preterm infants is well documented (7–12). The importance of this area is underlined by the frequent GIT diseases in the neonatal period of both preterm infants and animals. The most serious of these, necrotizing enterocolitis

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Table 1. Parameters of GIT Function in Preterm Infants and Preterm Domestic Animals at a Comparable Stage of Maturity[a]

Gut function	Man and primates (70%–90% gestation)		Domestic animals[b] (88%–95% gestation)	
Gut structure, metabolism				
Mass, surface area, proliferation	– ↑	(48, 52)	– ↑	(31, 32, 39, 40, 50, 51, 58, 66)
Blood flow, nutrient metabolism	– ↑	(54, 56, 57, 186, 187)	– (↑)	(57, 72–74)
Digestive enzymes/secretions				
Lactase-phloridzin hydrolase	– ↑	(16, 45, 52, 53, 77, 85, 86)	– ↓	(32, 40, 58, 60, 66, 80)
Other disaccharidases/peptidases	(–)	(16, 76, 77, 79)	(–) ↑	(32, 40, 58, 60, 66, 80, 131)
Gastric/pancreatic hydrolases	(–)	(16, 45, 81–84)	– (↑)	(32, 58, 60, 93, 96, 126, 127)
Nutrient absorption				
Nutrient absorption	(–) ↑	(53, 85, 98, 99)	(–) ↓	(32, 51, 58, 100, 101, 107)
Macromolecule absorption	+ ↓	(102–104, 192)	– ↓	(32, 36, 38, 40, 107, 109)
Gut motility and regulation				
GIT motility, enteric nerves	– ↑	(46, 54, 112, 113, 116, 120)	– ↑	(58, 59, 121, 122)
Gut regulatory peptides	(–) ↑	(113, 117, 124, 132, 188)	(–) ↑	(24, 118, 126–131, 189, 190)
Gut microbiology and defense				
Bacterial diversity, fermentation	– (↑)	(90, 134, 136, 140, 141, 150)	– ↑	(60, 92)
Inflammatory reactions	+ ↑	(137, 158, 160)	(+) ↑	(40, 59, 60, 69, 92, 169)
Gut permeability, integrity	+ (↓)	(53, 104, 105, 148, 191)	+ ↓	(101, 107)

[a] "–/+" indicates reduced/increased value following preterm birth, relative to term birth; "↓/↑" indicates whether a given function in preterm neonates decreases or increases in response to enteral food. Parentheses denote inconsistent results. Reference numbers are given in parentheses.
[b] Sheep, cattle, pigs.

(NEC), is closely linked with both preterm birth and enteral feeding.

The lack of information about GIT development in preterm neonates, and the response to enteral food intake, arises partly from difficulties in performing well-controlled studies on this vulnerable population of infants. In addition, few studies have been performed with preterm animals, despite the fact that immature organ function at birth may play a major role for the large perinatal mortality (5%–20%) in the large domestic species (e.g., pig, sheep, cattle, horse) (13–15). In these animals, birth takes place just after the completion of some final maturational changes in essential organ systems, which makes domestic animals relatively susceptiple to prematurity at birth, even when birth occurs close to term. On the other hand, direct comparison between preterm infants and term animals is complicated by the differences in organ maturation at birth and by the endocrine, metabolic, and circulatory conditions specifically related to preterm birth. Recent studies in fetal and preterm newborn farm animals (piglets, calves, lambs) may help to delineate how gestational age at birth, the mode of delivery (caesarean or vaginal), and diet influence the response of the immature GIT to enteral food. In this review, new results from domestic animals are coupled with current literature from human infants to gain a better understanding of the interactions between GIT maturity and response to enteral food intake immediately before or after preterm delivery. The discussion focuses on the stage of development during which neonates generally survive without intensive, long-term clinical care, yet demonstrate significant organ immaturity. By these criteria, preterm birth takes place at 70%–90% gestation in humans (28–36 weeks gestation), at 88%–95% gestation in the large domestic species (pig: 102–109 days; cattle: 248–268 days; sheep: 130–140 days), and at 94%–97% gestation in rats (1 day before term at 22 days).

The first aim is to evaluate fetal age as a determinant of GIT maturation in preterm neonates. Knowledge about the progression of GIT structure and function in the perinatal period is important to validate animal models and to indicate limitations in extending results from newborn animals to preterm infants. The second aim is to show how the preterm GIT responds to enteral nutrition in infants and in some large domestic species. The results from both aims are summarized in Table 1, which shows some selected indices of mucosal structure, digestive function, nutrient absorption, gut motility and microbiology, their degree of immaturity at preterm birth, and their responses to enteral nutrition given just after birth. Following some introductory remarks to comparative research in the field of pediatric gastroenterology and nutrition, the review follows the structure outlined in Table 1.

Species-Dependent Pattern of GIT Maturation

Whereas primates are generally viable from about 70% of the length of gestation, species such as pigs, calves, sheep, dogs, and rats are poorly viable until 90% gestation, even with intensive neonatal care. This developmental pattern is reflected in the ontogeny of the GIT. In humans, the functional maturation of the GIT (e.g., digestive enzymes and absorptive function) starts relatively early (i.e., during the first part of gestation), and progresses

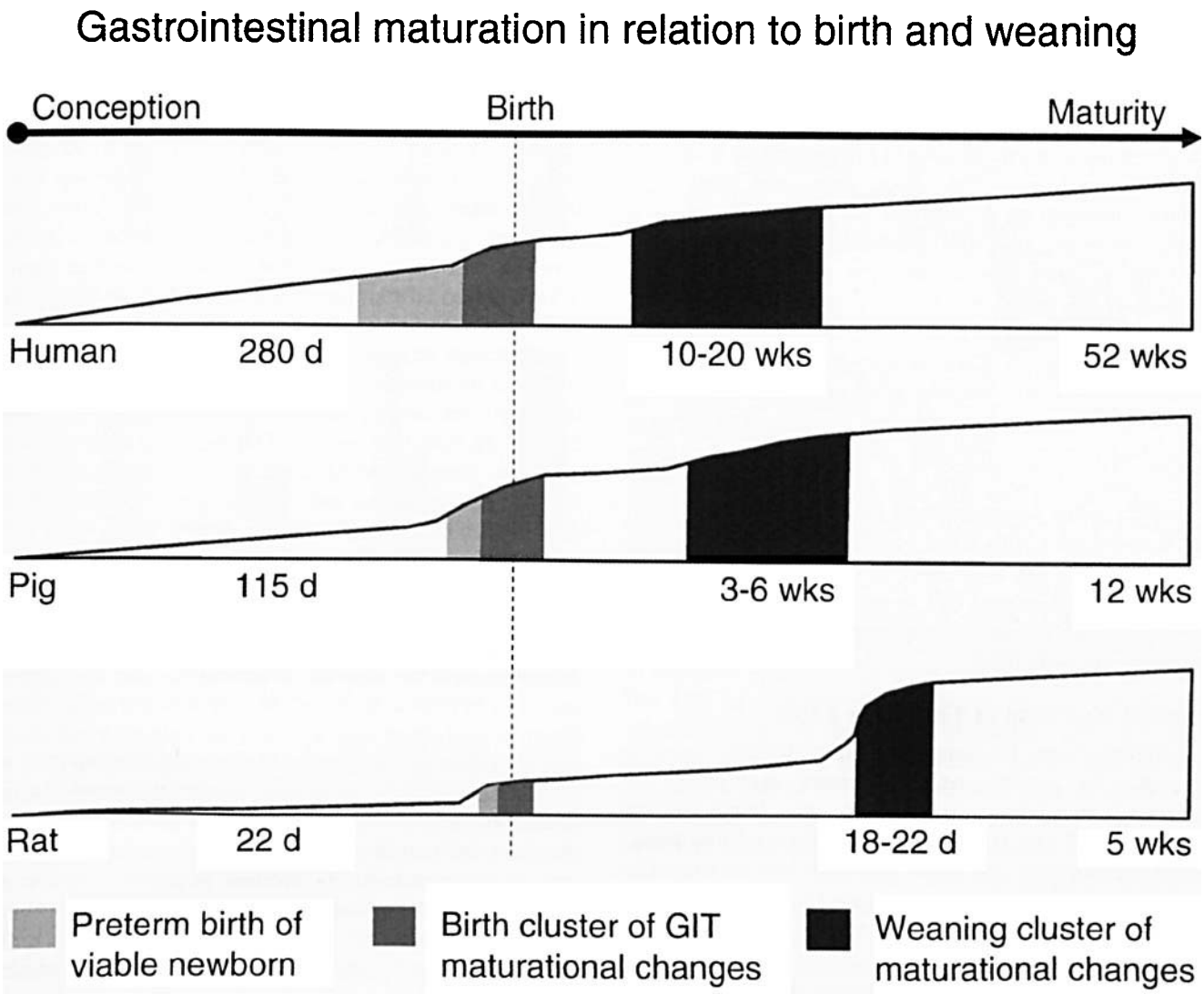

Figure 1. The timing of GIT maturation in three different groups of mammalian species. In humans and other primates, GIT development is slow and maturation starts early (in fetal life). In most small rodents and carnivorous species, the developmental changes occur relatively quickly and late (postnatally around weaning). GIT maturation in large domestic animals (pigs, sheep, cattle) is intermediate (i.e., maturation is rapid during the period from shortly before birth to shortly after weaning). Around weaning (black areas) and birth (dark grey areas), maturation is particularly rapid, resulting in a weaning cluster and a birth cluster of maturational changes. Birth of viable preterm neonates occurs over a wider range of gestational ages in humans compared with domestic animals like the pig (light grey areas).

relatively slowly (Fig. 1). At term birth, the infant GIT is thus sufficiently mature to digest significant amounts of nonmilk carbohydrates and proteins in addition to the nutrients contained in milk. From a "gut point of view," humans and primate species have a "precocious" mode of development. In contrast, most small rodents (e.g., rats and mice) and most carnivorous species (e.g., mink and cats) have very immature GITs at the time of birth, reflecting an "altricial" mode of GIT development. In these species, adult diets are poorly tolerated until relatively late into postnatal life, and adult-type GIT functions develop mainly postnatally and rapidly. In the pig, sheep, cattle, and horse, the timing and the rate of GIT maturation are intermediate, and major developmental events in the gut take place both pre- and postnatally (Fig. 1). The scientific evidence supporting these general contentions is derived from studies on GIT ontogeny during the last decades (16–24).

In addition to the gradual maturational process determined by the postconceptual age in each species, GIT maturation is particularly rapid at birth (a birth cluster of maturational changes) and at weaning (a weaning cluster of maturational changes). At these time points, the dietary habits change dramatically, and are accompanied by marked changes in the endocrinology, microbiology, and immunity of the GIT. A number of earlier studies have attempted to delineate the influences of external determinants of maturation at these two time-points (i.e., how endocrine factors like cortisol, or changes in diet at birth and weaning, modify the intrinsic GIT developmental program [16, 22–26]). From this information, it is evident that both diet and endocrine

factors play important roles, although the effects are both time- and species-specific. Hence, the birth cluster of maturational changes is more pronounced in species that are relatively mature at birth (e.g., large domestic species, guinea pigs, and nonhuman primates), whereas the weaning cluster of maturational changes is most important in altricial animal species (e.g., rats, mice, dogs, and cats). Less detailed information is available for human infants. It is likely, however, that GIT maturation around birth and weaning is more gradual and less dramatic in infants, because of the earlier and slower development of the GIT relative to that in the large domestic species and in altricial rodents (see Fig. 1). Little is known about the extent to which the birth and weaning clusters of maturation exist following preterm birth and preterm weaning. If an infant or an animal is born prematurely, the GIT has to adapt even more rapidly to cope with the challenge of oral foods, especially when continued total parenteral nutrition (TPN) for a period after birth is not possible. This GIT adaptation in preterm neonates may differ in timing and pattern from the normal birth–related GIT maturation. It may also differ markedly from the normal responses of the fetus to enteral intake of amniotic fluid.

