ANNUAL REVIEWS

Annual Review of Animal Biosciences

Translational Advances in Pediatric Nutrition and Gastroenterology: New Insights from Pig Models

Douglas Burrin,[1] Per Torp Sangild,[2] Barbara Stoll,[1] Thomas Thymann,[2] Randal Buddington,[3] Juan Marini,[1,4] Oluyinka Olutoye,[5] and Robert J. Shulman[1]

[1]USDA-ARS Children's Nutrition Research Center, Department of Pediatrics, Division of Gastroenterology, Hepatology, and Nutrition, Baylor College of Medicine, Houston, Texas 77030, USA; email: doug.burrin@usda.gov

[2]Comparative Pediatrics and Nutrition, University of Copenhagen, DK-1870 Frederiksberg C., Copenhagen, Denmark

[3]College of Nursing, University of Tennessee Health Science Center, Memphis, Tennessee 38163, USA

[4]Department of Pediatrics, Section of Critical Care Medicine, Baylor College of Medicine, Houston, Texas 77030, USA

[5]Division of Pediatric Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, Texas 77030, USA

Annu. Rev. Anim. Biosci. 2020. 8:321–54

The *Annual Review of Animal Biosciences* is online at animal.annualreviews.org

https://doi.org/10.1146/annurev-animal-020518-115142

Keywords

total parenteral nutrition, premature infants, necrotizing enterocolitis, short bowel syndrome, intestinal growth, glucagon-like peptide 2, parenteral lipid emulsions

Abstract

Pigs are increasingly important animals for modeling human pediatric nutrition and gastroenterology and complementing mechanistic studies in rodents. The comparative advantages in size and physiology of the neonatal pig have led to new translational and clinically relevant models of important diseases of the gastrointestinal tract and liver in premature infants. Studies in pigs have established the essential roles of prematurity, microbial colonization, and enteral nutrition in the pathogenesis of necrotizing enterocolitis. Studies in neonatal pigs have demonstrated the intestinal trophic effects of a

key gut hormone, glucagon-like peptide 2 (GLP-2), and its role in the intestinal adaptation process and efficacy in the treatment of short bowel syndrome. Further, pigs have been instrumental in elucidating the physiology of parenteral nutrition–associated liver disease and the means by which phytosterols, fibroblast growth factor 19, and a new generation of lipid emulsions may modify disease. The premature pig will continue to be a valuable model in the development of optimal infant diets (donor human milk, colostrum), specific milk bioactives (arginine, growth factors), gut microbiota modifiers (pre-, pro-, and antibiotics), pharmaceutical drugs (GLP-2 analogs, FXR agonists), and novel diagnostic tools (near-infrared spectroscopy) to prevent and treat these pediatric diseases.

INTRODUCTION

In the past 30 years, the pig has become a well-recognized model for human pediatric nutrition at all phases of early development, including fetal, premature, newborn, neonatal, and weanling ages. From a comparative biology perspective, pigs offer many advantages compared with other widely used animal models, mainly mice and rats, to support their validity as a model of normal, healthy human infant anatomy, physiology, and metabolism (1–6). As the use of pigs in human biomedical research has expanded, new frontiers have been established for their use in modeling fundamental principles in emerging areas of nutrition and gut microbiota development and host immune function, as well as the pathology and therapeutic approaches for treatment of complex human diseases that originate during infancy. This review describes how recent research using pigs has advanced our understanding of the role of nutrition and metabolism in the physiology and pathogenesis of several important diseases in pediatric gastroenterology that occur in premature infants.

Challenges of Nutritional Support of Premature Human Infants

Prematurity and low birth weight continue to be significant challenges in the care and nutritional support of human infants in the United States and worldwide (7–9). These infants have an immature gastrointestinal tract and innate immunity that increase morbidity and metabolic stress and lead to increased incidence of diseases such as necrotizing enterocolitis (NEC), short bowel syndrome (SBS), and parenteral nutrition–associated liver disease (PNALD) (10–12) (**Figure 1**). These three conditions form a cascade of disease risks that face premature infants, and some incur more than one of these in the first weeks and months of life. These underlying conditions create a critical need to provide the optimal amount and composition of nutritional support to sustain normal growth, organ development, and function. However, despite the significant advancements and improved survival rates that have occurred in the past three decades in human perinatal clinical medicine, many premature and very-low-birth-weight (VLBW) infants still experience poor growth and neurodevelopmental outcomes (13–16).

NEC: necrotizing enterocolitis

SBS: short bowel syndrome

PNALD: parenteral nutrition–associated liver disease

VLBW: very low birth weight

Given the continuous challenge of prematurity to human pediatric nutrition and gastrointestinal disease and the ethical limitations of human investigation, it has become increasingly important to study the biology of premature and VLBW infants using translational animal models. The pig presents key advantages, including the ability for viable preterm delivery by cesarean section at late stages of gestation, which allows for various clinically relevant interventions, such as parenteral nutrition (PN), surgical intestinal resection, and orogastric tube feeding (**Figure 2**). This key feature, coupled with many of the previously established advantages, such as close comparative physiology

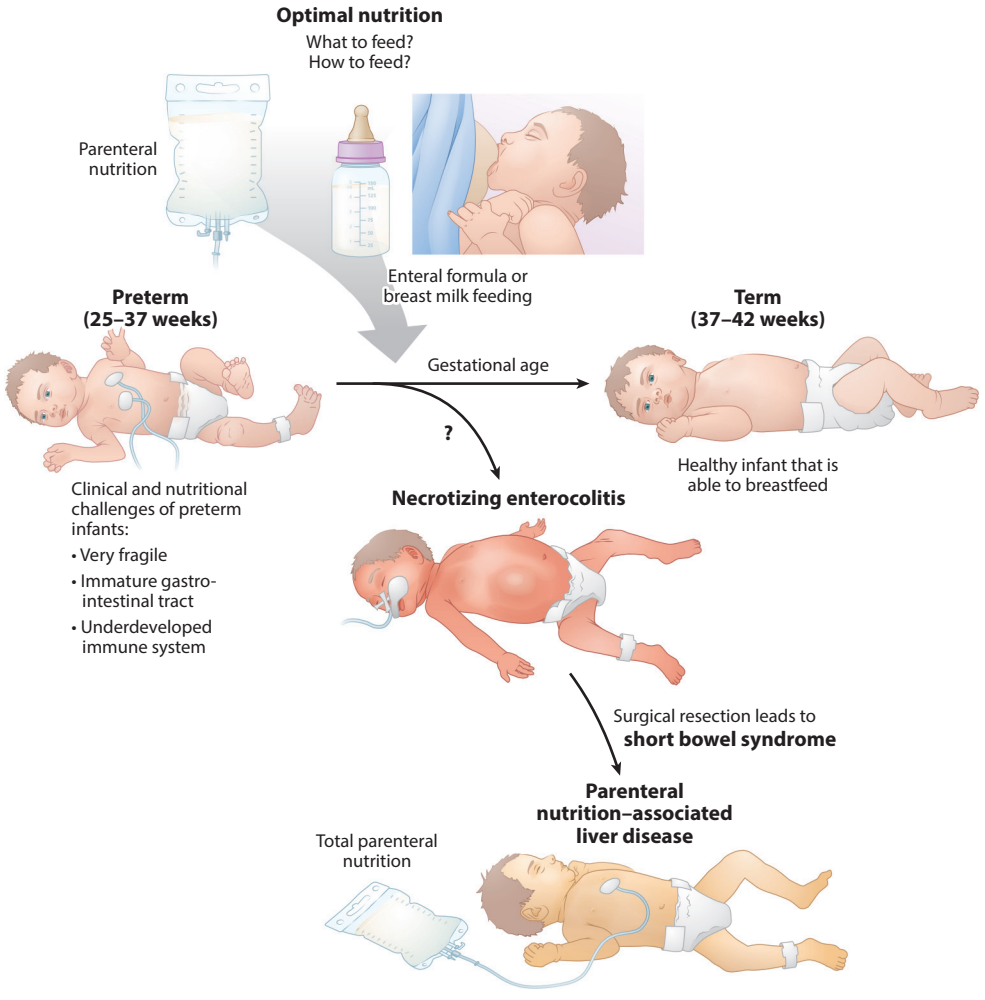


Figure 1

Clinical and nutritional challenges of preterm infants. Shown is an overview of the fundamental choices of how and what to feed preterm compared to term infants. These choices include intravenous parenteral nutrition (i.e., TPN) or enteral nutrition composed of infant formula or breast milk. The combination of immature intestinal digestive and immune function increases the risk for NEC, which can lead to intestinal resection and SBS. Prolonged parenteral nutrition resulting from GI disease (SBS) or other clinical morbidities increases the risk for PNALD. Abbreviations: GI, gastrointestinal; NEC, necrotizing enterocolitis; PN, parenteral nutrition; PNALD, parenteral nutrition–associated liver disease; SBS, short bowel syndrome; TPN, total parenteral nutrition.

and anatomy of the gastrointestinal tract, omnivorous diet, milk composition, body composition, and litter bearing, makes the pig a unique and cost-effective model (versus nonhuman primates). The feature of prematurity provides an especially clinically relevant model of NEC that allows the investigation of host-related biology and how it contributes to disease pathogenesis.

An important question pertains to the relative stage of prematurity of pigs versus human infants given the difference in gestation length (115 versus 280 days, respectively). The gestational length of commercial pigs can vary from approximately 115 days for most US herds to more than

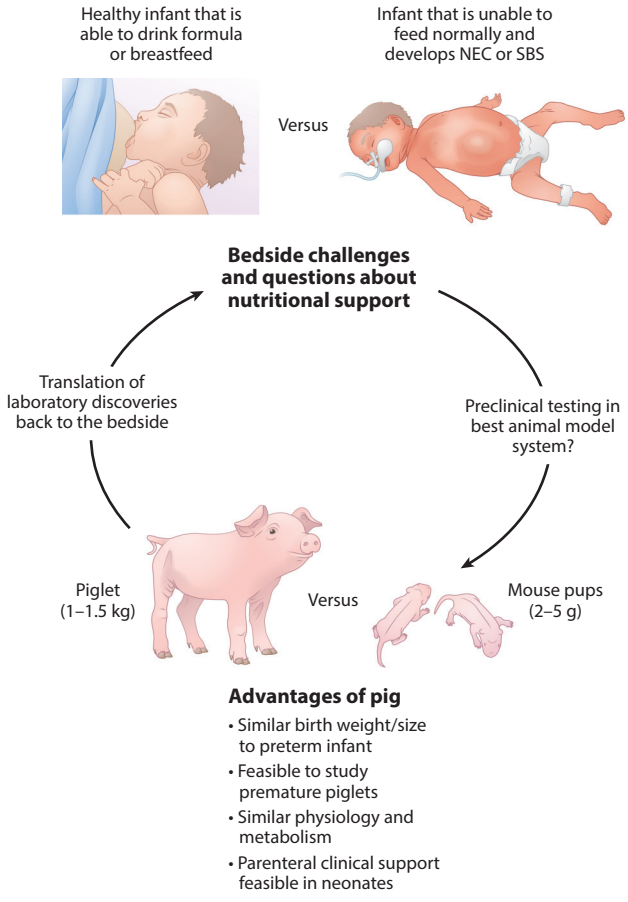


Figure 2

Translational relevance and advantages of the pig to investigate human pediatric nutrition and GI diseases. The similarities of the newborn pig to human infants in terms of body size, anatomy, and physiology provide a well-suited animal model to test questions that are not feasible or ethically possible in human infants. The pig also enables the use of clinical procedures and therapies (e.g., TPN, orogastric tube feeding, surgical intestinal resection) used in human infants that more closely simulate current medical practices. The advantages of newborn mice are lower cost and widely available genetic mouse models to test specific gene effects on GI and liver disease. Abbreviations: GI, gastrointestinal; NEC, necrotizing enterocolitis; PN, parenteral nutrition; SBS, short bowel syndrome; TPN, total parenteral nutrition.

117 days in Danish herds (4). Litter sizes also vary, being larger among Danish herds (~15–20 pigs) compared with those in the United States (10–12 pigs) as a result of genetic selection (17). The large litter size also results in varying birth weights, and pigs born with low birth weight or small for gestational age often occur spontaneously, which provides a model of natural intrauterine-growth retardation (18, 19). An assessment of the relative stage of prematurity in pigs compared with human infants is difficult, but our estimate is that preterm pigs born at 90% gestation are comparable to human preterm infants at 75% gestation (30–32 weeks) (4). This estimate is based on development of various organs; however, the gut is less mature and the brain more mature in pigs than in humans at an equivalent stage of gestation. Another difference is that newborn, term pigs are considered to be precocial, whereas term human infants are altricial; pigs are born with

locomotive ability even at one week preterm. As in humans, the key factor limiting the survival of preterm pigs is respiratory function, and recent evidence shows that pigs born at a gestational age of 102 days or earlier often die from respiratory distress (20, 21). Lung development of pigs born at 102 days of gestation is similar to that in 28–30-week infants with an intermediate stage of saccular alveoli development (20).

TPN: total parenteral nutrition

GLP-2: glucagon-like peptide 2

How, What, and When to Feed Preterm Infants

As a result of many of the underlying concerns noted above, basic questions continue to challenge clinicians, such as how, what, how much, and when to feed premature and VLBW infants. Owing to the immaturity in gastroduodenal motor function and the poorly developed ability to suck and swallow, nearly 70% of hospitalized premature VLBW infants experience enteral feeding intolerance and are nourished via PN for a substantial period after birth (22). In the past 50 years, PN has become a standard-of-care, lifesaving clinical support therapy for premature infants (23, 24). Despite the significant clinical benefit of PN, some clinical studies (25, 26) and many animal studies (27), including those in neonatal pigs (28–35), have shown that exclusive or total parenteral nutrition (TPN) leads to intestinal atrophy, reduced gastrointestinal blood flow, digestive dysfunction, and delayed development of the mucosal epithelium and innate immune function (**Figure 3**). These neonatal pig studies showed that within 8–12 h of removal of enteral nutrition and maintaining TPN, portal venous and superior mesenteric atrial blood flow is reduced by ~50%, epithelial cell apoptosis increases, and villus atrophy occurs (32). As villus atrophy and loss of mucosal mass progress, there is a reduction in intestinal digestive enzyme activity and capacity for lactose digestion and hexose absorption (30). PN provision results in a lack of luminal stimulus of the gut by enteral nutrients and reduced gastrointestinal secretions. Moreover, as in human infants (36), studies in neonatal pigs showed that enteral nutrition is important for secretion of many gut peptide hormones and growth factors, especially glucagon-like peptide 2 (GLP-2) (29). Thus, the need to provide an early and safe enteral stimulus to the premature intestine led to the concept of minimal enteral nutrition that is now widely used in clinical practice (37–40). Minimal enteral nutrition, or trophic feeding, involves the enteral administration of small amounts of human milk or formula in the early days after birth, followed by a gradual advancement of enteral feeding volumes until infants reach full enteral feeding. Studies in pigs demonstrated the quantitative importance of enteral nutrition and the amount necessary to sustain intestinal growth (29, 41). The questions of timing of enteral feeding and rate of volume advancement are also important considerations in premature infants, and early versus late feeding has been shown to influence the incidence of intestinal dysfunction in pigs (42–44).