Enteral Nutrition of Fetuses *In Utero*

Before birth, the fetus, including its GIT, receives nutrients for growth and development mainly *via* the placental circulation, passing into the fetus *via* the hepatic–portal vein (i.e., parenteral nutrition). From midgestation, however, the fetus also receives considerable enteral nutrition by swallowing large amounts of amniotic fluid (about 20% of body weight per day during late gestation). Although the nutrient content is relatively low (~1% protein), nutrients in swallowed amniotic fluid has been estimated to contribute 10%–20% of the fetal energy demands (27). Studies on enteral nutrition in fetal animals *in utero* may help to differentiate between the factors of birth and ontogenetic immaturity. If the fetal GIT responds differently to enteral nutrients than the preterm newborn intestine, factors other than ontogenetic immaturity appear to determine enteral food responses. On the other hand, a series of physiologic conditions differ markedly between fetuses and preterm neonates (e.g., blood oxygenation, GIT blood flow, body temperature, parenteral nutrition, and microflora exposure). These factors, and the extensive surgical manipulation often required to do fetal studies, must be kept in mind when comparing results from *in utero* and *ex utero* studies, even at the same postconceptual age.

In large domestic species, major developmental changes take place in the gut following the onset of fetal swallowing (28–30), and amniotic fluid in the GIT lumen is important in maintaining and stimulating mucosal differentiation during the prenatal period. Fetal lambs, in which swallowing of amniotic fluid has been prevented by ligating the esophagus at 90 days of gestation, show both reduced growth and altered differentiation of the intestine by late gestation. Similar effects have been observed in fetal lambs, pigs, and rabbits after short-term esophageal ligation in late gestation (31–33), at which time-point body growth is significantly reduced after just 1–2 weeks of esophageal ligation. When luminal fluid input is restored after esophageal ligation in lambs at 80%–90% gestation (29), or when ligated fetuses are infused with amniotic fluid, milk whey, or colostrum whey (31), GIT growth is partly reversed. Also, in the fetal rabbit, the small intestine grows in response to enteral nutrients given in late gestation (34, 35).

Although amniotic fluid has an important role in the structural development of the small intestine, its effects on the functional development of the GIT is equivocal, and depends on both the specific GIT function and the stage of gestation. Enteral feeding experiments with pig fetuses at 87%–94% of gestation and sheep fetuses at 82%–87% of gestation show increased plasma gastrin levels after 6–7 days of intragastric infusion of amniotic fluid, milk whey, or colostral whey (28). In these studies, the effects of the three fluids on gastrin release were similar, despite large differences in their content of protein and growth factors. However, both the absolute concentrations and the relative increases in plasma gastrin during the infusion period were lower in fetal sheep than in fetal pigs, indicating effects of species and/or gestational age. Fetuses receiving no fluid at all following esophageal ligation showed decreased plasma gastrin levels. Both the lack of luminal nutrients (proteins, peptides) and gastrin-releasing substances in amniotic fluid may be responsible for the decrease in gastrin secretion in such fetuses. In the fetal pig, but not the fetal lamb, fluid infusions decreased gastric fluid pH at preterm birth (28), indicating that such luminal fluids may enhance maturation of immature parietal cells, which is consistent with the findings in fetal rabbits (27).

In fetal piglets, prevention of amniotic fluid intake at 80%–90% gestation results in lowered aminopeptidase activities in the distal small intestine (32). Lack of swallowed fluid has no negative effect on the ability to absorb glucose, amino acids, and dipeptides, and actually increases the activity of lactase in the small intestine (32), probably as a result of an associated rise in cortisol. Likewise, brush border enzyme activities have been little affected in studies on esophageal ligation in fetal lambs at 82%–87% gestation. Protein endocytosis remained low, with or without subsequent infusion of amniotic fluid, growth factor (gastrin releasing peptide), milk whey, or colostrum whey in these animals (36). Luminal fluids also have limited effects on fetal rabbit intestinal lactase at 77%–97% gestation (33), although infusion of glucose and amino acids increased nutrient absorptive capacity (34). The modulating effects of luminal factors on development of the fetal GIT may take longer to become expressed if luminal dietary manipulations are instituted before the immediate prenatal period. Enterocyte turnover is slower in

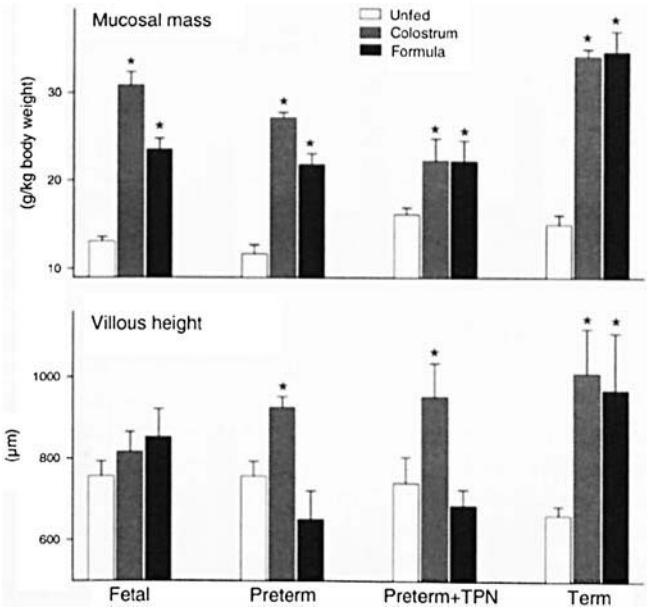

Figure 2. The GIT trophic response to enteral food in fetal and newborn pigs. Bars (mean ± SEM) show small intestinal mucosal mass (Upper panel) and villous height (Lower panel) before and after feeding colostrum or formula (15 ml/kg/3 h) for 1–2 d. Significantly different value in fed pigs vs. unfed pigs ($P < 0.05$). Results are compiled from different enteral feeding experiments in fetuses and neonates (preterm, preterm TPN-fed, and term) (40, 59–61).

the fetal intestine than in the postnatal intestine (30), and molecular mechanisms linking enteral food with mucosal differentiation may be immature. It has not yet been tested whether amniotic fluid has trophic and anti-inflammatory effects on the small intestine of preterm infants, but preliminary trials suggest that amniotic fluid protects against NEC in preterm pigs (37).

The lack of effects of amniotic fluid and other exogenous luminal fluids on the functional characteristics of the immature intestinal enterocyte at 80% gestation contrasts with the results of studies on milk infusions into fetal pigs later in gestation for either 1 week (38, 39) or just 1 day (40). In both of these series of experiments, significant maturational effects of milk infusion were observed for brush border enzymes and the ability to absorb protein macromolecules by endocytosis. We therefore conclude that the trophic and maturational effects of enteral food on the immature GIT do not depend on birth and the associated endocrine (e.g., cortisol increase), metabolic (e.g., placental separation), and environmental (e.g., microbiological) influences. However, a normal GIT response to introduction of oral food may not occur until relatively close to term, concomitant with the time in gestation when the fetus can survive without extensive long-term clinical care. It is conceivable that this conclusion is valid for both human and animal fetuses. The time in gestation when the immature GIT starts to respond normally to enteral food depends on the developmental timing of birth in each species, as indicated in Figure 1.

Mucosal Growth and Metabolism in Preterm Neonates

In term neonates, the GIT has a marked trophic response to the introduction of oral food, forming a key aspect of the adaptation to life and nutrition *ex utero*. A wealth of information on this trophic response has been obtained from pigs (41–44), and it has been generally assumed that a similar GIT tissue response is present in term and preterm human infants. Because limited direct evidence is available from preterm infants, results from suckling animals have been used to support the important clinical concepts of "minimal enteral nutrition" and "trophic feeding" of preterm infants (43, 45, 46). In preterm infants, the intestine is relatively short (47, 48), and may have a reduced absorptive area, consistent with studies in pigs and calves (32, 49–51) (Table 1). In infants, the apparent surface area of the GIT increases with pre- and postnatal age and amount of enteral feeding (48, 52), whereas TPN induces mucosal atrophy and increased permeability (53).

Intestinal blood flow and nutrient metabolism may be key determinants of the trophic response to enteral feeding. In preterm infants, intolerance to oral food is associated with an impaired ability to increase mesenteric blood flow (54). The GIT in suckling pigs normally depends heavily on substrates such as glutamate, glutamine, aspartate, and glucose for oxidation (55). In preterm infants, this nutrient metabolism may be altered toward more glucose, relative to amino acids, as substrate for oxidation (56). Furthermore, intestinal amino acid metabolism differs between preterm and term birth, potentially making preterm neonates deficient in amino acids such as arginine, which are important for mucosal blood perfusion, growth, and immunity (57). Enterocytes synthesize citrulline and arginine, which are crucial to maintain arginine homeostasis in the fetus and neonate. Synthesis of citrulline from glutamine or proline is low, and there is little conversion of citrulline into arginine in enterocytes of preterm neonates (57).

Relatively little is known about the trophic response to enteral diets during the immediate postnatal period in preterm infants. Results from preterm animal models provide information on variables such as gestation length, mode of birth, and types of feeding and diets. Studies in newborn, caesarean-delivered, preterm pigs and calves suggest that the trophic response to mother's milk is similar, although not identical, in preterm and term neonates. Despite being relatively small, the small intestine, with its underdeveloped villous structure, shows a 50%–80% increase in mass during the first 1–2 days in milk-fed neonates, similar to the changes observed in term neonates (39, 49, 50, 58; Fig. 2). This marked GIT trophic response is indeed induced by the exposure to luminal milk nutrients, and is not a result of birth *per se*, as shown by the lack of GIT and pancreatic growth in piglets fed TPN after birth (58). In addition, the small intestine of fetal pigs (92% gestation) responds rapidly to the

introduction of oral colostrum or milk formula with large increases (+50%–75%) in intestinal weight (Fig. 2), similar to those in preterm and term newborn pigs receiving sow's colostrum (40). However, only after birth is colostrum ingestion associated with increased villous height (Fig. 2). Thus, the GIT trophic response to the first oral food is certainly present following preterm birth, at least when birth occurs at a time when the neonate is normally viable with moderate clinical support (Table 1).

Regardless of the marked trophic responses in preterm animals, there are also indications that the preterm GIT reacts differently to enteral food than the term GIT. For example, the absence of enteral food with TPN feeding does not induce a marked decrease in the mucosal weight and villous height in preterm pigs (58, 59) like that in newborn and suckling pigs born at term (60–64). Furthermore, when oral milk diets are given to 3-day-old TPN-fed preterm pigs, the mucosal trophic response is clearly diminished relative to that in newborn preterm pigs without a TPN period (59; Fig. 2). Preterm-delivered calves and pigs also differ from term neonates in their intestinal cell proliferative and apoptotic responses to oral diets (65, 66).

The differences in the trophic responses between preterm and term neonates are most pronounced when using diets other than mother's milk. In preterm pigs and, to a lesser degree, fetal pigs, formula feeding is associated with a diminished GIT trophic response relative to colostrum (40, 44, 59, 60). Feeding an infant milk replacer to preterm newborn pigs leads to a marked atrophy of the mucosal surface, particularly in the distal small intestine and colon, a response not seen when using natural sow's colostrum. Such diet-dependent mucosal atrophy may play a causative role in NEC in preterm infants (67), and term newborn pigs and rats also develop mucosal atrophy and NEC when aggressive formula feeding is combined with hypoxia or hypothermia (68–70). Mucosal atrophy and inflammation prior to NEC may result from a partial inability of the enterally fed intestine to increase cell proliferation, decrease apoptosis, and control GIT blood flow (68, 71, 72). A diminished intestinal synthesis of arginine, an important nitric oxide (NO) precursor, may increase the problems of poor intestinal immunity and NO-induced tissue perfusion in preterm neonates (57). On the other hand, the preterm pig intestine maintains GIT blood flow surprisingly well during hypoxia and hypothermia (73, 74). Likewise, preterm rats are even more resistant to intestinal injury following reperfusion injury than are corresponding term rats (75). Together, these results make it questionable that immature regulation of mesenteric blood flow alone is a key factor leading to mucosal atrophy and dysfunction in preterm neonates following introduction of suboptimal enteral diets.