Most premature and VLBW infants are started on PN immediately after birth, and the standard recommendation is to introduce minimal enteral feeding using human milk, either mother's own breast milk or donor human milk, owing to its well-established benefits for health and outcome (45–48). For many preterm infants, however, human milk is not available, and infant formula is fed instead. The underlying biological mechanisms to explain the health benefits of human milk versus formula are ethically difficult to study in human infants, yet studies in term and preterm pigs have revealed important clues. Our early studies in term, newborn pigs showed that pig colostrum and mature milk provide an important trophic stimulus to the gut, skeletal muscle, and brain and improve digestive function compared with formula (49–52). More recent studies in preterm pigs have shown that natural milks from pigs, humans, and cows provide a greater intestinal trophic stimulus and function advantage compared with infant formulas (53–57), findings that are reflected in our studies in preterm infants (39, 40). The increasing evidence to promote human milk feeding has prompted greater efforts to develop human milk banking to provide donor human milk

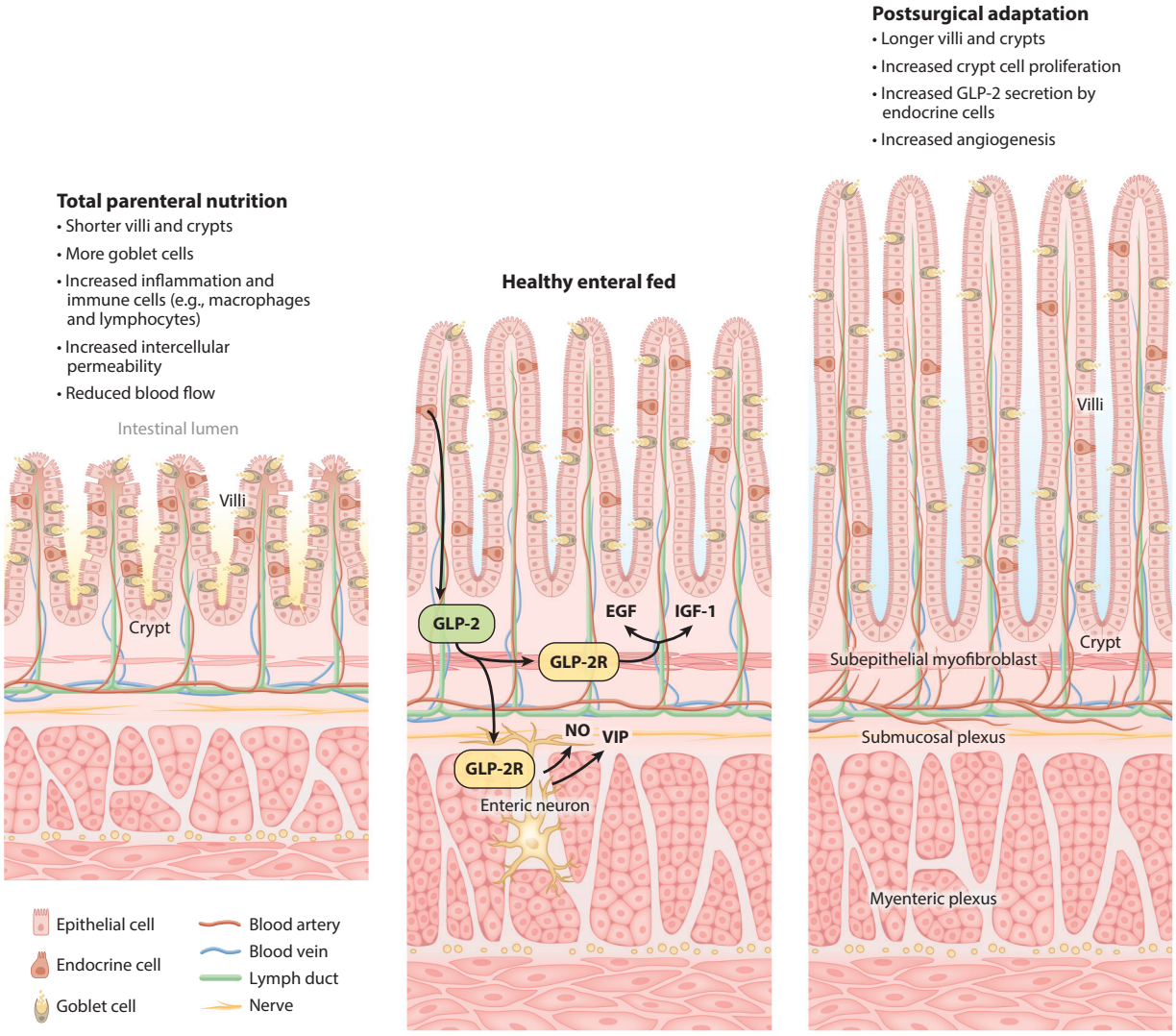


Figure 3

Intestinal mucosal adaptation to TPN and intestinal resection. Illustrated is the influence of TPN, a common clinical practice in hospitalized preterm infants, which deprives the gut lumen of enteral nutrition and results in mucosal villus atrophy, deterioration of intestinal barrier function, and infiltration of immune cells. Also shown is the influence of surgical resection of intestine, which occurs due to congenital and acquired GI diseases, that results in activation of adaptive processes that promote mucosal growth, such as increased GLP-2 secretion, crypt cell proliferation, and blood flow. GLP-2 is a key gut hormone that functions to activate mucosal enteric neuron release of NO and VIP as well as subepithelial fibroblast release of EGF and IGF-1. Abbreviations: EGF, epidermal growth factor; GI, gastrointestinal; GLP-2, glucagon-like peptide 2; GLP-2R, glucagon-like peptide 2 receptor; IGF-1, insulin-like growth factor 1; NO, nitric oxide; TPN, total parenteral nutrition; VIP, vasoactive intestinal peptide.

to preterm infants. Thus, the biological value of various forms of human milk, such as banked donor milk, banked mother's own milk, or expressed and breastfed mother's milk, and how they are processed has become a hot topic in pediatric nutrition (58). Recent studies in preterm pigs suggest that the method of pasteurization may impact the protective role of donor human milk (59). Also important is the fact that the content of some key nutrients in human milk fails to meet

preterm infant nutrient needs, and thus fortification of human milk with supplements is necessary (60). A key question regarding human milk fortification is whether the supplements should be derived from human or bovine milk. The nutritional value of human milk fortifiers has been investigated in preterm pigs, and recent evidence suggests that fortification of human milk with bovine colostrum provides improved growth and intestinal function compared with commercial bovine milk formula-based fortifiers (61). Importantly, this latter result in preterm pigs may be translationally relevant given recent preliminary results from a stepwise safety study suggesting that pasteurized bovine colostrum was safe and well tolerated in premature infants (62).

MODELING NECROTIZING ENTEROCOLITIS IN PRETERM PIGS

In the past several decades, NEC has been the leading cause of infant death from gastrointestinal disease, affecting 5–10% of those born premature in North America (11, 63, 64). The disease has a mortality as high as 50%, and surgical intervention is necessary in 20–40% of cases, leading to increased morbidity. The incidence of NEC is directly related to the degree of prematurity. Thus, with improved clinical care and increased survival of preterm, extremely low-birth-weight infants, this disease will continue to be a challenge in pediatric nutrition. For several decades, rodents have been the primary model used to study the pathology of NEC (63, 65, 66). The advantage of rodent models is their lower cost, short gestation period, and relatively large litters, as well as the ease of housing and maintaining rodent colonies. An especially important advantage of mouse models is the ability to examine single gene mechanisms using mouse genetics and commercially available transgenic and knockout models. The main limitations of the rodent models include the difficulties owing to their small size and fragility and the challenges in gavage feeding. Most rodent models of NEC evolved from the studies by Barlow et al. (67), in which histological changes representative of NEC were induced in newborn rats by using formula feeding, adding bacteria, hypoxia, and/or hypothermia. Since then, modifications have been made in some mouse models that also incorporate prematurity by delivering the pups by cesarean section 12–24 h prior to term (65, 68). Studies in rats and mice have been important in highlighting some of the key factors that contribute to the incidence and pathogenesis of NEC, namely enteral feeding, bacterial colonization, and host factors of immunology and vascular physiology.

Early pig studies investigating NEC imposed asphyxia, hypoxia, and modified dietary approaches (69–73) mostly in term, newborn pigs. However, in the past decade, several important advancements in pig studies have created a more clinically relevant model of NEC (4, 6). The premature pig model of NEC reproduces key features whereby, like in the human infant, the NEC incidence in preterm pigs is markedly higher than in term pigs (53). The pig model reproduces all the hallmark clinical and histological features of NEC in human infants, including abdominal distension, cyanosis, radiographic evidence of pneumatosis intestinalis, tissue coagulation necrosis, villus sloughing, mucosal tissue edema, and leukocyte infiltration. These histological signs of NEC also localize to the distal intestine and colon, as in humans, but early onset in the pig (<24 h) results in fulminant disease from the stomach to the large intestine. Whereas the rodent models require treatment with hypoxia, hypothermia, endotoxin, or bacteria, the preterm piglet develops NEC spontaneously within 2–4 days of feeding infant formulas or a combination of PN and formula—analogous to the preterm infant. Thus, the pig model recapitulates NEC disease using the standard clinical nutrition support given to human preterm infants. The pig NEC model diverges from clinical observations in humans in that disease develops sooner (2–4 days versus 2–4 weeks postnatally) and at higher incidence (30–80% versus 5–10%) than in the preterm infant, respectively.

An experimental approach that may hold promise for NEC research is the use of ex vivo intestinal enteroids that are being used to model host epithelial–pathogen interactions (74). A

recent report showed the enteroids isolated from intestine from early- and late-stage fetuses and adults showed differential gene expression profiles and responses to lipopolysaccharide (LPS) (75). Gonzalez and colleagues (76–78) adapted the enteroid model using pigs and showed that enteroids isolated from intestine subjected to ischemic damage exhibit a blunted response compared with those derived from healthy intestine. We recently demonstrated the developmental changes in citrulline production capacity of preterm and term intestinal enteroids (79). Thus, enteroids appear to recapitulate the developmental program and responsiveness to injury observed in host intestine during NEC.

LPS: lipopolysaccharide

EGF: epidermal growth factor

Role of Enteral Feeding and Diet in Necrotizing Enterocolitis Pathogenesis

Early clinical observations of NEC led to the hypothesis that enteral feeding triggers the disease. This thinking has led to the clinical practice of minimal enteral feeding (discussed above), whereby preterm infants at risk of NEC are given small-volume nasogastric feeds in the first few days after birth, which are increased depending on feeding tolerance. This practice prompted many physicians to adopt delayed introduction of enteral feeds (e.g., early versus delayed) and varying rates of enteral feed advancement (e.g., slow versus rapid) to reduce the incidence of NEC in preterm infants. The evidence is inconclusive regarding whether these practices are effective in preventing NEC in preterm infants (43, 65, 80). Our studies in preterm pigs showed that delayed initiation, but not gradual advancement of enteral feeding, is protective against NEC (42). The alternative to the idea that enteral feeding promotes NEC is that TPN or partial PN can prevent NEC. However, as noted above, it is well established in neonatal pig studies that TPN results in delayed intestinal growth and development marked by mucosal atrophy, compromised barrier, reduced digestive and transport function, and increased local inflammation (29–32).

The practice of feeding breast milk or human milk is the most well-established dietary approach to prevent NEC (45, 46, 58, 81). The promotion of human milk feeding to preterm infants has increased the interest in whether banked human donor breast milk is as effective in NEC prevention as mother's own milk. Several studies in preterm pigs have shown that milk from various species, including swine colostrum and mature milk, bovine colostrum, and donor human milk, protects against NEC (53, 55, 82). These pig studies also showed that the efficacy of unprocessed donor human milk in preventing NEC seems to slightly diminish with increased processing methods, such as pasteurization and spray drying. The advantage of breast milk versus formula has been linked to the many growth factors and immune-related proteins in breast milk, such as insulin, insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), lactoferrin, and transforming growth factor β (2, 83, 84). Clinical studies suggest that enteral lactoferrin may prevent NEC (85) and that oral insulin may improve feeding tolerance and enhance lactase activity in preterm infants and in pediatric SBS (86, 87). Studies examining formula supplementation with these milk-borne factors in term pigs produced mixed results, with some showing intestinal trophic effects (88–90) and protection against pathogen infection but limited efficacy in improving digestive function and prevention of NEC (91–93).

Enteral feeding as a causal factor in NEC has been linked to the idea that immature or insufficient digestive capacity of the premature intestine leads to malabsorption and bacterial overgrowth. Lactose is the most abundant carbohydrate in human milk, as it is in other mammalian species. Yet, studies in premature infants demonstrated that some degree of lactose malabsorption and colonic fermentation occur and that both lactose digestion and glucose absorption are increased with gestational age (94–97). Studies in preterm and term pigs support clinical findings and show that glucose transport and sodium-dependent, glucose transporter expression are upregulated in late gestation (98). Studies in preterm pigs also showed that lactose digestive capacity is reduced in formula- versus colostrum-fed pigs (52). Clinical studies have suggested that

preterm infants have poor lactose digestive capacity and that low-lactose formulas reduce feeding intolerance (39, 99, 100). These findings led to concerns that lactose could predispose to NEC, yet the clinical evidence to support this concern was limited (101). Studies in preterm infants showed that the capacity for digestion and absorption of glucose polymers was greater than that for lactose (96), consistent with the developmental pattern of disaccharidase enzymes in human infants (102). The concerns about lactose malabsorption and risk of NEC raised by clinical studies were followed by changes in the formulations of commercial preterm formulas. Current preterm infant formulas contain lactose and maltodextrins or corn syrup solids, a partially digested form of starch, at a ratio of approximately 40:60, respectively. Recent studies in preterm pigs show that formulas containing maltodextrin or corn syrup solids result in significantly higher rates of NEC compared with lactose-containing formulas (103–106). The inclusion of corn syrup solids with up to 50–100% total carbohydrate content leads to a threefold increase in NEC incidence. It should be noted that pigs have a much higher capacity to digest lactose than maltose or glucose polymers, and this is reflected in malabsorption of maltodextrin in preterm pigs (103). The addition of corn syrup solids did not markedly change the gut microbiome profile but resulted in distinct gut metabolomic signatures that clustered with NEC disease (106). These findings imply that the addition of maltodextrin or corn syrup solids may not alter the microbial community structure but rather leads to markedly different products of microbial fermentation compared with lactose. The adverse proinflammatory effects of additive maltodextrins observed in preterm pigs are consistent with other reports in mice showing that maltodextrin promotes depletion of the intestinal mucus layer, enhances the formation of pathogen biofilms, and increases endoplasmic reticulum stress in gut epithelial cells (107–109). These findings raise the question of what is the most appropriate carbohydrate composition in preterm infant formulas. Human milk does not contain maltodextrin or corn syrup solids; thus, the addition of these carbohydrates to current preterm infant formulas is at odds with the desire to model the composition of human milk.