Nutrient Hydrolysis in Preterm Neonates

Digestive function is thought to be immature in preterm infants, and this has formed the rationale behind the widespread use of partially hydrolyzed milk diets and a delayed enteral food introduction. Nevertheless, the extent to which the preterm infant intestine is immature with regard to digestive function remains unclear. In baboons (nonhuman primates), intestinal lactase and peptidase activities appear only slightly reduced in preterm relative to term neonates, and sucrase and maltase activities are well developed (76, 77). The latter two enzymes have often been used as marker proteins for the development of mature brush border function in mammals (17, 78). Their development during the early fetal period in man and other nonhuman primates (16, 79), during the perinatal period of the large domestic species (80), and during the weanling period of altricial rodents (17) supports the species-specific developmental trends presented in Figure 1. Other hydrolytic enzymes, such as gastric pepsin and pancreatic hydrolases, have also been reported to be immature following preterm birth, although they are responsive to enteral food intake (particularly lipases), but these data are

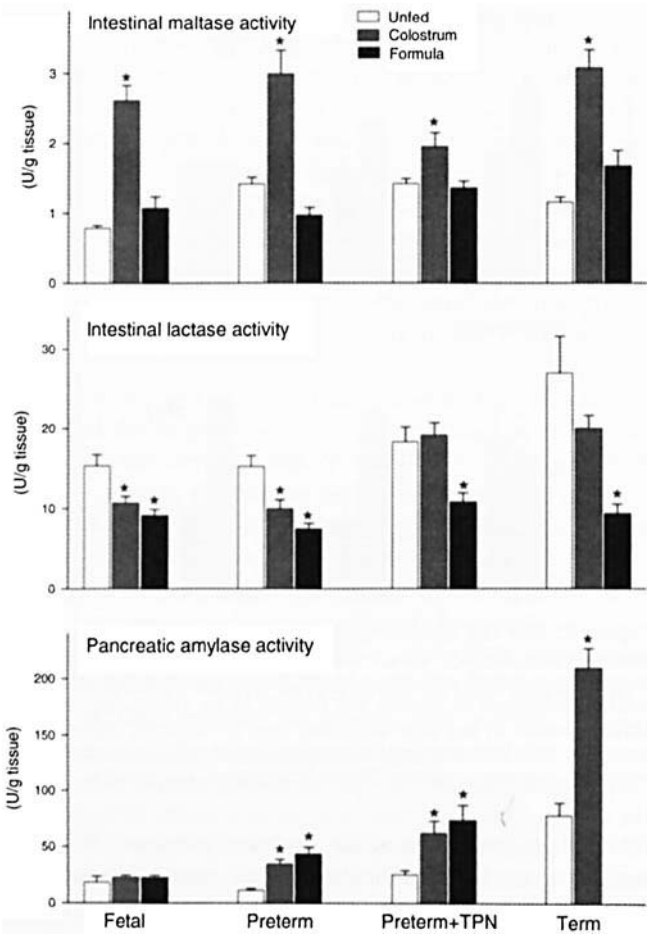

Figure 3. Digestive enzyme responses to oral food introduction in newborn pigs. Bars (mean ± SEM) show activity of intestinal maltase (Upper panel), intestinal lactase (Middle panel), and pancreatic amylase (Lower panel) before and after feeding colostrum or formula (15 ml/kg/3 h) for 1–2 days. * Significantly different value in fed pigs vs. unfed pigs ($P < 0.05$). Results are compiled from different enteral feeding experiments in fetuses and neonates (preterm, preterm TPN-fed, and term; Refs. 40, 60, 61, 92).

not consistent (81–84). More consistent are reports on decreased brush border function in preterm infants, indicated by reduced lactase activity and lactose hydrolysis (52, 53, 79, 85). Lactase activity is stimulated by enteral food, especially mother's milk (45, 86, 87), and this may benefit carbohydrate digestibility (88, 89). On the other hand, an increase in dietary lactose content does not neccessarily result in maldigestion and excessive colonic nutrient fermentation in preterm infants (90, 91).

Studies employing controlled diet regimens in preterm domestic animals provide insights into a wider range of digestive enzymes at more specific gestational ages. Intestinal lactase activity reaches a maximum before birth in lambs, whereas in pigs and calves, a peak is reached at term. In all three genera, the prenatal developmental changes are stimulated by cortisol (23, 80). Both short-term (1 day) and longer-term (1 week) infusion of colostrum into fetal or preterm newborn pigs markedly increase the activity of maltase and aminopeptidases, whereas lactase activity remains stable or decreases slightly (39, 40, 60). Although these responses are generally similar to those in term pigs (42, 44) there are also a number of differences, consistent with findings in preterm and term calves (66). Hence, the postnatal rise in maltase activity in colostrum-fed preterm pigs is only temporary, and lactase activity at 1 week of age tends to be lower than in corresponding term piglets (58). Preterm pigs deprived of enteral food and given 3 days of TPN also respond to oral colostrum with a much lower increase in maltase activity compared with newborn preterm pigs (Fig. 3; Ref. 92). Finally, the enzymic responses to the first enteral food in preterm pigs are highly diet dependent in that the increase in maltase activity is absent, and the decrease in lactase activity is more rapid, following formula feeding compared with colostrum feeding (Fig. 3). Collectively, these results show that brush border enzyme maturation in domestic animals is highly responsive to introduction of oral food (Table 1), but that the responses depend on TPN, enteral diet, and gestational age at birth.

In the stomach and pancreas of the fetal pig and lamb, the concentration of hydrolytic enzymes (e.g., pepsin, chymosin, amylase, trypsin, and chymotrypsin) increases markedly during the weeks before term (32, 93–95). Correspondingly, preterm-delivered animals have significantly lower levels of such enzymes (Table 1). Immediately after birth, the enzyme contents of exocrine cells in the gastric mucosa and pancreas decrease in most species investigated, but this may reflect a birth-associated emptying of secretory granules rather than decreased enzyme biosynthesis (94). In the days following birth, the enzyme concentrations generally increase, and this increase is in part stimulated by enteral feeding. In particular, amylase concentrations undergo a marked diet-independent increase in response to enteral feeding relative to TPN, and this response is very similar in preterm and term pigs (58), but is absent in fetal pigs (Fig. 3; Ref. 40).

The lack of amylase responsiveness to the introduction of oral food in pig fetuses (Fig. 3) may be explained not only by ontogenetic immaturity of the exocrine pancreas, but also by the lack of exposure to a perinatal, birth-associated rise in cortisol levels. This hypothesis is supported by the maturational effects of oral diets infused into pig and lamb fetuses. Neither plasma cortisol nor pancreatic amylase levels were increased by 1 week of milk or colostrum whey infusion into lamb fetuses at 83%–87% gestation (96). When similar studies were performed in pig fetuses at 88%–95% gestation (closer to the time of the normal prenatal cortisol surge), infusion of oral amniotic fluid and milk diets were associated with increases in both cortisol and pancreatic amylase concentrations (96). Amylase concentrations and a series of other cortisol-sensitive GIT enzymes were also elevated in fetuses prevented from receiving any luminal fluid after surgical esophageal ligation (32, 97). For digestive functions that are cortisol sensitive around birth (e.g., intestinal lactase, aminopeptidases and gastric/pancreatic chymosin, amylase, and acid secretion), induction of GIT maturation by enteral feeding depends on the perinatal cortisol surge (23, 28). In summary, gastric, pancreatic, and intestinal hydrolytic enzymes are immature in the immediate postnatal period of infants and domestic animals. The introduction of enteral food increases enzyme activity, but the responses are enzyme-specific and differ in timing, extent, and direction from those in term neonates, particularly if the time and mode of delivery (e.g., elective caesarean section) results in low cortisol levels.

Nutrient Absorption in Preterm Neonates

In noninvasive clinical studies, it is difficult to differentiate between the absorptive function of the small intestinal mucosa and the digestive (hydrolytic) function of the GIT. Nutrients transported across the brush border membrane by active, saturable, carrier-mediated mechanisms (e.g., glucose and galactose) could be affected by immature enterocyte function at the time of birth. Likewise, the ability of the enterocyte to take up protein macromolecules by endocytosis is likely to depend on the structural and functional maturation of the brush border membrane. Nutrients absorbed passively and in a concentration-dependent manner (e.g., fatty acids and fructose), or by both passive and active mechanisms (e.g., many amino acids), are less likely to be affected, although immature brush border membrane structure could still play a role. Fatty acid absorption is little affected by gestational age at birth in infants, and it increases in both preterm and term infants following enteral food intake (98). In contrast, carrier-mediated glucose absorption is relatively low in preterm infants, but again, absorption is enhanced by milk feeding and advancing pre- and postnatal age (Table 1; Refs. 53, 99). The weakness of these studies, like many others in preterm infants, is that corresponding measurements in term healthy infants have not been made. Thus, animal studies help to define more precisely how intestinal absorptive

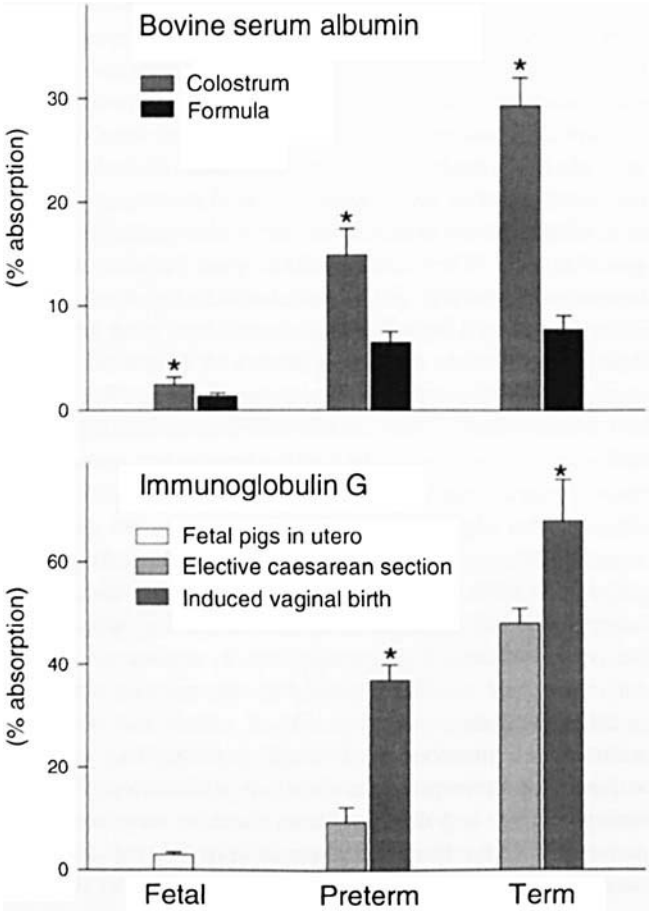

Figure 4. Absorption of intact protein (% absorption, mean ± SEM) during development in pigs. The upper panel shows absorption of bovine serum albumin (BSA) in fetal pigs (88% gestation) and in preterm and term caesarean-delivered pigs fed BSA-containing colostrum or formula. The lower panel shows absorption of IgG after feeding colostrum to fetal pigs *in utero* (88% gestation) or to preterm pigs (91% gestation) and term pigs delivered either by elective caesarean section or following induced vaginal birth. * Significantly higher value in pigs fed colostrum vs. other diets (Upper panel) or in pigs delivered vaginally vs. by caesarean section (Lower panel) ($P < 0.05$). Results are compiled from a series of different experiments (36, 38, 44, 107).

capacity reacts to enteral food, and how this response may differ from that in term neonates.

In pigs, the absorption of nutrients as measured by the uptake of monosaccharides and amino acids by the intestinal mucosa is low during the first half of gestation, but increases rapidly thereafter (100). During the final weeks of gestation, there is a rapid increase in the tissue-specific capacity for glucose uptake, whereas the ability of the mucosa to absorb most amino acids, remains largely unchanged (32, 51, 101). The low glucose absorption in newborn preterm pigs (32) is compensated by an increase in transport function within the first week after birth, even in TPN-fed preterm pigs (58). In contrast, the ability of intestinal tissue to transport glucose decreases postnatally in term pigs, although to a lesser degree in enterally fed pigs than in TPN-fed pigs (58, 64). These results suggest that the feeding-induced decrease in glucose absorptive function at the tissue level (Table 1) may not be as pronounced for preterm neonates as it is for term neonates (Table 1), and that the TPN-induced intestinal absorptive dysfunction is less pronounced in preterm than in term piglets. It should be noted, however, that the results reflect only the tissue-specific uptake of glucose per gram of intestine, whereas the actual total *in vivo* uptake capacity for nutrients is determined also by factors such as intestinal motility and total mucosal mass. Regardless, these results clearly demonstrate that studies in term animals do not necessarily reflect the physiologic conditions present in animals or infants delivered prematurely.

Preterm infants absorb more intact proteins, such as lactalbumins and oligosaccharides (e.g., lactulose), than term infants, but the absorbed amounts remain small (<1%) (102–105). Following the initiation of feeding, the GIT permeability to large molecules decreases in both term and preterm infants (Table 1; Refs. 53, 105). In the large domestic species, the conditions for intestinal macromolecule uptake differ markedly from those in humans because of the dependence on absorbing bulk amounts of Igs from colostrum immediately after birth, and the lack of prenatal placental transfer of maternal Igs. Hence, the uptake of macromolecules by the newborn domestic animal is not merely a result of "an immature or leaky epithelium," but reflects a specific maturational process that involves both a specific IgG receptor and nonspecific endocytosis (106). As such, intact protein absorption in domestic animals is a sensitive marker of enterocyte function around the time of birth, both term and preterm.