Following preterm birth, the enteral swallowing of amniotic fluid is replaced with variable combinations of PN and advancing volumes of enteral milk diet. Similar to breast milk, amniotic fluid may contain important immunomodulatory, growth, and antimicrobial factors and exosomes that may act similarly to facilitate tolerance to bacterial antigens and dampen excessive Toll-like receptor (TLR)-mediated responses in the perinatal period (110–112). Studies in preterm pigs and mice have shown that feeding amniotic fluid postnatally reduces intestinal inflammation and NEC (113, 114).

Role of the Microbiota in Necrotizing Enterocolitis Pathogenesis

The role of the microbiota in the development and function of the infant gastrointestinal tract is one of the most rapidly advancing areas of research owing to the development of genomic sequencing approaches to identify microbes in different body sites and fluids. Recent reports of microbial DNA in placenta, amniotic fluid, and meconium samples from preterm infants have challenged the idea that the fetal environment is sterile (115). The presence of milk-associated microbiota has been implicated as another factor in the protective effects of breastfeeding on the human infant. In the context of NEC, there is evidence that the microbiota communities are characterized by high levels of facultative anaerobes and abundance of *Proteobacteria* and low levels of *Bifidobacterium* and *Bacteroides* (116).

The evidence linking bacteria in the pathogenesis of NEC includes signs of luminal bowel gas production (e.g., pneumatosis intestinalis), products of bacterial fermentation (e.g., short-chain fatty acids), and epidemic outbreaks of NEC (63, 65). Early studies in gnotobiotic quail, a lactase-deficient species, colonized with infant fecal bacteria and fed a lactose-based formula produced

NEC-like lesions (6). In the past decade, several studies in pigs have provided strong evidence that colonization of the preterm gut with microbes is a necessary element in NEC pathogenesis. Cesarean-derived, newborn, preterm pigs fed infant formula developed a markedly higher NEC rate (57%) when reared conventionally versus in germ-free isolators. Another study showed that in utero, esophageal infusion of infant formula to fetal pigs at 105 days of gestation failed to induce NEC, whereas littermate preterm pigs delivered by cesarean 24 h later and fed formula for 24 h experienced a 38% NEC rate (4). Subsequent studies with preterm pigs given antibiotics that are frequently administered to human preterm infants (ampicillin, gentamicin, and metronidazole) showed that oral, but not parenteral, treatment reduced gut bacterial colonization, inflammation, and NEC lesions (4, 117, 118). Further evidence suggests that oral antibiotic administration prevented NEC by enhancing maturation of blood neutrophils and by diminishing gut colonization, permeability, and translocation of gram-positive bacteria. These pig studies strongly link gut bacterial colonization to NEC pathogenesis; however, the precise mechanism remains unclear. The evidence in pigs suggests that antibiotic treatment is an effective preventive against NEC, but major concerns for antibiotic resistance have limited the adoption of this practice. In fact, evidence in human studies is mixed as to whether antibiotic exposure creates a dysbiosis that may predispose to NEC (119, 120).

HMOs: human milk oligosaccharides

Prebiotics and Probiotics in Necrotizing Enterocolitis Prevention

Human milk oligosaccharides (HMOs) are another important carbohydrate component of human breast milk that has been implicated in the protection against NEC. Mammalian milks contain a variety of oligosaccharides that are free glycan molecules composed of different monosaccharides (glucose, galactose, *N*-acetylglucosamine, fucose, and *N*-acetylneuraminic acid) linked to a lactose core (121–124). The concentration in mammalian milks varies among species, being the highest in colostrum and decreased in mature milk. Human milk oligosaccharide concentration decreases from 24 to 12 g/L, whereas that in bovine and porcine milk decreases from approximately 1 to 0.05 g/L and 12 to 7 g/L, respectively. Various studies have shown that HMOs have multiple direct beneficial functions in the neonatal gut, including increased mucosal growth, barrier function, immunomodulation, and pathogen defense (121). In addition to direct host action, milk oligosaccharides function as prebiotics because they are resistant to gut enzymatic digestion and pass into the colon, where they are readily fermented by select groups of microbes. HMOs are an important prebiotic substrate for the growth of *Bifidobacterium* species that commonly colonize and represent the dominant genus in breastfed term infants but are less abundant in preterm infants. These and other bacterial species that grow readily on HMOs lower gut pH by producing short-chain fatty acids. In addition, conditioned media from *Bifidobacterium infantis* fermentation suppress the release of proinflammatory cytokines in intestinal epithelial cell cultures. Several prebiotic oligosaccharides, including galactooligosaccharides, fructooligosaccharides, oligofructose and inulin, and polydextrose, have been produced commercially and shown to increase the abundance of bifidobacteria and lactobacilli, similar to HMOs (125).

Many recent studies have examined the nutritional function of oligosaccharides in the diet of term and preterm neonatal pigs under normal healthy conditions and when challenged with a viral pathogen (121, 126–128). The oligosaccharides of pig milk are highly sialyated, being more similar to those in bovine than in human milk (129). The ex vivo treatment with HMOs increased proliferation and IL-10 production of cultured peripheral blood mononuclear cells isolated from sow-fed and formula-fed term neonatal pigs (130). Studies in neonatal pigs challenged with rotavirus showed that feeding formula supplemented with HMOs or mixtures of prebiotic oligosaccharides reduced the duration of diarrhea and enhanced T helper type 1 interferon-gamma and

IL-10 cytokines in the ileum (131). Moreover, HMO-fed pigs have twice as many natural killer cells and 36% more mesenteric lymph node effector memory T cells, suggesting improved mucosal immune function (132). Feeding neonatal pigs formula supplemented with polydextrose reduced pH and increased lactic acid in cecal and colonic contents in association with lowered ileal proinflammatory cytokine expression (133). Other pig studies with mixtures of polydextrose and galactooligosaccharides have shown changes in lactobacilli without impacting gut barrier function (121).

FMT: fecal microbiome transfer

In the past 20 years, several randomized controlled trials and cohort studies in preterm infants have demonstrated that probiotic treatment prevents NEC (134). These clinical studies have used different bacterial species, with oral doses of bacteria usually higher than a billion organisms per day. The prevention of NEC seems most effective when infants are given both *Bifidobacterium* and *Lactobacillus* species. Fewer randomized controlled trials in preterm infants have investigated the effect of prebiotics in the prevention of NEC (135). These studies show that prebiotics are safe and can decrease the incidence of sepsis and mortality but not NEC. Most of the prebiotics tested in preterm infant studies include short-chain galactooligosaccharides/long-chain fructooligosaccharides and pectin-derived acidic oligosaccharides. An important point and potential limitation of these studies is that many of the prebiotics tested in clinical trials are neither derived from nor present in human milk and thus may have different functions than HMOs normally consumed by infants.

There are several studies of both probiotics and prebiotics in preterm pigs, and these studies have produced mixed results regarding protection against NEC. Treatment with a mixture of probiotic species, including *Bifidobacterium animalis* and different lactobacilli, such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus pentosus*, *Lactobacillus plantarum* (10^9 CFU each species/each feeding) (136), or a single probiotic species, *Lactobacillus rhamnosus* (10^{11} CFU/day) (137), generally reduced the incidence of NEC. An exception was the increased incidence of NEC in preterm pigs inoculated with *Lactobacillus paracasei*, *B. animalis*, and *Streptococcus thermophilus* (10^{10} CFU/day). Few studies have examined the prebiotic protective effects in prevention of NEC, but all of these studies have tested HMOs rather than non-HMOs. A study comparing a mixture of 4 versus 25 different HMO blends found that these HMOs suppressed intestinal epithelial cell proliferation and had a modest immunomodulatory effect in the intestine in vivo but did not prevent NEC or diarrhea when added to formula and fed to preterm pigs for 5–11 days after birth (126). Addition of mixtures of bovine milk oligosaccharides was well tolerated but did not improve any clinical outcomes in preterm pigs (138). Studies in preterm pigs also tested whether adding the HMO α-1,2-fucosyllactose (2′-FL) was protective against NEC and challenge with enterotoxigenic *Escherichia coli* F18 because 2′-FL has structural homology to bacterial adhesion sites in the intestine (127, 128). Both of these studies showed that addition of 2′-FL to formula-fed preterm pigs did not reduce or prevent diarrhea or NEC incidence in the first week of life. The lack of 2′-FL effect in these pig studies may be due to the fact that pigs produce a relative abundance of α-1,2′-fucose in the intestine and thus may not be reliant on exogenous 2′-FL in the diet. These studies also suggest that dietary 2′-FL may not be critical in the immediate postnatal period of preterm neonates, when gut colonization and intestinal immunity are still immature. Taken together, the studies in neonatal pigs suggest that prebiotics, both human milk derived and nonhuman milk derived, have a greater influence on intestinal immune function in term compared with preterm pigs. The lack of response to HMOs in preterm pigs maybe due to the immaturity of the mucosal and systemic innate immunity or the relatively short duration of the studies.

In addition to therapeutic approaches using defined probiotic preparations, fecal microbiome transfer (FMT) has rapidly emerged as a tool to demonstrate the sufficiency of the microbiota to

transfer the donor phenotype to the recipient host (115, 139). This approach has become a standard of care in the treatment of recurrent *Clostridium difficile* infection in humans (140). The use of FMT has recently emerged as an approach to protect against NEC and modify the gut microbiome in mice, rats, and preterm pigs (141–144). Interestingly, the study in preterm pigs showed that rectal compared with oral FMT administration may be a safer and more tolerable route in the preterm host, in which innate immunity and intestinal barrier function are poorly developed (141). These early studies using FMT in neonates raise several important questions as to the source and amount of donor fecal samples, as well as the route (oral, orogastric tube feeds, rectal) and frequency of administration. The use of preterm pigs will be a useful model for investigating these questions.

TLR4: Toll-like receptor 4

NF-κB: nuclear factor-κB

Host Factors in Necrotizing Enterocolitis Pathogenesis

Intestinal immunity. The fetal intestine develops with very limited exposure to microbes and is bathed in amniotic fluid that is swallowed during gestation. The abrupt transition to the extrauterine environment after birth requires the innate immune system for protection from infection (10). The mucosal epithelium is a major element of innate immunity and a key interface between the neonatal host and gut microbiota after birth. A major function of the mucosal epithelium is to facilitate cross talk between the commensal microbiota and the host immune system to prevent inappropriate inflammatory responses and enable tolerance. It has become evident that the premature exposure of the mucosal epithelium to bacteria or their products (e.g., LPS) before normal term birth induces an excessive mucosal inflammatory response (145, 146). One key molecular mechanism that mediates the premature inflammatory response to LPS involves increased expression and signaling via the Toll-like receptor 4 (TLR4) pathway (147). Recent animal studies confirm this hypothesis and show that exposure of the perinatal intestine to LPS after birth induces rapid loss of LPS responsiveness and nuclear factor-κB (NF-κB) activation in epithelial cells. This appears to be mediated by posttranscriptional downregulation of the interleukin 1 receptor–associated kinase 1, which is essential for epithelial TLR4 signaling. This pattern of epithelial immune maturation is analogous to the well-known immunological development of the lung epithelium following experimental prenatal gram-negative bacterial LPS or chorioamnionitis, a common cause of preterm delivery (148, 149). These results suggest that perinatal development of inflammatory tolerance to bacterial endotoxins occurs after an initial limited pre-exposure, and there is supporting evidence for this phenomenon with other bacterial TLR ligands, including flagellin (150, 151).

Intestinal blood flow, mucosal perfusion, and ischemia. Intestinal microcirculatory dysfunction leading to ischemia appears to play a central role in the establishment and progression of NEC (71, 76, 152, 153). In an excellent review, Nowicki (71) addresses the central questions regarding the role of ischemia in the pathogenesis of NEC, namely, where, when, and how it occurs. Studies from this group using pigs provided important information about developmental changes in neonatal intestinal blood flow and mucosal perfusion. The dominant factor in intestinal blood flow is vascular resistance, and the main sites of resistance are in submucosal arterioles. The high metabolic demand of the intestinal epithelium for digestive and absorptive processes, particularly after enteral feeding, together with the vascular architecture of the villus creates an oxygen gradient that makes the villus tip hypoxic under normal conditions (154). This "physiological hypoxia" has an even steeper gradient in the distal parts of the intestine (155). Early studies in pigs showed that asphyxia induced intestinal ischemic injury that resembled NEC (69, 156). However, the magnitude of the insult together with the cellularity of the intestinal mucosal damage did not

match the development of NEC in infants, because intrapartum asphyxia and occurrence of NEC during the first week of life are rare (157–159). Attempts to link NEC to derangements in macrocirculatory intestinal blood flow, i.e., the superior mesenteric artery, have yielded mixed results (153).

ET-1: endothelin-1

NO: nitric oxide

There are important differences among species regarding villus microvascular architecture (76); whereas pigs and humans have a single central arteriole arborizing at the tip of the villus and converging into one or two venules, rodent architecture is quite different. In rats, the single central arteriole does not branch until it reaches the tip of the villus, where it forms a capillary network (160). In contrast, mice have two arterioles that provide oxygenated blood to the villus, which after division into a capillary network converge into a single venule that extends from the tip to the base of the villus (76). In rats, newborns have a simpler villous plexus than adults (160), and this may contribute to their inability to vasodilate and maintain perfusion, thereby increasing the susceptibility to mucosal damage during periods of decreased perfusion (161).