Intestinal macromolecular uptake is present *in utero* during the last 2 weeks of gestation, but is markedly lower in the pig fetus during late gestation (88%–95% gestation) compared with the neonate (Fig. 4; Ref. 38). At 80%–85% gestation, the protein absorptive function in the fetal lamb intestine is barely detectable (36), whereas newborn lambs have a large capacity to take up intact macromolecules. These results, coupled with those of studies on fetal tissues *in vitro* (32) and postnatal *in vivo* studies (58, 101, 107), confirm that the endocytotic capacity of the developing intestine in domestic animals reaches its maximum at term or a few days before term. Correspondingly, the capacity for Ig absorption is severely depressed in caesarean-delivered, preterm piglets (Fig. 4), calves, and lambs (Table 1; Refs. 32, 36, 58, 107–109). In addition to ontogenetic immaturity of enterocyte function, altered metabolic or endocrine parameters (hypoxia, acidemia, lack of cortisol) may prevent the intestinal enterocytes in preterm neonates from reaching their full endocytotic potential. Changes in such factors may explain why induced vaginal birth partly prevents the reduction in protein endocytotic capacity observed after preterm caesarean section (Fig. 4).

Studies in fetal and neonatal pigs (38, 44, 110) indicate that colostrum itself plays a key role in the endocytotic capacity and induction of intestinal closure. Experimental colostrum infusion into the prenatal pig intestine (88%–93%

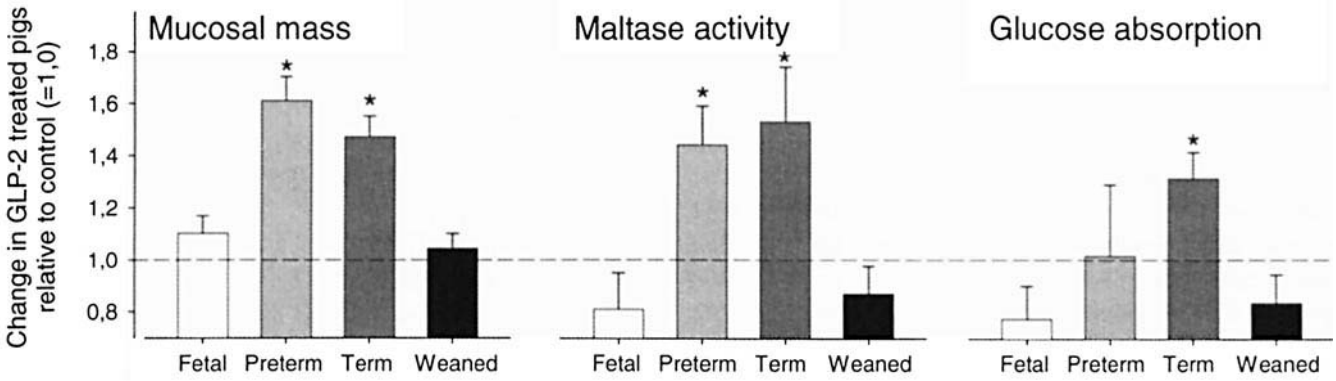

Figure 5. Relative change (means ± SEM) in intestinal mucosal mass, maltase activity, and glucose absorption following GLP-2 treatment in fetal pigs (87%–92% gestation), preterm, TPN-fed pigs (93% gestation), term TPN-fed pigs (100% gestation), and weanling pigs (31 days postnatally). * Significant increase ($P < 0.05$) expressed relative to the value in saline-treated control pigs (defined as 1.0). (Adapted, with permission, from data in Reference 133).

gestation) has shown a decreased Ig uptake, and a delayed intestinal closure (Table 1), relative to newborn pigs. Similar results are observed in preterm newborn pigs (40, 58). The ability to take up and transfer intact proteins from the epithelium into the circulation is therefore a highly specialized function that develops close to term. Colostrum itself not only provides the substrates for endocytosis (Igs) but also stimulates the endocytotic capacity by mechanisms that are as yet unknown. The dramatic reductions in the intestinal protein absorptive capacity following feeding with noncolostrum diets take place to a similar extent in fetal, preterm, and term newborn pigs (Fig. 4). Thus, the detrimental effect of formula on endocytotic function is determined by the chemical nature of the diet rather than by the developmental stage of the intestinal epithelium (40, 44).

Gut Motility and Gut Regulatory Peptides in Preterm Neonates

Numerous hormones and regulatory factors are produced by the cells of the GIT mucosa and from the enteric nervous system (ENS) (e.g., gastrin, motilin, neurotensin, pancreatic polypeptide (PP), glucose-insulinotropic peptide (GIP), somatostatin, vasoactive intestinal polypeptide, gastrin releasing peptide, cholecystokinin, secretin, and many others). Some of these regulators are produced and released specifically in response to oral food intake, and play well-defined roles in the digestive processes, including GIT motility, exocrine secretion, and absorptive function. Until now, gut regulatory peptides in preterm neonates have received little attention, but a few examples indicate how immaturity of gut regulatory peptide synthesis and secretion may affect the response to enteral food in preterm neonates. In infants, there has been a focus on gut peptides in relation to the development of GIT motility. In combination with locally released nonpeptide components from the ENS (e.g., NO, cholinergic substances), certain regulatory peptides act to control GIT motility *via* endocrine, paracrine, as well as neurocrine pathways. Normal patterns of intestinal motility develop during the last 10% of gestation, as least in rabbits (111), and immature gut motility and stasis, therefore, contributes to development of enteric disease in preterm infants (46, 111). Also in preterm piglets, bowel movements are not well developed during the first days of enteral feeding (58), probably because of dysfunctional enteric neurons (59), and this may lead to to stasis, inappropriate colonization, nutrient fermentation, and NEC (40, 59, 60). Early feeding of preterm neonates, even with small amounts of milk, is believed to decrease food transit time and induce maturation of GIT motility patterns, probably *via* increased endocrine release of gut hormones, such as motilin and gastrin (46, 112–116), and increased number of nitrergic neurons (Table 1; Ref. 59).

The enteroendocrine cells of the gut, and the circulating levels of gut regulatory peptides, develop relatively early in gestation in both humans and domestic animals (24, 117–119). Likewise, the ENS, and its neuronal production of regulatory peptides, has an early (fetal) structural development in both humans (120) and pigs (121, 122). Preterm infants respond to enteral food intake with large increases in many gut regulatory peptides (motilin, gastrin, PP, neurotensin, GIP, and enteroglucagon), and the increases are generally comparable with those in term infants, with similar responses occurring for breast milk and formula diets (123, 124). This does not preclude, however, the possibility that a functional immaturity of such cells, and their target GIT receptors, contribute to a disturbed GIT response to enteral food in preterm neonates (125).

Probably the best example of a GIT regulatory peptide hormone showing immature synthesis, secretion, and effects in preterm neonates is gastrin. This hormone is produced by the enteroendocrine G-cells of the stomach, and is known to have trophic effects on, and stimulate acid secretion by, the gastric mucosa. The circulating levels reach peak values in neonatal pigs and infants, concomitantly with a relatively low gastric acid secretion and tissue synthesis of hormone (126, 127). In late gestation, fetal pigs and lambs have gastrin and gastric acid secretions that are immature, despite

the fact that the release of these hormones is responsive to infusion of enteral diets (97, 128). In the fetus and neonate, gastrin concentrations are high in the stomach lumen and responsive to enteral food intake, indicating that, during early development, the G-cells may function not only in an endocrine, but also an exocrine, manner (97, 126, 127). Thus, ingestion of amniotic fluid plays a role in prenatal gastrin development in the pig, despite the fact that interaction with other endocrine determinants (e.g., the prenatal cortisol surge) may be even more important (28, 126).

In contrast to gastrin, there are other gut regulatory peptides that show peak levels in plasma in the days after birth, and for these it is more likely that the first enteral food intake stimulates peptide secretion. One example of such a gut regulatory peptide is glucagon-like peptide 2 (GLP-2), an enterotropic hormone that is produced in the ileal L-cells and is released in response to a meal rich in fat and carbohydrate. Circulating GLP-2 levels are much lower in late-gestation pig fetuses (5 ± 2 pM) compared with newborn pigs (17 ± 3 pM) or pigs receiving luminal nutrients for 1 week (60 ± 10 pM) (129). To support the hypothesis that GLP-2 is a key nutrient-responsive growth factor for the intestinal mucosa just after birth, the effects of exogenous GLP-2 infusion into TPN-fed fetal, premature neonatal, and term neonatal pigs have been compared. The results show that exogenous GLP-2 has a significant effect on mucosal growth just after birth (even after preterm delivery), but has little, if any, effect on intestinal mucosal growth *in utero* (65, 129–131). Although enteral diets induce large increases in GLP-2 secretion in pig fetuses, and GLP-2 receptors are present both before and after birth (129), the release of GLP-2 in response to the first intake of enteral food appears to play a trophic and functional role for neonatal gut adaptation only after birth. Also, in sick preterm infants, the circulating GLP-2 levels are well correlated with the ability to tolerate oral food (132), and these data make GLP-2 one of the most promising candidates for a regulatory signal between enteral food intake and GIT adaptation in preterm neonates. Whether this knowledge can be used therapeutically in preterm neonates unable to tolerate oral food remains to be investigated, but studies in preterm pigs suggest that exogenous GLP-2 and epidermal growth factor (EGF) both fail to prevent NEC development (60). It is also noteworthy that the GIT adaptation seen in TPN-fed, GLP-2–treated piglets differs structurally and functionally from that which occurs after oral milk intake (65, 131). Figure 5 summarizes some results from studies on exogenous GLP-2 treatment in developing pigs, suggesting that GLP-2 may mediate at least some of the intestinotropic and enterocyte maturational effects of enteral nutrition in preterm and term neonatal pigs, whereas GLP-2 may have more limited effects before birth and after weaning (133).

Gut Microflora and Inflammatory Responses in Preterm Neonates

The postnatal development of bacterial assemblages in the GIT involves progressive changes in the type and number of species that may differ between preterm and term neonates. Commensal genera, such as *Bifidobacterium* and *Lactobacillus*, take longer to become established in the GIT lumen, and the flora is less diverse in preterm than in term infants (134). In healthy preterm infants, a slower colonization pattern is followed by a lower production of short chain fatty acids in the GIT lumen (90). The use of broad-spectrum antibiotics and parenteral nutrition further delay colonization (92, 135, 136), and could make the immature GIT more susceptible to bacterial overgrowth with pathogenic strains (e.g., *Escherichia coli*, *Clostridium*, *Staphylococcus*, *Klebsiella*, and *Bacterioides*) (134, 137, 138). When luminal substrates are made available following maldigestion, excessive bacterial fermentation and luminal distension may damage the immature GIT mucosa (139, 140) and start the inflammatory cascade of NEC. Detailed studies of the microflora in relation to infant NEC is complicated by the limited access to luminal contents and mucosal tissue prior to clinical evidence of mucosal atrophy, inflammation, and necrosis. There is, however, increasing evidence that nonenteropathogenic bacteria added to mother's milk or formula can safely alter the GIT colonization pattern in preterm neonates and thereby reduce NEC by suppressing the pathogen load (141–145).

The differences in bacterial assemblages between term and preterm neonates may be related to immaturity of the GIT epithelium. Among the intrinsic developmental factors that predispose to a suboptimal bacterial colonization is an immature GIT motility that allows potentially harmful bacteria to proliferate and ferment nutrients in the GIT lumen, and attach to the mucosa at the expense of beneficial bacteria (139, 140). Production of mucous, including the protective trefoil factor-3 (146), and gastric acid and proteases, are reported to be immature in preterm infants (82, 83, 137, 147), leading to a diminished barrier function, impaired mucosal repair, and lowered intraluminal degradation of bacterial toxins. In preterm pigs and lambs, gastric acid and protease secretions are severely compromised, particularly following caesarean section (93, 126, 127). Impaired systemic immunity in preterms (5, 58), as well as lower levels of secretory IgA and gut B and T lymphocytes, may facilitate increased bacterial adherence to the intestinal mucosa and infection (137, 148). Coupled with the increased intestinal mucosal permeability in preterms (53, 105), this impairment leads to the delivery of whole bacteria and toxins deep into the mucosa, or even translocation to the blood stream.