Studies with pigs born at term and studied up until 35 days of age show that vascular resistance is low at birth and increases with age. The dominant factors that regulate the balance between vasoconstriction (mediated mainly by endothelin-1, ET-1) and vasodilation (mediated mainly by nitric oxide, NO) are tilted toward vasodilation in term newborn pigs. In pigs, vasoconstriction occurs in neonates after moderate ischemia/reperfusion (but not in older animals) and can be attenuated by blocking the ET-1 receptor type (ET_A) (162). These results mimic observations in humans in which the concentration of ET-1 was greater in necrotic areas compared with apparently healthy tissue and inhibition of the receptor (ET_A) resulted in vasodilation of submucosal arterioles (163).

In pigs, NO-mediated vasodilation was shown to be greater in newborns than in older subjects (152). However, submucosal arterioles from infants that developed NEC showed reduced production of NO and as a consequence failed to dilate in response to blood flow (164). In addition, low arginine availability seems to be another factor in the reduced NO response in neonates. Arginine is the precursor for NO production, and arginine supplementation has been shown to prevent NEC in preterm infants (165). The endogenous production of arginine relies on the production of citrulline by the gut, which is then converted into arginine by the kidney (166). Premature pigs showed a reduced enteral citrulline production that preceded the onset of NEC (79), thus reducing arginine availability in these animals. Despite the reports that arginine protects against NEC, studies in term, neonatal pigs have shown that enteral arginine did not increase superior mesenteric arterial blood flow (167).

Changes in gut tissue oxygenation are compounded by the activation and recruitment of innate immune cells, leading to inflammation (168). Premature pigs display immature gut morphology and digestive function (169, 170), which can lead to bacterial overgrowth (53). Activation of TLR4 by LPS results in the release of critical proinflammatory cytokines that have been shown to increase ET-1 expression and disrupt mucosal barrier function (171). Studies in preterm pigs showed that the transition from TPN to enteral feeding of formula, but not porcine colostrum, triggered an increase in intestinal TLR4 and proinflammatory cytokine expression (172). The combined early activation of TLR4-mediated inflammation in intestinal epithelial cells and microcirculatory endothelial cells leads to reduced intestinal perfusion and hypoxia that initiate the pathogenesis of NEC (63). Evidence in mice further supports the protective role of NO, showing that endothelial nitric oxide synthase knockout increases, but sildenafil and sodium nitrate treatment reduces, the severity of NEC (63). In contrast, activation of TLR9 by unmethylated bacterial CpG DNA seems to inhibit LPS-mediated TLR4 signaling (173). This may be the mechanism of action by which the administration of an ultraviolet-inactivated *L. rhamnosus* attenuated the severity of NEC in premature pigs (137).

NIRS: near-infrared spectroscopy

The use of near-infrared spectroscopy (NIRS) allows for the continuous, noninvasive monitoring of tissue oxygenation in neonates (174). Initially developed to monitor cerebral oxygenation (175), NIRS has also been used to monitor other regions of the body, including splanchnic organs (176). The size of the neonatal premature pig is similar to that of a human neonate, which has allowed for the use of abdominal NIRS (aNIRS) in pig models of NEC. Measurements of splanchnic oxygen saturation using aNIRS have been validated in anesthetized pigs by altering oxygen concentration in the anesthesia circuit. A high correlation between the aNIRS determinations and the oxygen saturation in mesenteric blood (177) or portal blood (178) supports the validity of this noninvasive tool to monitor intestinal perfusion. The continuous NIRS monitoring from birth has shown that premature pigs with lower aNIRS were more susceptible to developing NEC once enteral feeds were introduced (177, 179). For this reason, aNIRS may be useful in identifying those neonates susceptible to NEC prior to clinical manifestation of the disease (180).

MODELING INTESTINAL ADAPTATION AND SHORT BOWEL SYNDROME IN PIGS

SBS is a clinical condition that results from surgical resection, congenital defect, or disease-associated loss of absorption, leading to an inability to maintain nutrient balance when fed a normal diet (3). The incidence of SBS is twice as high in pediatrics (24.5 versus 9/100,000 live births) as in adults. The SBS incidence is approximately 100-fold higher in preterm versus term infants, mainly related to the higher incidence of NEC in preterm infants compared with other congenital causes, such as gastroschisis and intestinal atresia (181). The condition of SBS results from surgical resection of intestine, which triggers a process in the remnant intestine known as intestinal adaptation, which involves a coordinated increase in gut hormone secretion, mucosal growth, and secretory function (**Figure 3**). Many of these physiological processes that occur during intestinal adaptation were described in early studies with rat models (182), and some groups have extended this to mice (3). Importantly, the adaptation process depends on the subtypes of surgical resection that occur, either proximal intestine (jejunal) resection, ileal-colonic resection, or distal bowel resection with jejunostomy.

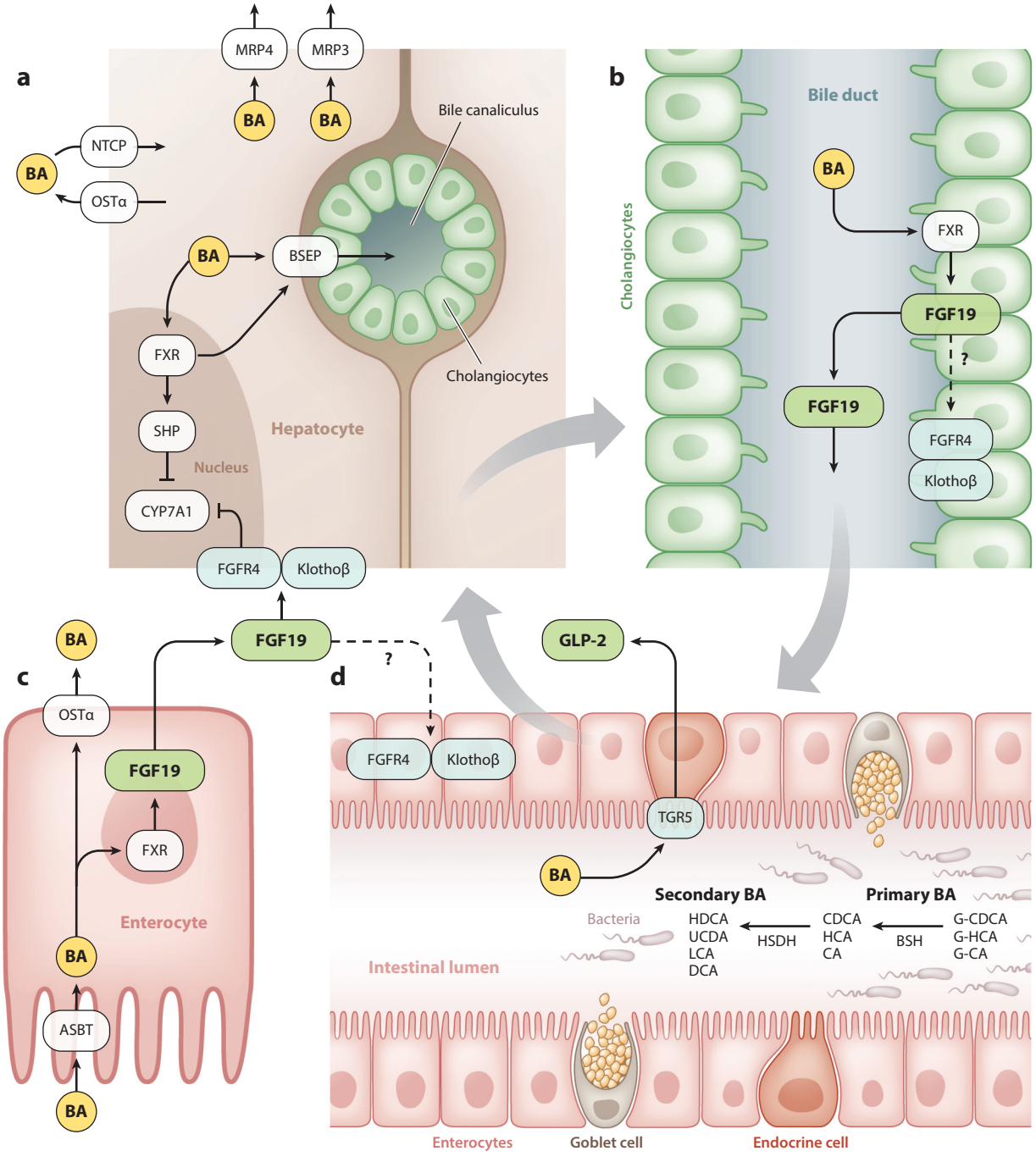
Models of SBS and intestinal adaptation have been developed in pigs of various ages, including preterm and term newborn pigs, juvenile pigs, and adult pigs (3, 183–188). Because SBS incidence is highest in infants, there is a need to understand how organ immaturity and stage of development affect the response to intestinal resection. Most investigators have performed a mid-intestinal, jejuno-ileal resection where the remaining small intestine consists of equal parts of jejunum and ileum. The postresection period has generally been longest (4–6 weeks) in studies on older pigs (183, 189). In contrast, only short-term (one-week) responses to resection have been studied in pigs younger than one week of age (185, 190, 191), except the studies on PN-fed term pigs (184, 192). The pig model of SBS has many advantages over rodent models in that repeated measurements, such as stool/ostomy output, plasma GLP-2 and citrulline, and clinical chemistry values, can be measured longitudinally to monitor clinical adaptation response in SBS pigs, especially in long-term studies (193). Additionally, other in vivo measurements of gut function can be made in pigs, such as nutrient uptake or nutrient digestibility, permeability using xylose absorption and portal blood flow, and nutrient flux. Another advantage of pigs that is ethically difficult in human SBS patients is that tissue samples can be collected at study endpoints to directly measure intestinal tissue growth and functional activity, such as villus height, crypt depth, crypt cell proliferation, apoptosis, and disaccharidase activity in the remnant intestine.

The pig model has been important in investigating the trophic effects of enteral nutrition and gut growth factors, especially GLP-2, in the intestinal adaptation response during SBS. In the

context of pediatric SBS, nutrition is vital not only as a stimulus of local intestinal growth but also for body growth of the developing infant. Enteral feeding is preferred to reduce the detrimental consequences of PN. Luminal nutrients are the most potent stimulus for intestinal adaptation and act directly to provide metabolic substrates for enterocytes and to trigger the increase in pancreatic-biliary secretions, neural factors, intestinal blood flow, and release of intestinal hormones and growth factors such as GLP-2 and EGF (29, 194). In the early phase after surgical resection, infants with SBS initially are fed small volumes of enteral nutrition along with PN. There is limited evidence as to the best source of enteral nutrition for SBS patients. Human milk is the recommended diet for infants, including those with SBS, and is becoming more widely used with the increasing availability of human milk banks, yet infants often receive formulas. As discussed above, studies in healthy, newborn, term pigs indicate that natural milks and colostrum (porcine, bovine, and human) have a greater trophic stimulus for intestinal growth than infant formulas. In studies with SBS pigs, however, the results show that the trophic effect of natural milk versus formula is mixed and not uniformly beneficial (195, 196).

Intestinal Trophic Action of GLP-2

The endogenous secretion and therapeutic effects of several hormones and growth factors, including GLP-2, EGF, growth hormone, IGF-1, and insulin, have been examined in the context of SBS mainly in animal models (3). The most well studied of these trophic factors in pigs is GLP-2, which is the only gut hormone that is approved for therapeutic use in treatment of SBS in children and adults. Studies in adult and pediatric SBS patients have shown clinical benefits of treatment with the long-acting GLP-2 analog (teduglutide, GATTEX®) (197, 198) (**Figure 4**). GLP-2 is a key trophic factor in intestinal adaptation. Infusion of GLP-2 in parenterally fed pigs acutely increased superior mesenteric arterial and portal venous blood flow, and these vascular effects were NO dependent (199–201). Infusion of GLP-2 in parenterally fed pigs also prevents mucosal atrophy by maintaining increased rates of intestinal crypt cell proliferation and protein synthesis and suppressing apoptosis (202–204). The trophic effects of GLP-2 infusion maintain intestinal digestive and absorptive function in TPN-fed pigs (205). Chronic GLP-2 treatment for six weeks after birth also increased intestinal growth in pigs nourished on the sow for three weeks and then weaned and fed a commercial swine diet (206). Studies in neonatal pigs and preterm infants show that enteral nutrition triggers secretion of GLP-2, whereas PN leads to low circulating concentrations (29, 207). A series of studies in SBS neonatal pigs showed that circulating GLP-2 increases following bowel resection, but this response required distal bowel continuity; furthermore, enteral nutrition augmented the intestinal trophic response (188, 190, 192, 208). More recent studies in neonatal SBS pigs treated with the natural peptide and long-acting analogs of human GLP-2, including teduglutide and apraglutide, demonstrated intestinal trophic effects that were enhanced by enteral nutrition (190, 191, 209–212); other long-acting GLP-2 analogs also are being developed (213). Studies in mice show that the trophic effects of GLP-2 are mediated by local paracrine secretion of EGF and IGF-1 from subepithelial myofibroblasts (214, 215). Recent studies in SBS pigs treated with GLP-2 or EGF alone and in combination demonstrated that the trophic effects of GLP-2 are greater than those of EGF, but combined treatment increased intestinal length, suggesting synergy between GLP-2 and EGF (216). New approaches have been tested in pigs to increase endogenous GLP-2 secretion using enteral administration of a natural agonist to Takeda G-protein-coupled receptor 5 (188, 217). Both the primary bile acid, chenodeoxycholic acid (CDCA), and the triterpenoid, ursolic acid, potently increased GLP-2 secretion in parenterally fed pigs, but their capacity to augment GLP-2 secretion above that triggered by enteral nutrition in conditions of SBS was limited (188).



(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

Enterohepatic cell and molecular mechanisms of BA transport and signaling. Illustrated are the interorgan and intercellular transport of BAs and their signaling pathways in the liver and gut. BAs function as hormonal signaling molecules that activate FXR and TGR5 in the liver, biliary ducts, and intestinal endocrine cells. FXR is a master BA–sensing nuclear receptor that regulates expression of genes in BA synthesis and transport in the liver and BA transport in the intestine. BA activation of FXR induces secretion of FGF19 mainly in intestinal enterocytes, but also in hepatocytes and cholangioctyes. BA activation of TGR5 induces secretion of GLP-1/GLP-2 in intestinal endocrine cells. Gut bacteria shape the profile of BA species via enzymatic conversion via BSH and HSDH. Abbreviations: ASBT, apical sodium-dependent bile acid transporter; BA, bile acid; BSH, bile salt hydrolase; BSEP, bile salt export pump; CA, cholic acid; CDCA, chenodeoxycholic acid; CYP7A1, cytochrome P450 family 7 subfamily A member 1; DCA, deoxycholic acid; FGF19, fibroblast growth factor 19; FGFR4, fibroblast growth factor receptor 4; FXR, farnesoid X receptor; G-CA, glyco-cholic acid; G-CDCA, glyco-chenodeoxycholic acid; G-HCA, glyco-hyocholic acid; GLP, glucagon-like peptide; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; HSDH, hydroxysteroid dehydrogenase; LCA, lithocholic acid; MRP3, multidrug resistance associated protein 3; MRP4, multidrug resistance protein 4; NTCP, Na^+-taurocholate cotransporting polypeptide; OSTα, organic solute transporter α; SHP, small heterodimer partner; TGR5, Takeda G-protein receptor 5.