The initial colonization pattern in preterm infants may be closely related to environmental factors in addition to the deficient host-associated defense mechanisms (136, 149, 150). Particularly following caesarean section, the neonate is prevented from the normal inoculation with maternal

rectal and vaginal flora during the birth process. Coupled with decreased gastric acid secretion relative to vaginal birth (127, 151), this may lead to an abnormal colonization pattern for caesarean-delivered infants (150, 152). On the other hand, it has not been possible to demonstrate a consistent relation between NEC and delivery method in preterm infants (150) or pigs (153). Inoculation with the maternal vaginal/rectal flora, therefore, does not seem to play a crucial role in securing a normal response to the first enteral food in preterm neonates.

The nature of the enteral diet, formula or mother's milk, plays a role in determining not only mucosal structure and function (see earlier), but also the pattern of bacterial colonization in preterm infants (135, 137) and pigs (89, 121). Interestingly, the detrimental effects of formula feeding to immature pigs depend on the presence of a GIT microflora, as shown in studies on gnotobiotic (germ-free) preterm pigs or pig fetuses (40, 60). Regardless, spontaneous development of NEC has not yet been associated with any single pathogen or group of pathogens, either in infants or in animals. The carbohydrate-fermenting *Clostridium* have played a predominant role among the bacterial species that colononize the mucosa during spontaneous NEC in preterm infants (138), preterm pigs (60, 92), and newborn quails (154). Conversely, experimental models of NEC in newborn term rats or pigs have used gram-negative endotoxemia, in combination with hypoxic and ischaemic insults (139, 155–157), to provide evidence that NEC results from a disordered enterocyte signaling to bacterial toxins *via* the release of proinflammatory mediators (e.g., platelet activating factor, interleukin (IL)-1β, IL-6, IL-8). These mediators activate p38 kinase, cyclooxygenase-2, and transcriptional nuclear factor κB (NF-κB) signaling pathways in intestinal cells leading to necrosis (135, 137, 156–158). Further studies will show whether these proposed molecular pathways of NEC explain spontaneous development of NEC in preterm neonates not being exposed to the artificial aggressive insults used in experimental NEC models. The nature, localization, and inflammatory potential of the GIT microflora prior to NEC need further study in preterm animal models. Regardless, the available literature show that the resident microflora do not act independently of other related GIT problems in preterm neonates (e.g., maldigestion, ischemia, hypoxia) to produce NEC.

One of the components that have received most attention in relation to the escalating feeding-induced inflammatory responses in preterm neonates is NO. Moderate levels of NO play an important role in maintaining GIT blood flow, provide enteric smooth muscle relaxation, and protect the mucosa from injury. This constitutive NO production is controlled by the activity of NO synthetase (NOS) in the endothelium (endothelial NOS) and ENS (neuronal NOS [nNOS]). During states of mucosal inflammation, including that in NEC, constitutive NO release is replaced by an excessive NO production facilitated by escalating inducible NOS (iNOS) activity in villous enterocytes, leading to oxidative damage and inflammation (159).

In both preterm and term pigs, formula feeding induces a marked upregulation of iNOS activity relative to mother's milk feeding, but only in preterm neonates is the increased NO production associated with NEC (60, 61, 92). The lack of a normal birth signal in caesarean-derived preterm pigs (e.g., lowered prostaglandin, cortisol secretion) may play a role, as an artificial induction of parturition reduces the intestinal proinflammatory effects of excessive NOS production in preterm piglets (69). Elevated iNOS activities in intestinal tissue have been observed in infants with NEC (160) and, conversely, stimulation of constitutive NOS activity in preterm pigs decreases the formula-induced mucosal injury (161). On the other hand, preterm pigs showed increased (rather than decreased) expression of nNOS-containing neurons prior to feeding-induced NEC development (59). These results contrast with the decreasing NOS neuron density observed after feeding in term neonatal pigs (121), and suggest a functional basis for the disturbed GIT motility in preterm neonates. Defective mechanisms in the antioxidant systems of the small intestine (159), or the lack of dietary antioxidants (60, 92), may render the mucosal cells more sensitive to elevated NO production and, thus, contribute to the diet- and colonization-dependent development of NEC in preterm neonates.

Bioactive Milk Factors for Preterm Neonates

As indicated above, the local GIT response to the first intake of enteral food and bacteria differs markedly between preterm and term neonates, despite the fact that many indices of GIT morphology and nutritient digestion and aborption respond similarly. The elevated sensitivity to inflammatory insults, and the apparent immaturity of the immune system (5), may explain why the supply of anti-inflammatory factors in mother's milk is particularly important following preterm delivery. Milk contains a large number of immunologic factors that help to protect the immature intestine from bacterial overgrowth and inflammatory responses, and the contents of such factors may be even higher in preterm milk (10, 162–164).

The main bacteriostatic function of milk could be to prevent mucosal adherence of pathogenic bacteria and tissue damage by mucosal infiltration and toxin release (60, 135). Oligosaccharides may function as receptor analog decoys, binding bacteria before they adhere to glycoconjugates on the microvillus membrane. Hence, improved colonization with a beneficial microflora has been observed in formula-fed preterm infants following dietary oligosaccharide supplementation (165–168). More specific immunity could be provided by maternal milk in the form of lymphocytes and secretory polymeric IgA specifically directed against enteric pathogens to which the mother has previously been exposed (169). Milk also contains nucleotides and cytokines that may aid in the protection from chemical injury in

immature enterocytes (170, 171), and, likewise, high amounts of antioxidants (e.g., vitamins E and C) help to protect the preterm intestine from the damaging actions of reactive oxygen free radicals, also suggested from studies in preterm pigs (40, 60). The molecular mechanisms of the milk effects remain poorly understood, but *in vitro* studies indicate that milk suppresses the IL-1β–induced activation of the NEC-like inflammatory cascade by inhibiting the activation pathway of NF-κB (172).

The concentrations of some peptide growth factors, like EGF, insulin, and insulin-like growth factor, are particularly high in the first milk, even when birth occurs prematurely (173–177). Because high concentrations of receptors for milk-borne growth factors have been found for both preterm and term pigs and calves (129, 178–180), and luminal proteolysis is decreased in preterm neonates (181, 182), it is plausible that milk-borne growth factors play a particularly important role in GIT maturation in preterm neonates. This hypothesis is supported by several reports on the beneficial effects of mother's milk in preventing enteric disease (e.g., NEC) in preterm infants and pigs (40, 60, 67). Provision of single milk growth factors or regulatory peptides have often failed to affect term neonates (41, 183, 184), but the potential remains that the immature intestine is particularly responsive to luminal growth factor stimulation, provided that epithelial growth factor receptors are present and functional. Transforming growth factor β (TGF-β), an important anti-inflammatory cytokine in milk, did not activate TGF receptors to the same extent in milk-fed fetal or preterm pigs (unpublished observations) compared with term newborn pigs (185). Regardless, the identification of regulatory factors in milk that mediate maturational changes in the immature GIT remains an attractive goal. Supplementation with a combination of such factors, during TPN or formula feeding, may mimic the effects of bioactive components normally present in mother's milk.

Conclusion

Studies in both infants and large farm animals show that enteral food, particularly mother's milk, induces rapid maturation of the gut mucosa, some hydrolytic enzymes, nutrient absorption, gut motility, and microbial colonization in preterm neonates. Together, these results provide strong support for the concept of minimal enteral feedingto improve the adaptation of the immature intestine to oral food (43, 45). The evidence for GIT immaturity following preterm birth, and the maturational responses to enteral nutrition, are summarized for infants and the large domestic species in Table 1. Although many GIT responses to enteral nutrition are similar between preterm and term neonates, there are also marked differences in the timing and the nature the responses. This makes preterm neonates more sensitive to serious feeding-induced GIT complications, such as NEC. Immature digestive, absorptive, and immunologic functions lead to nutrient fermentation, bacterial overgrowth, and mucosal inflammation. The possible hypoxia, hypothermia, intestinal ischemia, altered endocrine and metabolic status, and inappropriate bacterial colonization related to preterm birth may add to this increased susceptibility to enteric disease. All these clinically relevant factors are difficult to study without the use of animal models that allow easy experimental manipulation of physiologic conditions before birth (e.g., growth retardation and access to luminal diets), at birth (time and mode of delivery), and shortly after birth (e.g., TPN versus enteral nutrition, hypoxia, hypothermia, and bacterial colonization). An animal model of nutrition in preterm infants should ideally have a GIT structure and a dietary habit in the adult animal reflecting those in adult humans. The preterm piglet fulfills all these requirements. In addition, the size, large number of offspring per litter, and apparent clinical and developmental similarity with preterm infants make the pig an attractive animal model for futher studies on the many physiologic and clinical complications related to preterm birth.

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1. Kramer MS. The epidemiology of adverse pregnancy outcomes: an overview. J Nutr(5 Suppl 2)133:1592S–1596S, 2003.
2. Mattison D, Damus K, Fiore E, Petrini J, Alter C. Preterm delivery: a public health perspective. Paediatr Perinat Epidemiol 15:7–16, 2001.
3. Romero R, Mazor M, Munoz H, Gomez R, Galasso M, Sherer D. The preterm labor syndrome. Ann N Y Acad Sci 697:9–27, 1994.
4. WHO. Maternal anthropometry and pregnancy outcomes: WHO collaborative study. Bull World Health Organ 73:1–68, 1995.
5. Ashworth A. Effects of intrauterine growth retardation on mortality and morbidity in infants and young children. Eur J Clin Nutr 52(Suppl 1): S34–41, 1998.
6. Johnston RJ, Williams M, Hogue C, Mattison D. Overview: new perspectives on the stubborn challenge of preterm birth. Paediatr Perinat Epidemiol 15:3–6, 2001.
7. Picaud JC. Formula-fed preterm neonates. Minerva Pediatr 55:217–229, 2003.
8. Lee JS, Polin RA. Treatment and prevention of necrotizing enterocolitis. Semin Neonatol 8:449–459, 2003.
9. McGuire W, Henderson G, Fowlie PW. Feeding the preterm infant. Br Med J 329:1227–1230, 2004.
10. Walker WA. The dynamic effects of breastfeeding on intestinal development and host defense. Adv Exp Med Biol 554:155–170, 2004.
11. Reber KM, Nankervis CA. Necrotizing enterocolitis: preventative strategies. Clin Perinatol 31:157–167, 2004.
12. Berseth CL. Feeding strategies and necrotizing enterocolitis. Curr Opin Pediatr 17:170–173, 2005.
13. van der Lende T, Knol EF, Leenhouwers JI. Prenatal development as a