MODELING PARENTERAL NUTRITION–ASSOCIATED LIVER DISEASE IN PIGS

PN is a lifesaving nutritional support for thousands of premature and term hospitalized infants in the United States annually. Many premature infants are nourished solely by PN following the surgical removal of the gastrointestinal tract owing to congenital or acquired disease-related causes, mainly NEC. A challenge in the care of premature infants is to provide sufficient nutrition to meet their high metabolic needs for growth, but at the same time minimize the risk for PNALD (218, 219). Cholestasis in premature infants, a key element of PNALD, presents clinically as increased serum biochemical markers, such as direct bilirubin, bile acids, and liver transaminases (22, 220). Steatosis may occur in many patients, but it often goes undetected because liver biopsy, with its attendant risk, is required for definitive diagnosis (221). The incidence of cholestatic liver disease can be as high as 50% in premature infants given PN for prolonged periods. These high-risk premature infants on prolonged PN often have concurrent intestinal disease, resulting from surgical bowel resection as described above for SBS; this condition has been termed intestinal failure–associated liver disease (IFALD) (220). There is also epidemiological evidence that the incidence of PNALD/IFALD is higher in infants who have suffered NEC (222). PNALD occurs mainly in infancy, yet the ability to model PNALD in neonatal animals is limited by the ability to surgically implant and maintain venous catheters for administration of PN solutions. Thus, most studies of PNALD in neonatal animals have been conducted using pigs, at both term (223–226) and preterm (227–229) stages of development. The neonatal guinea pig also has been used to effectively model infant PNALD and the role of oxidative stress (230). The mouse has been used to model PNALD but at older ages of adolescent and adult stages (8–12 weeks) (231–234). PNALD pathogenesis is thought to involve several factors, including PN nutrient composition, inflammation, gestational age, and lack of enteral feeding as a stimulus of the gut.

New-Generation Lipid Emulsions May Prevent Parenteral Nutrition–Associated Liver Disease: Role of Phytosterols and Vitamin E

Lipids make a significant contribution to the energy and essential fatty acid needs of parenterally fed infants. Since the introduction of parenteral lipid emulsions decades ago, the first lipid emulsion approved for pediatric use has been Intralipid®, a soybean oil emulsion. However, in recent years, new-generation lipid emulsions have been developed containing pure olive oil (Clinoleic®); pure fish oil (Omegaven®); or various blends of soy, olive, safflower, medium-chain triglyceride, and fish oil [Lipofundin®, Liposyn II®, SMOFlipid® (SMOF), Lipoplus®] that have been approved or are pending approval for pediatric use. The link between PNALD and lipid

emulsions became evident from clinical studies in pediatric PNALD patients demonstrating the efficacy of Omegaven (235, 236) to treat PNALD in infants previously nourished with soybean oil emulsions. More recent randomized controlled trials have shown that SMOFlipid compared with soybean oil emulsion reduced markers of cholestasis in infants with IFALD (237) but did not reduce PNALD in otherwise healthy extremely low-birth-weight infants (238).

FXR: farnesoid X receptor

Studies in neonatal preterm and term pigs support the observations that both Omegaven and SMOFlipid can reduce or prevent PNALD (225, 228). Interestingly, SMOFlipid also improves insulin sensitivity and promotes higher lean body mass compared with Intralipid in preterm TPN-fed pigs (239). The mechanism that explains the protective effect of Omegaven and SMOF against PNALD is not clear, but phytosterols have been implicated. Phytosterols are cholesterol-like molecules that are enriched in plant-based oils, such as soybean oil, whereas their concentration in SMOF is relatively low, and they are absent in Omegaven. Infants given soybean oil emulsions for prolonged periods (longer than a month) are at increased risk for developing PNALD, and this is associated with phytosterol accumulation in plasma and liver tissue (240). A molecular mechanism implicated in the cause of PNALD is that plant phytosterols disrupt the function of the liver farnesoid X receptor (FXR) (**Figure 5**). FXR is a nuclear receptor and is the primary sensor of bile acids that controls the molecular regulation of target genes involved in bile acid homeostasis. Our studies with cultured piglet hepatocytes, and those of others using mouse and human hepatocytes, show that phytosterols antagonize FXR (228, 241). A study in TPN-fed mice showed that addition of the phytosterol stigmasterol to a fish oil emulsion (Omegaven) induced PNALD, but only in the presence of intestinal injury and inflammation (231). In contrast, our study in TPN-fed pigs showed that addition of phytosterols to the fish oil emulsion, Omegaven, did not induce PNALD (227). A hypothesis put forth based on mouse PNALD studies (220) is that the adverse effect of hepatic phytosterol accumulation requires a concurrent inflammatory costimulation from gut-derived LPS that functions as a second hit to trigger activation of proinflammatory cytokines, such as IL-1β, which in turn suppresses the expression of hepatobiliary transporters for bile acids (bile salt exporter pump, BSEP) and phytosterols (ATP-binding cassette transporter G5/8, ABCG5/8). Our studies in preterm TPN-fed pigs confirm that LPS treatment in vivo induces proinflammatory cytokine secretion and suppresses the hepatic expression of both BSEP and ABCG5/8 (242). In the two-hit model of PNALD, hepatic Kupffer cells are thought to be the source of local IL-1β. Studies in cultured neonatal pig hepatocytes suggest that phytosterol accumulation alone does not induce IL-1β. Yet in neonatal pig Kupffer cells, costimulation with LPS coupled with phytosterol accumulation synergistically maximizes the inflammatory response (242). Studies in mice also suggest that blockade of IL-1β/NF-κB signaling can prevent PNALD (243). Thus, the studies in TPN-fed neonatal pigs confirm the clinical evidence that lipid emulsions that are low in phytosterols protect against PNALD. Further studies are necessary to demonstrate that accumulation of phytosterols in vivo directly causes PNALD in neonatal pigs and show that this is associated with reduced FXR target gene function.

Vitamin E or tocopherol is another key nutrient that has been implicated in PNALD because the composition of various tocopherols varies widely among different parenteral lipid emulsions (244). Vitamin E protects against fatty liver diseases, such as nonalcoholic fatty liver disease (NAFLD), because it functions as a natural, lipid-soluble antioxidant that reduces cellular oxidative stress in vivo, as well as reducing lipid peroxidation and maintaining the stability of parenteral lipid solutions. A variety of tocopherol isoforms with different biological activities exist in parenteral lipid emulsions depending on the source of lipid. The vitamin E present in most commercial soybean oil lipid emulsions is mainly the γ-tocopherol isoform, whereas in several new-generation emulsions (i.e., Omegaven and SMOFlipid), vitamin E is added in the more bioactive α-tocopherol isoform (244). The differences in vitamin E content in Intralipid, Omegaven, and SMOFlipid

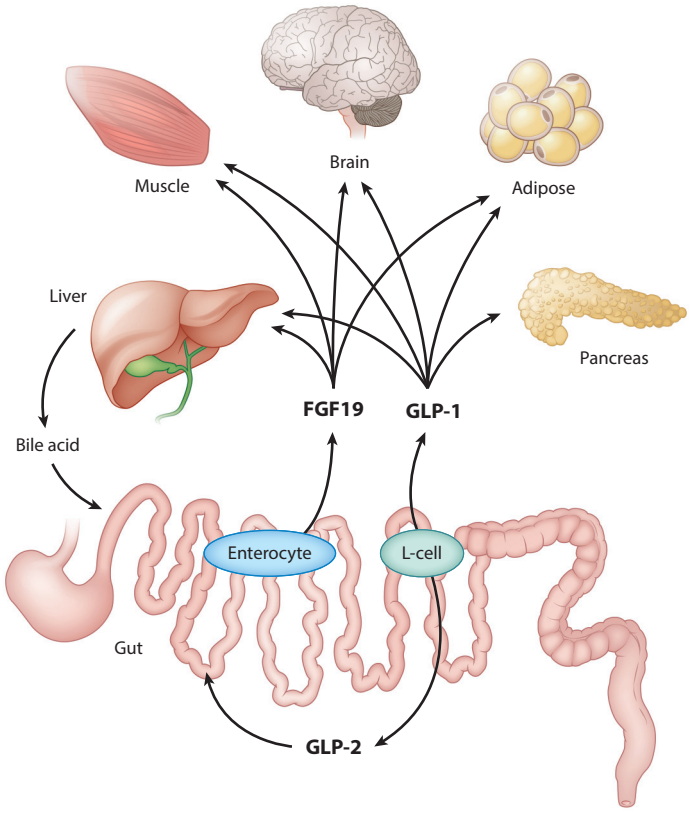


Figure 5

Key gut hormone signals that regulate metabolism. Overview of key hormones produced in the gut that sense dietary nutrients and bile acids. These hormones signal the level of nutrient availability in the gut lumen to the liver and pancreas, but also to peripheral tissues in the body. The metabolic effects of GLP-1/GLP-2 and FGF19 and their role in adult diseases (e.g., diabetes, nonalcoholic steatohepatitis, cholestasis) is an active area of scientific research, but their role in pediatric nutrition and GI diseases is poorly understood. Abbreviations: FGF19, fibroblast growth factor 19; GI, gastrointestinal; GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2; L-cell, enteroendocrine L cell.

given to preterm TPN-fed pigs are reflected in the plasma and tissue tocopherol concentrations. Furthermore, the addition of α-tocopherol to Intralipid reduced serum markers of PNALD in preterm TPN-fed pigs, supporting its protective role (227). Recent studies in term TPN-fed pigs, however, showed that added vitamin E is not protective under conditions of increased parenteral soybean-oil lipid intakes and high phytosterol loads (226). These studies suggest that some of the protection against PNALD observed with new-generation lipid emulsions may be mediated by vitamin E when given at relatively low lipid loads with reduced phytosterol content.

Fibroblast Growth Factor 19: A Potential Mechanistic Link to Enterohepatic Bile Acid Signaling and Parenteral Nutrition–Associated Liver Disease

The lack of enteral feeding and associated luminal gut stimulus has been linked to the pathogenesis of PNALD. Attention has been focused on gut hormones that are triggered with enteral feeding, namely cholecystokinin, given its function in promoting gallbladder contraction and

FGF19: fibroblast growth factor 19

CYP7A1: cholesterol 7-α-monooxygenase

bile secretion. However, clinical studies suggest that cholecystokinin treatment does not prevent PNALD in infants (245). Recent attention has focused on fibroblast growth factor 19 (FGF19), a gut-derived enterokine hormone secreted in response to feeding and the presence of bile acids in the gut lumen. FGF19 is one of the 22 members of the fibroblast growth factor (FGF) family (246), most of which are paracrine growth factors (**Figure 4**). The FGF19 protein is part of the FGF19 subfamily (FGF15/19, FGF21, and FGF23), members of which function as endocrine hormones. FGF19 activates cell function by binding to a membrane localized tyrosine kinase receptor, FGF receptor 4, and the heterodimeric partner, β-klotho. Importantly, humans and pigs produce FGF19 in the intestine and liver, whereas mice and rats express the human ortholog FGF15, and only in the intestine (247), thus making the pig a relevant model of human FGF19 biology. FGF19 is most well-known for its role as a downstream target of FXR, and studies in mice first showed that bile acid stimulation induces intestinal expression of FGF15 (248). FGF19 produced in the small intestine circulates into portal blood and leads to suppression of the rate-limiting enzyme in bile acid synthesis, cholesterol 7-α-monooxygenase (CYP7A1), in hepatocytes via activation of extracellular-signal-regulated kinase (ERK) mitogen-activated kinase pathways. Thus, bile acid activation of intestinal FXR-FGF19 is a key negative feedback signal to suppress hepatic bile acid synthesis. In support of this FXR-FGF19-mediated mechanism, we first showed that plasma FGF19 is suppressed by TPN and that enteral treatment with CDCA induces circulating FGF19 and prevents cholestasis (217). Our unpublished results show that a selective and potent FXR agonist, obeticholic acid (OCA), prevents cholestasis by activating FXR-target genes involved in hepatobiliary bile acid transport, preventing loss of bile duct structure, and inducing a robust dose-dependent increase in circulating FGF19. OCA is a potent, first-in-class FXR agonist derived from CDCA that was recently Food and Drug Administration approved under the name Ocaliva® to treat primary biliary cholangitis and is being developed to treat NAFLD and biliary atresia (249). These new promising results point to multiple mechanisms whereby therapeutic use of enteral OCA may prevent PNALD.

The primary factor that stimulates intestinal FGF19 secretion appears to be bile acids secreted into the gut lumen after oral feeding. This explains why chronic parenteral rather than enteral nutrition results in reduced FGF19 secretion (217). There is limited information about the ontogeny and developmental regulation of FGF19, yet one report showed that in human infants, the plasma FGF19 concentration increased tenfold between birth and 4 months of age and was lower in growth-restricted infants (250). A recent study in preterm infants showed that advancing gestational age correlated with plasma FGF19 and the bile acid synthesis marker 7α-hydroxy-4-cholestene-3-one. This marker has been used as a surrogate for activity of CYP7A1, the rate-limiting enzyme in the classic bile acid synthesis pathway (251). This study found that CYP7A1 activity is upregulated with gestational age and volume of enteral feeding, but in contrast, circulating FGF19 concentrations are higher in preterm infants and decline with advancing age. We postulate that FGF19 secretion increases with stage of development as enteral feeding, and bile acid synthesis and secretion, begins after birth. Consistent with Sánchez-Infantes et al.'s (250) findings in infants, but in contrast to those of Memon et al. (251), our unpublished results show that the plasma concentration and intestinal mRNA expression of FGF19 in preterm pigs are markedly lower than in term pigs and increase during the neonatal period. The similarity between pigs and humans with regard to the gut–liver axis and FXR–FGF19 signaling makes pigs a useful model to investigate the significance of gestational age and factors that regulate FGF19 function in early development.