predisposing factor for perinatal losses in pigs. Reprod Suppl 58:247–261, 2001.
14. Bjorklund NE, Svendsen J, Svendsen LS. Histomorphological studies of the perinatal pig: comparison of five mortality groups with unaffected pigs. Acta Vet Scand 28:105–116, 1987.
15. Johanson JM, Berger PJ. Birth weight as a predictor of calving ease and perinatal mortality in Holstein cattle. J Dairy Sci 86:3745–3755, 2003.
16. Grand RJ, Watkins JB, Torti FM. Development of the human gastrointestinal tract: a review. Gastroenterology 70:790–810, 1976.
17. Henning SJ. Ontogeny of enzymes in the small intestine. Annu Rev Physiol 47:231–245, 1985.
18. Lebenthal E, Leung YK. Feeding the premature and compromised infant: gastrointestinal considerations. Pediatr Clin North Am 35:215–238, 1988.
19. Henning SJ, Rubin, DC, Shulman RJ. Ontogeny of the intestinal mucosa. In: Johnson LR, Ed. Physiology of the Gastrointestinal Tract (3rd ed.). New York: Raven, pp571–610, 1994.
20. Lebenthal A, Lebenthal E. The ontogeny of the small intestinal epithelium. J Parenter Enteral Nutr 23(Suppl 5):S3–S6, 1999.
21. Montgomery RK, Mulberg AE, Grand RJ. Development of the human gastrointestinal tract: twenty years of progress. Gastroenterology 116: 702–731, 1999.
22. Sangild PT, Fowden AL, Trahair JF. How does the fetal gastrointestinal tract develop in preparation for enteral nutrition after birth? Livest Prod Sci 66:141–155, 2000.
23. Sangild PT, Xu RJ, Trahair JF. Maturation of intestinal function: the role of birth and cortisol. In: Zabielski R, Lesnewski V, Weström BR, Pierzynowski SR, Eds. Biology of the Small Intestine in Growing Animals. Amsterdam: Elsevier, pp111–144, 2002.
24. Zabielski R, Le Huerou-Luron I, Guilloteau P. Development of gastrointestinal and pancreatic functions in mammalians (mainly bovine and porcine species): influence of age and ingested food. Reprod Nutr Dev 39:5–26, 1999.
25. Lebenthal E, Lee PC. Review article. Interactions of determinants in the ontogeny of the gastrointestinal tract: a unified concept. Pediatr Res 17:19–24, 1983.
26. Lamers WH, Mooren PG, Charles R. Perinatal development of the small intestine and pancreas in rat and spiny mouse: its relation to altricial and precocial timing of birth. Biol Neonate 47:153–162, 1985.
27. Mulvihill SJ, Stone MM, Debas HT, Fonkalsrud EW. The role of amniotic fluid in fetal nutrition. J Pediatr Surg 20:668–672, 1985.
28. Trahair JF, Sangild PT. Systemic and luminal influences on the perinatal development of the gut. Equine Vet J 24 Suppl:40–50, 1997.
29. Trahair JF, Harding R. Restitution of fetal swallowing restores intestinal growth after mid-gestation esophageal obstruction. J Pediatr Gastroenterol Nutr 20:156–161, 1995.
30. Trahair JF, Sangild PT. Structural development of the fetal gastrointestinal tract. In: Zabielski R, Lesnewski V, Weström BR, Pierzynowski SR, Eds. Biology of the Small Intestine in Growing Animals. Amsterdam: Elsevier, pp1–54, 2002.
31. Trahair JF, Sangild PT. Fetal organ growth in response to infusion of amniotic fluid, colostrum, milk, or gastrin-releasing peptide; a study in fetal sheep. Reprod Fert Dev 12:87–95, 2000.
32. Sangild, PT, Schmidt M, Elnif J, Bjornvad CR, Buddington RK. Prenatal development of the gastrointestinal tract in pigs and the effect of fetal gut obstruction. Pediatr Res 52:416–424, 2002.
33. Buchmiller TL, Gregg J, Rivera FA Jr, Diamond JM, Fonkalsrud EW. Effect of esophageal ligation on the development of fetal rabbit intestinal lactase. J Pediatr Surg 28:1473–1477, 1993.
34. Buchmiller TL, Fonkalsrud EW, Kim CS, Chopourian HL, Shaw KS, Lam MM, Diamond JM. Upregulation of nutrient transport in fetal rabbit intestine by transamniotic substrate administration. J Surg Res 52:443–447, 1992.
35. Buchmiller TL, Kim CS, Chopourian HL, Fonkalsrud EW. Transamniotic fetal feeding: enhancement of growth in a rabbit model of intrauterine growth retardation. Surgery 116:36–41, 1994.
36. Sangild PT. Uptake of passive immunity by the compromized newborn animal. Acta Vet Scand 98(Suppl):105–122, 2003.
37. Siggers JL, Siggers, RH, Schmidt M, Boye M, Sangild PT. Amniotic fluid decreases the incidence of necrotizing enterocolitis in preterm pigs. Scand J Food Nutr 50(Suppl 1):50–51, 2006.
38. Sangild PT, Trahair JF, Loftager MK, Fowden AL. Intestinal macromolecule absorption in the fetal pig after infusion of colostrum *in utero*. Pediatr Res 45:595–602, 1999.
39. Sangild PT, Silver M, Schmidt M, Fowden AL. The perinatal pig in pediatric gastroenterology. In: Tumbleson ME, Schnook L, Eds. Advances in Swine in Biomedical Research. New York: Plenum Press, pp745–756, 1996.
40. Bjornvad CR, Schmidt M, Petersen YM, Jensen SK, Offenberg H, Elnif J, Sangild PT. Preterm birth makes the immature intestine sensitive to feeding-induced intestinal atrophy. Am J Physiol Regul Integr Comp Physiol 289:R1212–R1222, 2005.
41. Xu RJ. Development of the newborn GI tract and its relation to colostrum/milk intake: a review. Reprod Fertil Dev 8:35–48, 1996.
42. Zhang H, Malo C, Buddington RK. Suckling induces rapid intestinal growth and changes in brush border digestive functions of newborn pigs. J Nutr 127:418–426, 1997.
43. Burrin DG, Stoll B, Jiang R, Chang X, Hartmann B, Holst JJ, Greeley GH Jr, Reeds PJ. Minimal enteral nutrient requirements for intestinal growth in neonatal piglets: how much is enough? Am J Clin Nutr 71: 1603–1610, 2000.
44. Jensen AR, Elnif J, Burrin DG, Sangild PT. Development of intestinal immunoglobulin absorption and enzyme activity in neonal pigs is diet-dependent. J Nutr 131:3259–3265, 2001.
45. McClure RJ, Newell SJ. Randomized controlled study of digestive enzyme activity following tropic feeding. Acta Paediatr 91:292–296, 2002.
46. Berseth CL, Bisquera JA, Paje VU. Prolonging small feeding volumes early in life decreases the incidence of necrotizing enterocolitis in very low birth weight infants. Pediatrics 111:529–534, 2003.
47. Shanklin DR, Cooke RJ. Effects of intrauterine growth on intestinal length in the human fetus. Biol Neonate 64:76–81, 1993.
48. Weaver LT, Austin S, Cole TJ. Small intestinal length: a factor essential for gut adaptation. Gut 32:1321–1323, 1991.
49. Sangild PT, Schmidt M, Jacobsen H, Fowden AL, Forhead A, Avery B, Greve T. Blood chemistry, nutrient uptake and organ weights of fetal and newborn calves derived from *in vitro* produced embryos. Biol Reprod 62:1495–1504, 2000.
50. Schmidt M, Sangild PT, Blum J, Andersen JB, Greve T. Glucocorticoid and ACTH administration improves survival and organ maturation in premature calves. Theriogenology 61:1729–1744, 2004.
51. Buddington RK, Elnif J, Puchal-Gardiner AA, Sangild PT. Intestinal apical amino acid absorption during development of the pig. Am J Physiol Regul Comp Integr Physiol 280:R241–R247, 2001.
52. Shulman RJ, Wong WW, Smith EO. Influence of changes in lactase activity and small-intestinal mucosal growth on lactose digestion and absorption in preterm infants. Am J Clin Nutr 81:472–479, 2005.
53. Rouwet EV, Heineman E, Buurman WA, ter Riet G, Ramsay G, Blanco CE. Intestinal permeability and carrier-mediated monosaccharide absorption in preterm neonates during the early postnatal period. Pediatr Res 51:64–70, 2002.
54. Robel-Tillig E, Knupfer M, Pulzer F, Vogtmann C. Blood flow parameters of the superior mesenteric artery as an early predictor of intestinal dysmotility in preterm infants. Pediatr Radiol 34:958–962, 2004.
55. Burrin DG, Stoll B. Key nutrients and growth factors for the neonatal gastrointestinal tract. Clin Perinatol 29:65–96, 2002.
56. van der Schoor SR, Stoll B, Wattimena DL, Buller HA, Tibboel D,

Burrin DG, van Goudoever JB. Splanchnic bed metabolism of glucose in preterm neonates. Am J Clin Nutr 79:831–837, 2004.
57. Wu G, Jaeger LA, Bazer FW, Rhoads JM. Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. J Nutr Biochem 15:442–451, 2004.
58. Sangild PT, Petersen YM, Schmidt M, Elnif J, Petersen TK, Buddington RK, Michaelsen KF, Greisen G, Burrin DG. Preterm birth affects the gastrointestinal responses to parenteral and enteral nutrition in the newborn pig. J Nutr 132:2673–2681, 2002.
59. Oste M, Van Ginneken C, Van Haver E, Bjornvad CR, Thymann T, Sangild PT. The intestinal trophic response to enteral food is reduced in parenterally fed preterm pigs and is associated with more nitrergic neurons. J Nutr 135:2657–2663, 2005.
60. Sangild PT, Siggers RH, Schmidt M, Elnif J, Bjornvad CR, Thymann T, Grondahl ML, Hansen AK, Jensen SK, Boye M, Moelbak L, Buddington RK, Westrom BR, Holst JJ, Burrin DG. Diet- and colonization-dependent intestinal dysfunction predisposes to necrotizing enterocolitis in preterm pigs. Gastroenterology 130:1776–1792, 2006.
61. Thymann T, Burrin DG, Tappenden KA, Bjornvad CR, Jensen SK, Sangild PT. Formula-feeding reduces lactose digestive capacity in neonatal pigs. Br J Nutr 95:1075–1081, 2006.
62. Niinikoski H, Stoll B, Guan X, Kansagra K, Lambert BD, Stephens J, Hartmann B, Holst JJ, Burrin DG. Onset of small intestinal atrophy is associated with reduced intestinal blood flow in TPN-fed neonatal piglets. J Nutr 134:1467–1474, 2004.
63. Conour JE, Ganessunker D, Tappenden KA, Donovan SM, Gaskins HR. Acidomucin goblet cell expansion induced by parenteral nutrition in the small intestine of piglets. Am J Physiol Gastrointest Liver Physiol 283:G1185–G1196, 2002.
64. Burrin DG, Stoll B, Chang X, Van Goudoever JB, Fujii H, Hutson SM, Reeds PJ. Parenteral nutrition results in impaired lactose digestion and hexose absorption when enteral feeding is initiated in infant pigs. Am J Clin Nutr 78:461–470, 2003.
65. Burrin DG, Stoll B, Jiang R, Petersen Y, Elnif J, Buddington RK, Schmidt M, Holst JJ, Hartmann B, Sangild PT. GLP-2 stimulates intestinal growth in premature TPN-fed pigs by suppressing proteolysis and apoptosis. Am J Physiol Gastrointest Liver Physiol 279:G1249–G1256, 2000.
66. Bittrich S, Philipona C, Hammon HM, Rome V, Guilloteau P, Blum JW. Preterm as compared with full-term neonatal calves are characterized by morphological and functional immaturity of the small intestine. J Dairy Sci 87:1786–1795, 2004.
67. McGuire W, Anthony MY. Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. Arch Dis Child Fetal Neonatal Ed 88:F11–F14, 2003.
68. Crissinger KD, Burney DL, Velasquez OR, Gonzalez E. An animal model of necrotizing enterocolitis induced by infant formula and ischemia in developing piglets. Gastroenterology 106:1215–1222, 1994.
69. Di Lorenzo M, Krantis A. Altered nitric oxide production in the premature gut may increase susceptibility to intestinal damage in necrotizing enterocolitis. J Pediatr Surg 36:700–705, 2001.
70. Hsueh W, Caplan MS, Qu XW, Tan XD, De Plaen IG, Gonzalez-Crussi F. Neonatal necrotizing enterocolitis: clinical considerations and pathogenetic concepts. Pediatr Dev Pathol 6:6–23, 2003.
71. Clark JA, Lane RH, Maclennan NK, Holubec H, Dvorakova K, Halpern MD, Williams CS, Payne CM, Dvorak B. Epidermal growth factor reduces intestinal apoptosis in an experimental model of necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 288: G755–G762, 2005.
72. Dyess DL, Peeples GL, Ardell JL, Tacchi EJ, Roberts WS, Ferrara JJ, Powell RW. Indomethacin-induced blood flow distribution in premature and full-term piglets. J Pediatr Surg 28:1396–1400, 1993.
73. Powell RW, Dyess DL, Collins JN, Roberts WS, Tacchi EJ, Swafford AN Jr, Ferrara JJ, Ardell JL. Regional blood flow response to hypothermia in premature, newborn, and neonatal piglets. J Pediatr Surg 34:193–198, 1999.
74. Hoang TV, Choe EU, Burgess RS, Cork RC, Flint LM, Ferrara JJ. Characterization of α-adrenoceptor activity in the preterm piglet mesentery. J Pediatr Surg 31:1659–1662, 1996.
75. Chan KL, Hui CW, Chan KW, Fung PC, Wo JY, Tipoe G, Tam PK. Revisiting ischemia and reperfusion injury as a possible cause of necrotizing enterocolitis: role of nitric oxide and superoxide dismutase. J Pediatr Surg 37:828–834, 2002.
76. Sangild PT, Berghorn KA, Nathanielsz PW. Precocious development of disaccharidases in the baboon intestine. Gastroenterology 118: A294, 2000.
77. Sangild PT, Nathanielsz PW, Berghorn KA, Fowden AL. Glucocorticoid treatment prior to preterm birth: effects on intestinal maturation in preterm lambs and baboons (abstract). Gastroenterology 126:A272, 2004.
78. Galand G. Brush border membrane sucrase-isomaltase, maltase-glucoamylase and trehalase in mammals: comparative development, effects of glucocorticoids, molecular mechanisms, and phylogenetic implications. Comp Biochem Physiol B 94:1–11, 1989.
79. Raul F, Lacroix B, Aprahamian M. Longitudinal distribution of brush border hydrolases and morphological maturation in the intestine of the preterm infant. Early Hum Dev 13:225–234, 1986.
80. Sangild PT, Sjöström H, Norén O, Fowden AL, Silver M. The prenatal development and glucocorticoid control of brush-border hydrolases in the pig small intestine. Pediatr Res 37:207–212, 1995.
81. Armand M, Hamosh M, Mehta NR, Angelus PA, Philpott JR, Henderson TR, Dwyer NK, Lairon D, Hamosh P. Effect of human milk or formula on gastric function and fat digestion in the premature infant. Pediatr Res 40:429–437, 1996.
82. Lindberg T, Engberg S, Sjoberg LB, Lonnerdal B. *In vitro* digestion of proteins in human milk fortifiers and in preterm formula. J Pediatr Gastroenterol Nutr 27:30–36, 1998.
83. Henderson TR, Hamosh M, Armand M, Mehta NR, Hamosh P. Gastric proteolysis in preterm infants fed mother's milk or formula. Adv Exp Med Biol 501:403–408, 2001.
84. Lindberg T, Engberg S, Jakobsson I, Lonnerdal B. Digestion of proteins in human milk, human milk fortifier, and preterm formula in infant rhesus monkeys. J Pediatr Gastroenterol Nutr 24:537–543, 1997.
85. Kien CL, McClead RE, Cordero L Jr. *In vivo* lactose digestion in preterm infants. Am J Clin Nutr 64:700–705, 1996.
86. Weaver LT, Laker MF, Nelson R. Neonatal intestinal lactase activity. Arch Dis Child 61:896–899, 1986.
87. Shulman RJ, Schanler RJ, Lau C, Heitkemper M, Ou CN, Smith EO. Early feeding, feeding tolerance, and lactase activity in preterm infants. J Pediatr 133:645–649, 1998.
88. Erasmus HD, Ludwig-Auser HM, Paterson PG, Sun D, Sankaran K. Enhanced weight gain in preterm infants receiving lactase-treated feeds: a randomized, double-blind, controlled trial. J Pediatr 141:532–537, 2002.
89. Shulman RJ. Effect of enteral administration of insulin on intestinal development and feeding tolerance in preterm infants: a pilot study. Arch Dis Child Fetal Neonatal Ed 86:F131–F133, 2002.
90. Favre A, Szylit O, Popot F, Catala I, Rondeau C, Maurage C, Gold F, Borderon JC, Butel MJ. Diet, length of gestation, and fecal short chain fatty acids in healthy premature neonates. J Parenter Enteral Nutr 26: 51–56, 2002.
91. Kwinta P, Mitkowska Z, Kruczek P, Tomasik T, Pietrzyk JJ. Influence of the lactose free and lactose containing diet on prevalence of gram-negative sepsis and feeding intolerance in very low birth weight infants: double-blind randomized trial. Przegl Lek 59(Suppl 1):63–66, 2002.
92. Bjornvad CR, Nielsen TT, Jensen BB, Jensen SK, Sangild PT. Total parenteral nutrition prior to oral feeding predispose to spontaneous

development of NEC in premature piglets (abstract). Gastroenterology 126(Suppl 2):A272, 2004.
93. Sangild PT, Weström BR, Silver M, Fowden AL. Maturational effects of cortisol on the exocrine abomasum and pancreas in fetal sheep. Reprod Fert Dev 7:655–658, 1995.
94. Sangild PT, Weström BR, Fowden AL, Silver M. Developmental regulation of the porcine exocrine pancreas by glucocorticoids. J Pediatr Gastroenterol Nutr 19:204–212, 1994.
95. Sangild PT, Silver M, Fowden AL, Turvey A, Foltman B. Adrenocortical stimulation of stomach development in the prenatal pig. Biol Neonate 65:378–389, 1994.
96. Sangild PT. Biology of the pancreas before birth. In: Zabielski R, Pierzynowski SR, Eds. Biology of the Pancreas in Growing Animals. Amsterdam: Elsevier, pp1–13, 1999.
97. Sangild PT, Bjornvad CR, Fowden AL. The immature intestine is highly responsive to enteral food before birth (abstract). Gastroenterology 126:A143, 2004.
98. Rings EH, Minich DM, Vonk RJ, Stellaard F, Fetter WP, Verkade HJ. Functional development of fat absorption in term and preterm neonates strongly correlates with ability to absorb long-chain fatty acids from intestinal lumen. Pediatr Res 51:57–63, 2002.
99. Shulman RJ. *In vivo* measurements of glucose absorption in preterm infants. Biol Neonate 76:10–18, 1999.
100. Buddington RK, Malo C. Intestinal brush-border membrane enzyme activities and transport functions during the prenatal development of pigs. J Pediatr Gastroenterol Nutr 23:51–64, 1996.
101. Sangild PT, Diemæs L, Christiansen IJ, Skadhauge E. Intestinal transport of sodium, glucose and immunoglobulin in neonatal pigs: effect of glucocorticoids. Exp Physiol 78:485–497, 1993.
102. Axelsson I, Jakobsson I, Lindberg T, Polberger S, Benediktsson B, Raiha N. Macromolecular absorption in preterm and term infants. Acta Paediatr Scand 78:532–537, 1989.
103. Boehm G, Jakobsson I, Mansson M, Raiha NC. Macromolecular absorption in small-for-gestational-age infants. Acta Paediatr 81:864–867, 1992.
104. Kuitunen OO, Savilahti E, Sarnesto A. Human α-lactalbumin and bovine β-lactoglobulin absorption in premature infants. Pediatr Res 35:344–347, 1994.
105. van Elburg RM, Fetter WP, Bunkers CM, Heymans HS. Intestinal permeability in relation to birth weight and gestational and postnatal age. Arch Dis Child Fetal Neonatal Ed 88:F52–F55, 2003.
106. Kacskovics I. Fc receptors in livestock species. Vet Immunol Immunopathol 28:351–362, 2004.
107. Sangild PT, Holtug K, Diemæs L, Schmidt M, Skadhauge E. Birth and prematurity influence intestinal function in the newborn pig. Comp Biochem Physiol A Physiol 118:359–361, 1997.
108. Johnston NE, Stewart JA. The effect of glucocorticoids and prematurity on absorption of colostral immunoglobulin in the calf. Aust Vet J 63:191–192, 1986.
109. Hough RL, McCarthy FD, Thatcher CD, Kent HD, Eversole DE. Influence of glucocorticoid on macromolecular absorption and passive immunity in neonatal lambs. J Anim Sci 68:2459–2464, 1990.
110. Weström BR, Ohlsson BG, Svendsen J, Tagesson C, Karlsson BW. Intestinal transmission of macromolecules (BSA and FITC–dextran) in the neonatal pig: enhancing effect of colostrum, proteins and proteinase inhibitors. Biol Neonate 47:359–366, 1985.
111. Oyachi N, Acosta R, Cho MH, Atkinson JB, Buchmiller-Crair TL, Ross MG. Ontogeny of cholinergic regulation of fetal upper gastrointestinal motility. J Matern Fetal Neonatal Med 14:102–106, 2003.
112. Milla PJ. Intestinal motility during ontogeny and intestinal pseudo-obstruction in children. Pediatr Clin North Am 43:511–532, 1996.
113. Berseth CL, Nordyke CK, Valdes MG, Furlow BL, Go VL. Responses of gastrointestinal peptides and motor activity to milk and water feedings in preterm and term infants. Pediatr Res 31:587–590, 1992.
114. Berseth CL, Nordyke C. Enteral nutrients promote postnatal maturation of intestinal motor activity in preterm infants. Am J Physiol Gastrointest Liver Physiol 264:G1046–G1051, 1993.
115. Weaver LT, Lucas A. Development of bowel habit in preterm infants. Arch Dis Child 68(Suppl):317–320, 1993.
116. McClure RJ, Newell SJ. Randomised controlled trial of trophic feeding and gut motility. Arch Dis Child Fetal Neonatal Ed 80:F54–F58, 1999.
117. Adrian TE, Soltesz G, MacKenzie IZ, Bloom SR, Aynsley-Green A. Gastrointestinal and pancreatic hormones in the human fetus and mother at 18–21 weeks of gestation. Biol Neonate 67:47–53, 1995.
118. Alumets J, Hakanson R, Sundler F. Ontogeny of endocrine cells in porcine gut and pancreas: an immunocytochemical study. Gastroenterology 85:1359–1372, 1983.
119. Guilloteau P, Huerou-Luron IL, Chayvialle JA, Toullec R, Zabielski R, Blum JW. Gut regulatory peptides in young cattle and sheep. Zentralbl Veterinarmed A 44:1–23, 1997.
120. Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res 319:367–382, 2005.
121. van Ginneken C, Van Meir F, Sommereyns G, Sys S, Weyns A. Nitric oxide synthase expression in enteric neurons during development in the pig duodenum. Anat Embryol (Berl) 198:399–408, 1998.
122. van Ginneken C, Verlinden K, Van Meir F, Sys S, Weyns A. A stereologic evaluation of glucagon-like peptide-1 (GLP-1) mucosal cells in the small intestine of the developing pig. Anat Embryol (Berl) 205:153–157, 2002.
123. Lucas A, Bloom SR, Aynsley-Green A. Postnatal surges in plasma gut hormones in term and preterm infants. Biol Neonate 41:63–67, 1982.
124. Tadokoro R, Shimizu T, Hosaka A, Kaneko N, Satoh Y, Yamashiro Y. Postnatal and postprandial changes in plasma concentrations of glicentin in term and preterm infants. Acta Paediatr 92:1175–1179, 2003.
125. Gounaris A, Alexiou N, Costalos C, Daniilidou M, Frangou E, Konstandellou E. Gut hormone concentrations in preterm infants with necrotizing enterocolitis. Acta Paediatr 86:762–763, 1997.
126. Sangild PT, Hilsted L, Nexø E, Fowden AL, Silver M. Secretion of acid, gastrin and cobalamin-binding proteins by the fetal pig stomach: developmental regulation by cortisol. Exp Physiol 79:135–146, 1994.
127. Sangild PT, Hilsted L, Nexø E, Fowden AL, Silver M. Vaginal birth versus elective caesarean section: effects on gastric function in the neonate. Exp Physiol 80:147–157, 1995.
128. Sangild PT, Hilsted L, Bjørnskov-Bartholdy L, Holst JJ, Trahair JF. Gastrin release in the fetal lamb in response to luminal infusion of amniotic fluid, milk or GRP (abstract). Regul Pept 64:167, 1996.
129. Petersen YM, Hartmann B, Holst JJ, Le Huerou-Luron I, Bjornvad CR, Sangild PT. Introduction of enteral food increases plasma GLP-2 and decreases GLP-2 receptor mRNA abundance during pig development. J Nutr 133:1781–1786, 2003.
130. Petersen YM, Burrin DG, Sangild PT. GLP-2 has differential effects on small intestine growth and function in fetal and neonatal pigs. Am J Physiol Regul Comp Integr Physiol 281:R1986–R1993, 2001.
131. Petersen YM, Schmidt M, Elnif E, Sangild PT. Glucagon-like peptide 2 enhances maltase-glucoamylase and sucrase-isomaltase gene expression and activity in parenterally fed premature neonatal pigs. Pediatr Res 52:498–503, 2002.
132. Sigalet DL, Martin G, Meddings J, Hartman B, Holst JJ. GLP-2 levels in infants with intestinal dysfunction. Pediatr Res 56:371–376, 2004.
133. Burrin D, Guan X, Stoll B, Petersen YM, Sangild PT. Glucagon-like peptide 2: a key link between nutrition and intestinal adaptation in neonates? J Nutr 133:3712–3716, 2003.
134. Schwiertz A, Gruhl B, Lobnitz M, Michel P, Radke M, Blaut M. Development of the intestinal bacterial composition in hospitalized