The most well-characterized function of FGF19 is its role in the regulation of enterohepatic bile acid homeostasis. However, additional reports show that FGF19 functions as a metabolic hormone with actions similar to insulin, such as suppression of endogenous glucose production,

gluconeogenesis, and lipolysis and increased insulin sensitivity and glycogen synthesis (252). There is emerging evidence that FGF19 also induces protein anabolism based on reports in mice showing increased liver protein synthesis and skeletal muscle hypertrophy via activation of both the ERK and the ribosomal protein S6 kinase pathways. These reports suggest that FGF19 activates similar signaling pathways to insulin and thus has overlapping metabolic actions. This is important because we have shown that the circulating FGF19 concentration as well as insulin sensitivity and lean tissue mass are greater in enteral versus TPN-fed pigs (217, 223). Thus, the intestinal enterokine FGF19 may function similar to insulin in response to oral feeding to enhance insulin sensitivity and promote protein synthesis and lean tissue growth.

CONCLUSIONS

Pigs have been valuable animal models for biomedical research for decades, but in recent years they have been refined and particularly relevant for pediatric nutrition, as well as gastrointestinal and liver diseases. Pigs enable approaches that are not feasible in rodent models, serving as an important translational model to bridge the gap between mechanistic mouse models and human clinical studies. This is especially important in the case of premature infants, whose physiological fragility and ethical considerations make it prohibitive to conduct certain experiments. The neonatal pig has become a popular preclinical model for testing of nutritional and therapeutic agents targeted at these pediatric gut and liver diseases.

Several groups use term neonatal pigs to model human infant intestinal development, as well as fundamental responses to diet (colostrum versus formula), probiotics, HMOs, and gut microbe colonization patterns. The PN-fed, neonatal pig has provided a clinically relevant pediatric model showing that lack of enteral nutrition leads to deterioration of the gut, marked by intestinal mucosal atrophy, reduced digestive function and maturation, and increased permeability. Studies in neonatal pigs demonstrate the importance of minimal enteral nutrition and its impact on triggering secretion of trophic intestinal gut hormones (e.g., GLP-2). Studies in SBS pigs revealed the safety and efficacy of GLP-2 and its long-acting analogs being developed for treatment of pediatric patients.

The preterm pig has gained acceptance as a clinically relevant NEC model that has established key features of disease pathogenesis, especially prematurity, the importance of the microbiota, and enteral feeding. The preterm pig will continue to be a valuable preclinical model to address important questions about the safety and efficacy of new therapeutic approaches to shaping the developing gut microbiota in infants, including probiotics, prebiotics, and microbiota transfer. Pigs also will be valuable to test emerging questions concerning the nutritional value of donor human milk and human milk fortifiers to support optimal preterm infant growth and prevent NEC.

The neonatal pig serves an important role in modeling PNALD in infants and demonstrating the protective effects of new-generation lipid emulsions. Further studies with pigs will be important to establish a direct causative role for phytosterols in PNALD. Finally, studies in pigs were first to link low-circulating FGF19 to PNALD. Thus, with the emergence of selective FXR agonists and FGF19 analogs, pigs will play a critical role in evaluating the efficacy of drugs to augment intestinal secretion and plasma levels of FGF19 in treatment and prevention of PNALD.

DISCLOSURE STATEMENT

D.B. has received grant support from Fresenius Kabi, Mead Johnson Nutrition, Lucta, and Danone Nutricia Research. O.O. has received grant support from Covidien Inc. and Mallinckrodt

Inc. The other authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported in part by federal funds from the US Department of Agriculture, Agricultural Research Service under Cooperative Agreement Number 3092-51000-060-01 and grants from the University of Copenhagen, Innovation Fund Denmark (NEOMUNE) and National Institutes of Health (DK-094616) (D.B.).

LITERATURE CITED

1. Odle J, Lin X, Jacobi SK, Kim SW, Stahl CH. 2014. The suckling piglet as an agrimedical model for the study of pediatric nutrition and metabolism. *Annu. Rev. Anim. Biosci.* 2:419–44
2. Donovan SM, Odle J. 1994. Growth factors in milk as mediators of infant development. *Annu. Rev. Nutr.* 14:147–67
3. Sangild PT, Ney DM, Sigalet DL, Vegge A, Burrin D. 2014. Animal models of gastrointestinal and liver diseases. Animal models of infant short bowel syndrome: translational relevance and challenges. *Am. J. Physiol. Gastrointest. Liver Physiol.* 307:G1147–68
4. Sangild PT, Thymann T, Schmidt M, Stoll B, Burrin DG, Buddington RK. 2013. Invited review: the preterm pig as a model in pediatric gastroenterology. *J. Anim. Sci.* 91:4713–29
5. Mudd AT, Dilger RN. 2017. Early-life nutrition and neurodevelopment: use of the piglet as a translational model. *Adv. Nutr.* 8:92–104
6. Oosterloo BC, Premkumar M, Stoll B, Olutoye O, Thymann T, et al. 2014. Dual purpose use of preterm pigs as a model of pediatric GI disease. *Vet. Immunol. Immunopathol.* 159:156–65
7. Anderson JG, Baer RJ, Partridge JC, Kuppermann M, Franck LS, et al. 2016. Survival and major morbidity of extremely preterm infants: a population-based study. *Pediatrics* 138:e20154434
8. Kozuki N, Katz J, Christian P, Lee AC, Liu L, et al. 2015. Comparison of US birth weight references and the International Fetal and Newborn Growth Consortium for the 21st century standard. *JAMA Pediatr.* 169:e151438
9. Glob. Burd. Dis. Pediatr. Collab., Kyu HH, Pinho C, Wagner JA, Brown JC, et al. 2016. Global and national burden of diseases and injuries among children and adolescents between 1990 and 2013: findings from the Global Burden of Disease 2013 study. *JAMA Pediatr.* 170:267–87
10. Kollmann TR, Kampmann B, Mazmanian SK, Marchant A, Levy O. 2017. Protecting the newborn and young infant from infectious diseases: lessons from immune ontogeny. *Immunity* 46:350–63
11. Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, et al. 2015. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993–2012. *JAMA* 314:1039–51
12. Wales PW, Christison-Lagay ER. 2010. Short bowel syndrome: epidemiology and etiology. *Semin. Pediatr. Surg.* 19:3–9
13. Hull MA, Fisher JG, Gutierrez IM, Jones BA, Kang KH, et al. 2014. Mortality and management of surgical necrotizing enterocolitis in very low birth weight neonates: a prospective cohort study. *J. Am. Coll. Surg.* 218:1148–55
14. Horbar JD, Ehrenkranz RA, Badger GJ, Edwards EM, Morrow KA, et al. 2015. Weight growth velocity and postnatal growth failure in infants 501 to 1500 grams: 2000–2013. *Pediatrics* 136:e84–92
15. Horbar JD, Carpenter JH, Badger GJ, Kenny MJ, Soll RF, et al. 2012. Mortality and neonatal morbidity among infants 501 to 1500 grams from 2000 to 2009. *Pediatrics* 129:1019–26
16. Horbar JD, Badger GJ, Carpenter JH, Fanaroff AA, Kilpatrick S, et al. 2002. Trends in mortality and morbidity for very low birth weight infants, 1991–1999. *Pediatrics* 110:143–51
17. Bjerre D, Mark T, Sorensen P, Proschowsky HF, Vernersen A, et al. 2010. Investigation of candidate regions influencing litter size in Danish Landrace sows. *J. Anim. Sci.* 88:1603–9

18. Che L, Thymann T, Bering SB, Le Huërou-Luron, D'Inca R, et al. 2010. IUGR does not predispose to necrotizing enterocolitis or compromise postnatal intestinal adaptation in preterm pigs. *Pediatr. Res.* 67:54–59
19. Ferenc K, Pietrzak P, Godlewski MM, Piwowarski J, Kilianczyk R, et al. 2014. Intrauterine growth retarded piglet as a model for humans—studies on the perinatal development of the gut structure and function. *Reprod. Biol.* 14:51–60
20. Caminita F, van der Merwe M, Hance B, Krishnan R, Miller S, et al. 2015. A preterm pig model of lung immaturity and spontaneous infant respiratory distress syndrome. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 308:L118–29
21. Eiby YA, Wright LL, Kalanjati VP, Miller SM, Bjorkman ST, et al. 2013. A pig model of the preterm neonate: anthropometric and physiological characteristics. *PLOS ONE* 8:e68763
22. Christensen RD, Henry E, Wiedmeier SE, Burnett J, Lambert DK. 2007. Identifying patients, on the first day of life, at high-risk of developing parenteral nutrition-associated liver disease. *J. Perinatol.* 27:284–90
23. Wilmore DW, Dudrick SJ. 1968. Growth and development of an infant receiving all nutrients exclusively by vein. *JAMA* 203:860–64
24. Embleton ND, Simmer K. 2014. Practice of parenteral nutrition in VLBW and ELBW infants. *World Rev. Nutr. Diet.* 110:177–89
25. Buchman AL, Moukarzel AA, Bhuta S, Belle M, Ament ME, et al. 1995. Parenteral nutrition is associated with intestinal morphologic and functional changes in humans. *JPEN* 19:453–60
26. Rossi TM, Lee PC, Young C, Tjota A. 1993. Small intestinal mucosa changes, including epithelial cell proliferative activity, of children receiving total parenteral nutrition (TPN). *Dig. Dis. Sci.* 38:1608–13
27. Johnson LR, Copeland EM, Dudrick SJ, Lichtenberger LM, Castro GA. 1975. Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats. *Gastroenterology* 68:1177–83
28. Shulman RJ. 1988. Effect of different total parenteral nutrition fuel mixes on small intestinal growth and differentiation in the infant miniature pig. *Gastroenterology* 95:85–92
29. Burrin DG, Stoll B, Jiang RH, Chang XY, Hartmann B, et al. 2000. Minimal enteral nutrient requirements for intestinal growth in neonatal pigs: How much is enough? *Am. J. Clin. Nutr.* 71:1603–10
30. Burrin DG, Stoll B, Chang X, van Goudoever JB, Fujii H, et al. 2003. Parenteral nutrition results in impaired lactose digestion and hexose absorption when enteral feeding is initiated in infant pigs. *Am. J. Clin. Nutr.* 78:461–70
31. Kansagra K, Stoll B, Rognerud C, Niinikoski H, Ou CN, et al. 2003. Total parenteral nutrition adversely affects gut barrier function in neonatal pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 285:G1162–G70
32. Niinikoski H, Stoll B, Guan X, Kansagra K, Lambert BD, et al. 2004. Onset of small intestinal atrophy is associated with reduced intestinal blood flow in TPN-fed neonatal pigs. *J. Nutr.* 134:1467–74
33. Ganessunker D, Gaskins HR, Zuckermann FA, Donovan SM. 1999. Total parenteral nutrition alters molecular and cellular indices of intestinal inflammation in neonatal pigs. *JPEN* 23:337–44
34. Conour JE, Ganessunker D, Tappenden KA, Donovan SM, Gaskins HR. 2002. Acidomucin goblet cell expansion induced by parenteral nutrition in the small intestine of pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283:G1185–G96
35. Deplancke B, Vidal O, Ganessunker D, Donovan SM, Mackie RI, Gaskins HR. 2002. Selective growth of mucolytic bacteria including *Clostridium perfringens* in a neonatal piglet model of total parenteral nutrition. *Am. J. Clin. Nutr.* 76:1117–25
36. Aynsley-Green A, Lucas A, Lawson GR, Bloom SR. 1990. Gut hormones and regulatory peptides in relation to enteral feeding, gastroenteritis, and necrotizing enterocolitis in infancy. *J. Pediatr.* 117:S24–32
37. Lucas A, Bloom SR, Aynsley-Green A. 1986. Gut hormones and 'minimal enteral feeding.' *Acta Paediatr. Scand.* 75:719–23
38. Berseth CL. 1995. Minimal enteral feedings. *Clin. Perinatol.* 22:195–205
39. Shulman RJ, Schanler RJ, Lau C, Heitkemper M, Ou CN, Smith EO. 1998. Early feeding, feeding tolerance, and lactase activity in preterm infants. *J. Pediatr.* 133:645–49

40. Shulman RJ, Schanler RJ, Lau C, Heitkemper M, Ou CN, Smith EO. 1998. Early feeding, antenatal glucocorticoids, and human milk decrease intestinal permeability in preterm infants. *Pediatr. Res.* 44:519–23
41. Stoll B, Chang X, Fan MZ, Reeds PJ, Burrin DG. 2000. Enteral nutrient intake level determines intestinal protein synthesis and accretion rates in neonatal pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279:G288–G94
42. Ghoneim N, Bauchart-Thevret C, Oosterloo B, Stoll B, Kulkarni M, et al. 2014. Delayed initiation but not gradual advancement of enteral formula feeding reduces the incidence of necrotizing enterocolitis (NEC) in preterm pigs. *PLOS ONE* 9:e106888
43. Morgan J, Young L, McGuire W. 2014. Slow advancement of enteral feed volumes to prevent necrotising enterocolitis in very low birth weight infants. *Cochrane Database Syst. Rev.* 8:CD001241
44. Morgan J, Young L, McGuire W. 2014. Delayed introduction of progressive enteral feeds to prevent necrotising enterocolitis in very low birth weight infants. *Cochrane Database Syst. Rev.* 12:CD001970
45. Schanler RJ, Shulman RJ, Lau C. 1999. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics* 103:1150–57
46. Cristofalo EA, Schanler RJ, Blanco CL, Sullivan S, Trawoeger R, et al. 2013. Randomized trial of exclusive human milk versus preterm formula diets in extremely premature infants. *J. Pediatr.* 163:1592–5.e1
47. Gartner LM, Morton J, Lawrence RA, Naylor AJ, O'Hare D, et al. 2005. Breastfeeding and the use of human milk. *Pediatrics* 115:496–506
48. Moro GE, Arslanoglu S, Bertino E, Corvaglia L, Montirosso R, et al. 2015. XII. Human milk in feeding premature infants: consensus statement. *J. Pediatr. Gastroenterol. Nutr.* 61(Suppl. 1):S16–19
49. Burrin DG, Shulman RJ, Reeds PJ, Davis TA, Gravitt KR. 1992. Porcine colostrum and milk stimulate visceral organ and skeletal muscle protein synthesis in neonatal pigs. *J. Nutr.* 122:1205–13
50. Burrin DG, Davis TA, Ebner S, Schoknecht PA, Fiorotto ML, et al. 1995. Nutrient-independent and nutrient-dependent factors stimulate protein synthesis in colostrum-fed newborn pigs. *Pediatr. Res.* 37:593–99
51. Burrin DG, Dudley MA, Reeds PJ, Shulman RJ, Perkinson S, Rosenberger J. 1994. Feeding colostrum rapidly alters enzymatic activity and the relative isoform abundance of jejunal lactase in neonatal pigs. *J. Nutr.* 124:2350–57
52. Thymann T, Burrin DG, Tappenden KA, Bjornvad CR, Jensen SK, Sangild PT. 2006. Formula-feeding reduces lactose digestive capacity in neonatal pigs. *Br. J. Nutr.* 95:1075–81
53. Sangild PT, Siggers RH, Schmidt M, Elnif J, Bjornvad CR, et al. 2006. Diet- and colonization-dependent intestinal dysfunction predisposes to necrotizing enterocolitis in preterm pigs. *Gastroenterology* 130:1776–92
54. Bjornvad CR, Thymann T, Deutz NE, Burrin DG, Jensen SK, et al. 2008. Enteral feeding induces diet-dependent mucosal dysfunction, bacterial proliferation, and necrotizing enterocolitis in preterm pigs on parenteral nutrition. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295:G1092–G103
55. Jensen ML, Sangild PT, Lykke M, Schmidt M, Boye M, et al. 2013. Similar efficacy of human banked milk and bovine colostrum to decrease incidence of necrotizing enterocolitis in preterm pigs. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305:R4–R12
56. Shen RL, Thymann T, Ostergaard MV, Stoy AC, Krych L, et al. 2015. Early gradual feeding with bovine colostrum improves gut function and NEC resistance relative to infant formula in preterm pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 309:G310–23
57. Rasmussen SO, Martin L, Ostergaard MV, Rudloff S, Li Y, et al. 2016. Bovine colostrum improves neonatal growth, digestive function, and gut immunity relative to donor human milk and infant formula in preterm pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 311:G480–91
58. Schanler RJ. 2007. Mother's own milk, donor human milk, and preterm formulas in the feeding of extremely premature infants. *J. Pediatr. Gastroenterol. Nutr.* 45(Suppl. 3):S175–77
59. Li Y, Nguyen DN, de Waard M, Christensen L, Zhou P, et al. 2017. Pasteurization procedures for donor human milk affect body growth, intestinal structure, and resistance against bacterial infections in preterm pigs. *J. Nutr.* 147:1121–30