preterm infants in comparison with breast-fed, full-term infants. Pediatr Res 54:393–399, 2003.
135. Caicedo RA, Schanler RJ, Li N, Neu J. The developing intestinal ecosystem: implications for the neonate. Pediatr Res 58:625–628, 2005.
136. Fanaro S, Chierici R, Guerrini P, Vigi V. Intestinal microflora in early infancy: composition and development. Acta Paediatr Suppl 91:48–55, 2003.
137. Claud EC, Walker WA. Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. FASEB J 15:1398–1403, 2001.
138. de la Cochetiere MF, Piloquet H, des Robert C, Darmaun D, Galmiche JP, Roze JC. Early intestinal bacterial colonization and necrotizing enterocolitis in premature infants: the putative role of Clostridium. Pediatr Res 56:366–370, 2004.
139. Chan KL, Ng SP, Chan KW, Wo YH, Tam PK. Pathogenesis of neonatal necrotizing enterocolitis: a study of the role of intraluminal pressure, age and bacterial concentration. Pediatr Surg Int 19:573–577, 2003.
140. Lin J. Too much short chain fatty acids cause neonatal necrotizing enterocolitis. Med Hypotheses 62:291–293, 2004.
141. Agarwal R, Sharma N, Chaudhry R, Deorari A, Paul VK, Gewolb IH, Panigrahi P. Effects of oral *Lactobacillus* GG on enteric microflora in low-birth-weight neonates. J Pediatr Gastroenterol Nutr 36:397–402, 2003.
142. Hammerman C, Bin-Nun A, Kaplan M. Germ warfare: probiotics in defense of the premature gut. Clin Perinatol 31:489–500, 2004.
143. Siggers RH, Siggers, JL, Leser T, Sangild PT. Probiotics improve gastrointestinal structure and function in preterm pigs. Scand J Food Nutr 50(Suppl 1):31–32, 2006.
144. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, Oh W. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics 115:1–4, 2005.
145. Martin R, Langa S, Reviriego C, Jiminez E, Marin ML, Xaus J, Fernandez L, Rodriguez JM. Human milk is a source of lactic acid bacteria for the infant gut. J Pediatr 143:754–758, 2003.
146. Vieten D, Corfield A, Carroll D, Ramani P, Spicer R. Impaired mucosal regeneration in neonatal necrotising enterocolitis. Pediatr Surg Int 21:153–160, 2005.
147. Omari TI, Davidson GP. Multipoint measurement of intragastric pH in healthy preterm infants. Arch Dis Child Fetal Neonatal Ed 88:F517–F520, 2003.
148. Kuitunen M, Savilahti E. Mucosal IgA, mucosal cow's milk antibodies, serum cow's milk antibodies and gastrointestinal permeability in infants. Pediatr Allergy Immunol 6:30–35, 1995.
149. Neely M, Toltzis P. Infection control in pediatric hospitals. Curr Opin Infect Dis 14:449–453, 2001.
150. Hallstrom M, Eerola E, Vuento R, Janas M, Tammela O. Effects of mode of delivery and necrotising enterocolitis on the intestinal microflora in preterm infants. Eur J Clin Microbiol Infect Dis 23:463–470, 2004.
151. Usowicz AG, Dab SB, Emery JR, McCann EM, Brady JP. Does gastric acid protect the preterm infant from bacteria in unheated human milk? Early Hum Dev 16:27–33, 1988.
152. Gronlund MM, Salminen S, Mykkanen H, Kero P, Lehtonen OP. Development of intestinal bacterial enzymes in infants: relationship to mode of delivery and type of feeding. APMIS 107:655–660, 1999.
153. Siggers RH, Thymann T, Sangild PT. Mode of delivery affects diet-induced small intestine atrophy and dysfunction in preterm pigs (abstract). Scand J Food Nutr 50(Suppl 1):51, 2006.
154. Waligora-Dupriet AJ, Dugay A, Auzeil N, Huerre M, Butel MJ. Evidence for clostridial implication in necrotizing enterocolitis through bacterial fermentation in a gnotobiotic quail model. Pediatr Res 58:629–635, 2005.
155. Ewer AK, Al-Salti W, Coney AM, Marshall JM, Ramani P, Booth IW. The role of platelet activating factor in a neonatal piglet model of necrotising enterocolitis. Gut 53:207–213, 2004.
156. Grishin AV, Wang J, Potoka DA, Hackam DJ, Upperman JS, Boyle P, Zamora R, Ford HR. Lipopolysaccharide induces cyclooxygenase-2 in intestinal epithelium *via* a noncanonical p38 MAPK pathway. J Immunol 176:580–588, 2006.
157. Chan KL, Ho JC, Chan KW, Tam PK. A study of gut immunity to enteral endotoxin in rats of different ages: a possible cause for necrotizing enterocolitis. J Pediatr Surg 37:1435–1440, 2002.
158. Romagnoli C, Frezza S, Cingolani A, De Luca A, Puopolo M, De Carolis MP, Vento G, Antinori A, Tortorolo G. Plasma levels of interleukin-6 and interleukin-10 in preterm neonates evaluated for sepsis. Eur J Pediatr 160:345–350, 2001.
159. Kelly N, Friend K, Boyle P, Zhang XR, Wong C, Hackam DJ, Zamora R, Ford HR, Upperman JS. The role of the glutathione antioxidant system in gut barrier failure in a rodent model of experimental necrotizing enterocolitis. Surgery 136:557–566, 2004.
160. Ford H, Watkins S, Reblock K, Rowe M. The role of inflammatory cytokines and nitric oxide in the pathogenesis of necrotizing enterocolitis. J Pediatr Surg 32:275–282, 1997.
161. Di Lorenzo M, Krantis A. Nitric oxide synthase isoenzyme activities in a premature piglet model of necrotizing enterocolitis: effects of nitrergic manipulation. Pediatr Surg Int 18:624–629, 2002.
162. Schanler RJ, Atkinson SA. Effects of nutrients in human milk on the recipient premature infant. J Mammary Gland Biol Neoplasia 4:297–307, 1999.
163. Gross SJ, Buckley RH, Wakil SS, McAllister DC, David RJ, Faix RG. Elevated IgA concentration in milk produced by mothers delivered of preterm infants. J Pediatr 99:389–393, 1981.
164. Goldman AS. The immunological system in human milk: the past—a pathway to the future. Adv Nutr Res 10:15–37, 2001.
165. Rueda R, Maldonado J, Narbona E, Gil A. Neonatal dietary gangliosides. Early Hum Dev 53(Suppl):S135–S147, 1998.
166. Miniello VL, Moro GE, Armenio L. Prebiotics in infant milk formulas: new perspectives. Acta Paediatr Suppl 91:68–76, 2003.
167. Boehm G, Jelinek J, Stahl B, van Laere K, Knol J, Fanaro S, Moro G, Vigi V. Prebiotics in infant formulas. J Clin Gastroenterol 38:S76–S79, 2004.
168. Kullen MJ, Bettler J. The delivery of probiotics and prebiotics to infants. Curr Pharm Des 11:55–74, 2005.
169. Tlaskalova-Hogenova H, Stepankova R, Hudcovic T, Tuckova L, Cukrowska B, Lodinova-Zadnikova R, Kozakova H, Rossmann P, Bartova J, Sokol D, Funda DP, Borovska D, Rehakova Z, Sinkora J, Hofman J, Drastich P, Kokesova A. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunol Lett 15:97–108, 2004.
170. Martinez-Augustin O, Boza JJ, Del Pino JI, Lucena J, Martinez-Valverde A, Gil A. Dietary nucleotides might influence the humoral immune response against cow's milk proteins in preterm neonates. Biol Neonate 71:215–223, 1997.
171. Maheshwari A, Christensen RD, Calhoun DA. ELR+CXC chemokines in human milk. Cytokine 24:91–102, 2003.
172. Minekawa R, Takeda T, Sakata M, Hayashi M, Isobe A, Yamamoto T, Tasaka K, Murata Y. Human breast milk suppresses the transcriptional regulation of IL-1β–induced NF-κB signaling in human intestinal cells. Am J Physiol 287:C1404–C1411, 2004.
173. Sangild PT, Xu RJ. Colostrum. In: Pond WG, Bell AW, Eds. Encyclopedia of Animal Science. New York: Marcel Dekker, pp1–3, 2004.
174. Berseth CL, Michener SR, Nordyke CK, Go VL. Postpartum changes in pattern of gastrointestinal regulatory peptides in human milk. Am J Clin Nutr 51:985–990, 1990.
175. Nagashima K, Itoh K, Kuroume T. Levels of insulin-like growth factor I in full- and preterm human milk in comparison to levels in cow's milk and in milk formulas. Biol Neonate 58:343–346, 1990.

176. Dvorak B, Fituch CC, Williams CS, Hurst NM, Schanler RJ. Increased epidermal growth factor levels in human milk of mothers with extremely premature infants. Pediatr Res 54:15–19, 2003.
177. Shehadeh N, Khaesh-Goldberg E, Shamir R, Perlman R, Sujov P, Tamir A, Makhoul IR. Insulin in human milk: postpartum changes and effect of gestational age. Arch Dis Child Fetal Neonatal Ed 88:F214–F216, 2003.
178. Wang T, Huo YJ, Shi F, Xu RJ, Hutz RJ. Effects of intrauterine growth retardation on development of the gastrointestinal tract in neonatal pigs. Biol Neonate 21:66–72, 2005.
179. Mei J, Zhang Y, King D, Sangild PT, Xu RJ. Distribution and developmental changes of transforming growth factor β receptors in the gastrointestinal tract of pigs. J Anim Vet Adv 3:89–106, 2003.
180. Georgieva TM, Georgiev IP, Ontsouka E, Hammon HM, Pfaffl MW, Blum JW. Abundance of message for insulin-like growth factors-I and -II and for receptors for growth hormone, insulin-like growth factors-I and -II, and insulin in the intestine and liver of pre- and full-term calves. J Anim Sci 81:2294–2300, 2003.
181. Sangild PT. Stimulation of gastric proteases in the neonatal pig by a rise in adrenocortical secretion at parturition. Reprod Fert Devel 7: 1293–1298, 1995.
182. Britton JR, Koldovsky O. Development of luminal digestion: implications for biologically active dietary polypeptides. J Pediatr Gastroenterol Nutr 9:144–162, 1989.
183. Burrin DG, Wester TJ, Davis TA, Amick S, Heath JP. Orally administered IGF-I increases intestinal mucosal growth in formula-fed neonatal pigs. Am J Physiol Regul Comp Integr Physiol 270:R1085–R1091, 1996.
184. Blum JW, Baumrucker CR. Colostral and milk insulin-like growth factors and related substances: mammary gland and neonatal (intestinal and systemic) targets. Domest Anim Endocrinol 23:101–110, 2002.
185. Mei J, Zhang Y, Wang T, Sangild PT, Xu RJ. Oral ingestion of colostrum alters intestinal transforming growth factor-beta receptor intensity in newborn pigs. Livest Sci (in press), 2006.
186. Fang S, Kempley ST, Gamsu HR. Prediction of early tolerance to enteral feeding in preterm infants by measurement of superior mesenteric artery blood flow velocity. Arch Dis Child Fetal Neonatal Ed 85:F42–F45, 2001.
187. Maruyama K, Koizumi T, Tomomasa T, Morikawa A. Intestinal blood-flow velocity in uncomplicated preterm infants during the early neonatal period. Pediatr Radiol 29:472–477, 1999.
188. Costalos C, Gounaris A, Sevastiadou S, Hatzistamatiou Z, Theodoraki M, Alexiou EN, Constandellou E. The effect of antenatal corticosteroids on gut peptides of preterm infants—a matched group comparison: corticosteroids and gut development. Early Hum Dev 74:83–88, 2003.
189. Shulkes A, Hardy KJ. Ontogeny of circulating gastrin and pancreatic polypeptide in the foetal sheep. Acta Endocrinol (Copenh) 100:565–572, 1982.
190. Guilloteau P, Le Huerou-Luron I, Le Drean G, Gestin M, Philouze-Rome V, Artiaga A, Bernard C, Chayvialle JA. Gut regulatory peptide levels in bovine fetuses and their dams between the 3rd and 9th months of gestation. Biol Neonate 74:430–438, 1998.
191. Shulman RJ, Schanler RJ, Lau C, Heitkemper M, Ou CN, Smith EO. Early feeding, antenatal glucocorticoids, and human milk decrease intestinal permeability in preterm infants. Pediatr Res 44:519–523, 1998.
192. Israel EJ, Simister N, Freiberg E, Caplan A, Walker WA. Immunoglobulin G binding sites on the human foetal intestine: a possible mechanism for the passive transfer of immunity from mother to infant. Immunology 79:77–81, 1993.