60. O'Connor DL, Kiss A, Tomlinson C, Bando N, Bayliss A, et al. 2018. Nutrient enrichment of human milk with human and bovine milk-based fortifiers for infants born weighing <1250 g: a randomized clinical trial. *Am. J. Clin. Nutr.* 108:108–16
61. Sun J, Li Y, Nguyen DN, Mortensen MS, van den Akker CHP, et al. 2018. Nutrient fortification of human donor milk affects intestinal function and protein metabolism in preterm pigs. *J. Nutr.* 148:336–47
62. Li Y, Juhl SM, Ye X, Shen RL, Iyore EO, et al. 2017. A stepwise, pilot study of bovine colostrum to supplement the first enteral feeding in preterm infants (Precolos): study protocol and initial results. *Front. Pediatr.* 5:42
63. Nino DF, Sodhi CP, Hackam DJ. 2016. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. *Nat. Rev. Gastroenterol. Hepatol.* 13:590–600
64. Yee WH, Soraisham AS, Shah VS, Aziz K, Yoon W, et al. 2012. Incidence and timing of presentation of necrotizing enterocolitis in preterm infants. *Pediatrics* 129:e298–304
65. Caplan MS, Fanaroff A. 2017. Necrotizing: a historical perspective. *Semin. Perinatol.* 41:2–6
66. Lu P, Sodhi CP, Jia H, Shaffiey S, Good M, et al. 2014. Animal models of gastrointestinal and liver diseases. Animal models of necrotizing enterocolitis: pathophysiology, translational relevance, and challenges. *Am. J. Physiol. Gastrointest. Liver Physiol.* 306:G917–28
67. Barlow B, Santulli TV, Heird WC, Pitt J, Blanc WA, Schullinger JN. 1974. An experimental study of acute neonatal enterocolitis—the importance of breast milk. *J. Pediatr. Surg.* 9:587–95
68. Premkumar MH, Sule G, Nagamani SC, Chakkalakal S, Nordin A, et al. 2014. Argininosuccinate lyase in enterocytes protects from development of necrotizing enterocolitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 307:G347–54
69. Touloukian RJ, Posch JN, Spencer R. 1972. The pathogenesis of ischemic gastroenterocolitis of the neonate: selective gut mucosal ischemia in asphyxiated neonatal pigs. *J. Pediatr. Surg.* 7:194–205
70. Di Lorenzo M, Bass J, Krantis A. 1995. An intraluminal model of necrotizing enterocolitis in the developing neonatal piglet. *J. Pediatr. Surg.* 30:1138–42
71. Nowicki PT. 2005. Ischemia and necrotizing enterocolitis: where, when, and how. *Semin. Pediatr. Surg.* 14:152–58
72. Cohen IT, Nelson SD, Moxley RA, Hirsh MP, Counihan TC, Martin RF. 1991. Necrotizing enterocolitis in a neonatal piglet model. *J. Pediatr. Surg.* 26:598–601
73. Crissinger KD, Burney DL, Velasquez OR, Gonzalez E. 1994. An animal model of necrotizing enterocolitis induced by infant formula and ischemia in developing pigs. *Gastroenterology* 106:1215–22
74. Yu H, Hasan NM, In JG, Estes MK, Kovbasnjuk O, et al. 2017. The contributions of human mini-intestines to the study of intestinal physiology and pathophysiology. *Annu. Rev. Physiol.* 79:291–312
75. Senger S, Ingano L, Freire R, Anselmo A, Zhu W, et al. 2018. Human fetal-derived enterospheres provide insights on intestinal development and a novel model to study necrotizing enterocolitis (NEC). *Cell. Mol. Gastroenterol. Hepatol.* 5:549–68
76. Gonzalez LM, Moeser AJ, Blikslager AT. 2015. Animal models of ischemia-reperfusion-induced intestinal injury: progress and promise for translational research. *Am. J. Physiol. Gastrointest. Liver Physiol.* 308:G63–G75
77. Gonzalez LM, Williamson I, Piedrahita JA, Blikslager AT, Magness ST. 2013. Cell lineage identification and stem cell culture in a porcine model for the study of intestinal epithelial regeneration. *PLOS ONE* 8:e66465
78. Stieler Stewart A, Freund JM, Blikslager AT, Gonzalez LM. 2018. Intestinal stem cell isolation and culture in a porcine model of segmental small intestinal ischemia. *J. Vis. Exp.* 135:e57647
79. Robinson JL, Smith VA, Stoll B, Agarwal U, Premkumar MH, et al. 2018. Prematurity reduces citrulline-arginine-nitric oxide production and precedes the onset of necrotizing enterocolitis in pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 315:G638–G49
80. Morgan J, Young L, McGuire W. 2015. Slow advancement of enteral feed volumes to prevent necrotising enterocolitis in very low birth weight infants. *Cochrane Database Syst. Rev.* 8:CD001241
81. Lucas A, Cole TJ. 1990. Breast milk and neonatal necrotising enterocolitis. *Lancet* 336:1519–23

82. Li Y, Jensen ML, Chatterton DE, Jensen BB, Thymann T, et al. 2013. Raw bovine milk improves gut responses to feeding relative to infant formula in preterm pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 306:G81–90
83. Donovan SM. 2016. The role of lactoferrin in gastrointestinal and immune development and function: a preclinical perspective. *J. Pediatr.* 173(Suppl.):S16–28
84. Burrin DG, Davis TA, Fiorotto ML, Reeds PJ. 1997. Role of milk-borne versus endogenous insulin-like growth factor I in neonatal growth. *J. Anim. Sci.* 75:2739–43
85. Pammi M, Suresh G. 2017. Enteral lactoferrin supplementation for prevention of sepsis and necrotizing enterocolitis in preterm infants. *Cochrane Database Syst. Rev.* 6:CD007137
86. Shamir R, Kolacek S, Koletzko S, Tavori I, Bader D, et al. 2009. Oral insulin supplementation in paediatric short bowel disease: a pilot observational study. *J. Pediatr. Gastroenterol. Nutr.* 49:108–11
87. Shulman RJ. 2002. 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:F131–33
88. Burrin DG, Wester TJ, Davis TA, Amick S, Heath JP. 1996. Orally administered IGF-I increases intestinal mucosal growth in formula-fed neonatal pigs. *Am. J. Physiol.* 270:R1085–91
89. Park YK, Monaco MH, Donovan SM. 1999. Enteral insulin-like growth factor-I augments intestinal disaccharidase activity in pigs receiving total parenteral nutrition. *J. Pediatr. Gastroenterol. Nutr.* 29:198–206
90. Shulman RJ. 1990. Oral insulin increases small intestinal mass and disaccharidase activity in the newborn miniature pig. *Pediatr. Res.* 28:171–75
91. Nguyen DN, Li Y, Sangild PT, Bering SB, Chatterton DE. 2014. Effects of bovine lactoferrin on the immature porcine intestine. *Br. J. Nutr.* 111:321–31
92. Reznikov EA, Comstock SS, Yi C, Contractor N, Donovan SM. 2014. Dietary bovine lactoferrin increases intestinal cell proliferation in neonatal pigs. *J. Nutr.* 144:1401–8
93. Burrin DG, Stoll B, Fan MZ, Dudley MA, Donovan SM, Reeds PJ. 2001. Oral IGF-I alters the post-translational processing but not the activity of lactase-phlorizin hydrolase in formula-fed neonatal pigs. *J. Nutr.* 131:2235–41
94. Kien CL. 1996. Digestion, absorption, and fermentation of carbohydrates in the newborn. *Clin. Perinatol.* 23:211–28
95. Shulman RJ. 1999. In vivo measurements of glucose absorption in preterm infants. *Biol. Neonate* 76:10–18
96. Shulman RJ, Feste A, Ou C. 1995. Absorption of lactose, glucose polymers, or combination in premature infants. *J. Pediatr.* 127:626–31
97. Shulman RJ, Wong WW, Smith EO. 2005. Influence of changes in lactase activity and small-intestinal mucosal growth on lactose digestion and absorption in preterm infants. *Am. J. Clin. Nutr.* 81:472–79
98. Buddington RK, Sangild PT, Hance B, Huang EY, Black DD. 2012. Prenatal gastrointestinal development in the pig and responses after preterm birth. *J. Anim. Sci.* 90(Suppl. 4):290–98
99. Griffin MP, Hansen JW. 1999. Can the elimination of lactose from formula improve feeding tolerance in premature infants? *J. Pediatr.* 135:587–92
100. Shulman RJ, Ou CN, Smith EO. 2011. Evaluation of potential factors predicting attainment of full gavage feedings in preterm infants. *Neonatology* 99:38–44
101. Kien CL. 1990. Colonic fermentation of carbohydrate in the premature infant: possible relevance to necrotizing enterocolitis. *J. Pediatr.* 117:S52–S58
102. Montgomery RK, Mulberg AE, Grand RJ. 1999. Development of the human gastrointestinal tract: twenty years of progress. *Gastroenterology* 116:702–31
103. Thymann T, Moller HK, Stoll B, Stoy AC, Buddington RK, et al. 2009. Carbohydrate maldigestion induces necrotizing enterocolitis in preterm pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 297:G1115–G25
104. Buddington RK, Bering SB, Thymann T, Sangild PT. 2008. Aldohexose malabsorption in preterm pigs is directly related to the severity of necrotizing enterocolitis. *Pediatr. Res.* 63:382–87
105. Buddington RK, Davis SL, Buddington KK. 2018. The risk of necrotizing enterocolitis differs among preterm pigs fed formulas with either lactose or maltodextrin. *J. Pediatr. Gastroenterol. Nutr.* 66:e61–e66

106. Call L, Stoll B, Oosterloo B, Ajami N, Sheikh F, et al. 2018. Metabolomic signatures distinguish the impact of formula carbohydrates on disease outcome in a preterm piglet model of NEC. *Microbiome* 6:111
107. Nickerson KP, Chanin R, McDonald C. 2015. Deregulation of intestinal anti-microbial defense by the dietary additive, maltodextrin. *Gut Microbes* 6:78–83
108. Nickerson KP, Homer CR, Kessler SP, Dixon LJ, Kabi A, et al. 2014. The dietary polysaccharide maltodextrin promotes *Salmonella* survival and mucosal colonization in mice. *PLOS ONE* 9:e101789
109. Laudisi F, Di Fusco D, Dinallo V, Stolfi C, Di Grazia A, et al. 2019. The food additive maltodextrin promotes endoplasmic reticulum stress-driven mucus depletion and exacerbates intestinal inflammation. *Cell. Mol. Gastroenterol. Hepatol.* 7:457–73
110. Dasgupta S, Jain SK. 2017. Protective effects of amniotic fluid in the setting of necrotizing enterocolitis. *Pediatr. Res.* 82:584–95
111. Good M, Siggers RH, Sodhi CP, Afrazi A, Alkhudari F, et al. 2012. Amniotic fluid inhibits Toll-like receptor 4 signaling in the fetal and neonatal intestinal epithelium. *PNAS* 109:11330–35
112. Zani A, Cananzi M, Fascetti-Leon F, Lauriti G, Smith VV, et al. 2014. Preventing necrotising enterocolitis with amniotic fluid stem cells. *Gut* 63:218–19
113. Siggers J, Ostergaard MV, Siggers RH, Skovgaard K, Molbak L, et al. 2013. Postnatal amniotic fluid intake reduces gut inflammatory responses and necrotizing enterocolitis in preterm neonates. *Am. J. Physiol. Gastrointest. Liver Physiol.* 304:G864–75
114. Ostergaard MV, Shen RL, Stoy AC, Skovgaard K, Krych L, et al. 2016. Provision of amniotic fluid during parenteral nutrition increases weight gain with limited effects on gut structure, function, immunity, and microbiology in newborn preterm pigs. *J. Parenter. Enter. Nutr.* 40:552–66
115. Charbonneau MR, Blanton LV, DiGiulio DB, Relman DA, Lebrilla CB, et al. 2016. A microbial perspective of human developmental biology. *Nature* 535:48–55
116. Pammi M, Cope J, Tarr PI, Warner BB, Morrow AL, et al. 2017. Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. *Microbiome* 5:31
117. Nguyen DN, Fuglsang E, Jiang P, Birck MM, Pan X, et al. 2016. Oral antibiotics increase blood neutrophil maturation and reduce bacteremia and necrotizing enterocolitis in the immediate postnatal period of preterm pigs. *Innate Immun.* 22:51–62
118. Birck MM, Nguyen DN, Cilieborg MS, Kamal SS, Nielsen DS, et al. 2016. Enteral but not parenteral antibiotics enhance gut function and prevent necrotizing enterocolitis in formula-fed newborn preterm pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 310:G323–33
119. Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, et al. 2009. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. *Pediatrics* 123:58–66
120. Greenberg RG, Chowdhury D, Hansen NI, Smith PB, Stoll BJ, et al. 2019. Prolonged duration of early antibiotic therapy in extremely premature infants. *Pediatr. Res.* 85:994–1000
121. Donovan SM, Comstock SS. 2016. Human milk oligosaccharides influence neonatal mucosal and systemic immunity. *Ann. Nutr. Metab.* 69(Suppl. 2):42–51
122. Smilowitz JT, Lebrilla CB, Mills DA, German JB, Freeman SL. 2014. Breast milk oligosaccharides: structure-function relationships in the neonate. *Annu. Rev. Nutr.* 34:143–69
123. Pacheco AR, Barile D, Underwood MA, Mills DA. 2015. The impact of the milk glycobiome on the neonate gut microbiota. *Annu. Rev. Anim. Biosci.* 3:419–45
124. Bode L, Jantscher-Krenn E. 2012. Structure-function relationships of human milk oligosaccharides. *Adv. Nutr.* 3:383S–91S
125. Davis EC, Wang M, Donovan SM. 2017. The role of early life nutrition in the establishment of gastrointestinal microbial composition and function. *Gut Microbes* 8:143–71
126. Rasmussen SO, Martin L, Ostergaard MV, Rudloff S, Roggenbuck M, et al. 2017. Human milk oligosaccharide effects on intestinal function and inflammation after preterm birth in pigs. *J. Nutr. Biochem.* 40:141–54
127. Cilieborg MS, Sangild PT, Jensen ML, Ostergaard MV, Christensen L, et al. 2017. α1,2-Fucosyllactose does not improve intestinal function or prevent *Escherichia coli* F18 diarrhea in newborn pigs. *J. Pediatr. Gastroenterol. Nutr.* 64:310–18

128. Cilieborg MS, Bering SB, Ostergaard MV, Jensen ML, Krych L, et al. 2016. Minimal short-term effect of dietary 2′-fucosyllactose on bacterial colonisation, intestinal function and necrotising enterocolitis in preterm pigs. *Br. J. Nutr.* 116:834–41
129. Tao N, Ochonicky KL, German JB, Donovan SM, Lebrilla CB. 2010. Structural determination and daily variations of porcine milk oligosaccharides. *J. Agric. Food Chem.* 58:4653–59
130. Comstock SS, Wang M, Hester SN, Li M, Donovan SM. 2014. Select human milk oligosaccharides directly modulate peripheral blood mononuclear cells isolated from 10-d-old pigs. *Br. J. Nutr.* 111:819–28
131. Li M, Monaco MH, Wang M, Comstock SS, Kuhlenschmidt TB, et al. 2014. Human milk oligosaccharides shorten rotavirus-induced diarrhea and modulate piglet mucosal immunity and colonic microbiota. *ISME J.* 8:1609–20
132. Comstock SS, Li M, Wang M, Monaco MH, Kuhlenschmidt TB, et al. 2017. Dietary human milk oligosaccharides but not prebiotic oligosaccharides increase circulating natural killer cell and mesenteric lymph node memory T cell populations in noninfected and rotavirus-infected neonatal pigs. *J. Nutr.* 147:1041–47
133. Herfel TM, Jacobi SK, Lin X, Fellner V, Walker DC, et al. 2011. Polydextrose enrichment of infant formula demonstrates prebiotic characteristics by altering intestinal microbiota, organic acid concentrations, and cytokine expression in suckling pigs. *J. Nutr.* 141:2139–45
134. Underwood MA. 2019. Probiotics and the prevention of necrotizing enterocolitis. *J. Pediatr. Surg.* 54:405–12
135. Chi C, Buys N, Li C, Sun J, Yin C. 2018. Effects of prebiotics on sepsis, necrotizing enterocolitis, mortality, feeding intolerance, time to full enteral feeding, length of hospital stay, and stool frequency in preterm infants: a meta-analysis. *Eur. J. Clin. Nutr.* 73:657–70
136. Siggers RH, Siggers J, Boye M, Thymann T, Molbak L, et al. 2008. Early administration of probiotics alters bacterial colonization and limits diet-induced gut dysfunction and severity of necrotizing enterocolitis in preterm pigs. *J. Nutr.* 138:1437–44
137. Good M, Sodhi CP, Ozolek JA, Buck RH, Goehring KC, et al. 2014. *Lactobacillus rhamnosus* HN001 decreases the severity of necrotizing enterocolitis in neonatal mice and preterm pigs: evidence in mice for a role of TLR9. *Am. J. Physiol. Gastrointest. Liver Physiol.* 306:G1021–32
138. Obelitz-Ryom K, Rendboe AK, Nguyen DN, Rudloff S, Brandt AB, et al. 2018. Bovine milk oligosaccharides with sialyllactose for preterm pigs. *Nutrients* 10:1489
139. Vaughn BP, Rank KM, Khoruts A. 2019. Fecal microbiota transplantation: current status in treatment of GI and liver disease. *Clin. Gastroenterol. Hepatol.* 17:353–61
140. Nicholson MR, Mitchell PD, Alexander E, Ballal S, Bartlett M, et al. 2019. Efficacy of fecal microbiota transplantation for *Clostridium difficile* infection in children. *Clin. Gastroenterol. Hepatol*. In press
141. Brunse A, Martin L, Rasmussen TS, Christensen L, Skovsted Cilieborg M, et al. 2019. Effect of fecal microbiota transplantation route of administration on gut colonization and host response in preterm pigs. *ISME J.* 13:720–33
142. Prado C, Michels M, Ávila P, Burger H, Milioli MVM, Dal-Pizzol F. 2019. The protective effects of fecal microbiota transplantation in an experimental model of necrotizing enterocolitis. *J. Pediatr. Surg.* 54:1578–83
143. Li X, Li X, Shang Q, Gao Z, Hao F, et al. 2017. Fecal microbiota transplantation (FMT) could reverse the severity of experimental necrotizing enterocolitis (NEC) via oxidative stress modulation. *Free Radic. Biol. Med.* 108:32–43
144. Yu Y, Lu L, Sun J, Petrof EO, Claud EC. 2016. Preterm infant gut microbiota affects intestinal epithelial development in a humanized microbiome gnotobiotic mouse model. *Am. J. Physiol. Gastrointest. Liver Physiol.* 311:G521–32
145. Claud EC, Walker WA. 2001. Hypothesis: Inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. *FASEB J.* 15:1398–403
146. Nanthakumar NN, Fusunyan RD, Sanderson I, Walker WA. 2000. Inflammation in the developing human intestine: a possible pathophysiologic contribution to necrotizing enterocolitis. *PNAS* 97:6043–48

147. Lotz M, Gutle D, Walther S, Menard S, Bogdan C, Hornef MW. 2006. Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. *J. Exp. Med.* 203:973–84
148. Kramer BW, Ikegami M, Moss TJ, Nitsos I, Newnham JP, Jobe AH. 2005. Endotoxin-induced chorioamnionitis modulates innate immunity of monocytes in preterm sheep. *Am. J. Respir. Crit. Care Med.* 171:73–77
149. Newnham JP, Moss TJ, Kramer BW, Nitsos I, Ikegami M, Jobe AH. 2002. The fetal maturational and inflammatory responses to different routes of endotoxin infusion in sheep. *Am. J. Obstet. Gynecol.* 186:1062–68
150. Abreu MT, Fukata M, Arditi M. 2005. TLR signaling in the gut in health and disease. *J. Immunol.* 174:4453–60
151. Sun J, Fegan PE, Desai AS, Madara JL, Hobert ME. 2007. Flagellin-induced tolerance of the Toll-like receptor 5 signaling pathway in polarized intestinal epithelial cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292:G767–G78
152. Reber KM, Nankervis CA, Nowicki PT. 2002. Newborn intestinal circulation: physiology and pathophysiology. *Clin. Perinatol.* 29:23–39
153. Watkins DJ, Besner GE. 2013. The role of the intestinal microcirculation in necrotizing enterocolitis. *Semin. Pediatr. Surg.* 22:83–87
154. Shepherd AP. 1982. Local control of intestinal oxygenation and blood flow. *Annu. Rev. Physiol.* 44:13–27
155. Zheng L, Kelly CJ, Colgan SP. 2015. Physiologic hypoxia and oxygen homeostasis in the healthy intestine. A review in the theme: cellular responses to hypoxia. *Am. J. Physiol. Cell Physiol.* 309:C350–C60
156. Alward CT, Hook JB, Helmrath TA, Mattson JC, Bailie MD. 1978. Effects of asphyxia on cardiac output and organ blood flow in the newborn piglet. *Pediatr. Res.* 12:824–27
157. Behrman RE, Stoll BJ, Kanto WP Jr., Glass RI, Nahmias AJ, Brann AW Jr. 1980. Epidemiology of necrotizing enterocolitis: a case control study. *J. Pediatr.* 96:447–51
158. Seeman SM, Mehal JM, Haberling DL, Holman RC, Stoll BJ. 2016. Infant and maternal risk factors related to necrotising enterocolitis-associated infant death in the United States. *Acta Paediatr. Int. J. Paediatr.* 105:e240–e46
159. Berkhout DJC, Klaassen P, Niemarkt HJ, De Boode WP, Cossey V, et al. 2018. Risk factors for necrotizing enterocolitis: a prospective multicenter case-control study. *Neonatology* 114:277–84
160. Chen YM, Zhang JS, Duan XL. 2003. Changes of microvascular architecture, ultrastructure and permeability or rat jejunal villi at different ages. *World J. Gastroenterol.* 9:795–99
161. Gosche JR, Harris PD, Garrison RN. 1993. Age-related differences in intestinal microvascular responses to low-flow states in adult and suckling rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 264:G447–G53
162. Nankervis CA, Schauer GM, Miller CE. 2000. Endothelin-mediated vasoconstriction in postischemic newborn intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279:G683–G91
163. Nowicki PT, Dunaway DJ, Nankervis CA, Giannnone PJ, Reber KM, et al. 2005. Endothelin-1 in human intestine resected for necrotizing enterocolitis. *J. Pediatr.* 146:805–10
164. Nowicki PT, Caniano DA, Hammond S, Giannone PJ, Besner GE, et al. 2007. Endothelial nitric oxide synthase in human intestine resected for necrotizing enterocolitis. *J. Pediatr.* 150:40–45
165. Shah PS, Shah VS, Kelly LE. 2017. Arginine supplementation for prevention of necrotising enterocolitis in preterm infants. *Cochrane Database Syst. Rev.* 4:CD004339
166. Marini JC, Agarwal U, Robinson JL, Yuan Y, Didelija IC, et al. 2017. The intestinal-renal axis for arginine synthesis is present and functional in the neonatal pig. *Am. J. Physiol. Endocrinol. Metab.* 313:E233–E42
167. Puiman PJ, Stoll B, van Goudoever JB, Burrin DG. 2011. Enteral arginine does not increase superior mesenteric arterial blood flow but induces mucosal growth in neonatal pigs. *J. Nutr.* 141:63–70
168. Colgan SP, Campbell EL, Kominsky DJ. 2016. Hypoxia and mucosal inflammation. *Annu. Rev. Pathol. Mech. Dis.* 11:77–100
169. Hansen CF, Thymann T, Andersen AD, Holst JJ, Hartmann B, et al. 2016. Rapid gut growth but persistent delay in digestive function in the postnatal period of preterm pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 310:G550–G60

170. Bjornvad CR, Schmidt M, Petersen YM, Jensen SK, Offenberg H, et al. 2005. Preterm birth makes the immature intestine sensitive to feeding-induced intestinal atrophy. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289:R1212–R22
171. Gribar SC, Anand RJ, Sodhi CP, Hackam DJ. 2008. The role of epithelial Toll-like receptor signaling in the pathogenesis of intestinal inflammation. *J. Leukoc. Biol.* 83:493–98
172. Siggers J, Sangild PT, Jensen TK, Siggers RH, Skovgaard K, et al. 2011. Transition from parenteral to enteral nutrition induces immediate diet-dependent gut histological and immunological responses in preterm neonates. *Am. J. Physiol. Gastrointest. Liver Physiol.* 301:G435–45
173. Gribar SC, Sodhi CP, Richardson WM, Anand RJ, Gittes GK, et al. 2009. Reciprocal expression and signaling of TLR4 and TLR9 in the pathogenesis and treatment of necrotizing enterocolitis. *J. Immunol.* 182:636–46
174. Garvey AA, Dempsey EM. 2018. Applications of near infrared spectroscopy in the neonate. *Curr. Opin. Pediatr.* 30:209–15
175. Wyatt JS, Delpy DT, Cope M, Wray S, Reynolds EOR. 1986. Quantification of cerebral oxygenation and haemodynamics in sick newborn infants by near infrared spectrophotometry. *Lancet* 328:1063–66
176. Martini S, Corvaglia L. 2018. Splanchnic NIRS monitoring in neonatal care: rationale, current applications and future perspectives. *J. Perinatol.* 38:431–43
177. Zamora IJ, Stoll B, Ethun CG, Sheikh F, Yu L, et al. 2015. Low abdominal NIRS values and elevated plasma intestinal fatty acid-binding protein in a premature piglet model of necrotizing enterocolitis. *PLOS ONE* 10:e0125437
178. Chen MW, Reyes M, Kulikowicz E, Martin L, Hackam DJ, et al. 2018. Abdominal near-infrared spectroscopy in a piglet model of gastrointestinal hypoxia produced by graded hypoxia or superior mesenteric artery ligation. *Pediatr. Res.* 83:1172–81
179. Gay AN, Lazar DA, Stoll B, Naik-Mathuria B, Mushin OP, et al. 2011. Near-infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature pigs. *J. Pediatr. Surg.* 46:1034–40
180. Patel AK, Lazar DA, Burrin DG, Smith EO, Magliaro TJ, et al. 2014. Abdominal near-infrared spectroscopy measurements are lower in preterm infants at risk for necrotizing enterocolitis. *Pediatr. Crit. Care Med.* 15:735–41
181. Squires RH, Duggan C, Teitelbaum DH, Wales PW, Balint J, et al. 2012. Natural history of pediatric intestinal failure: initial report from the Pediatric Intestinal Failure Consortium. *J. Pediatr.* 161:723–28.e2
182. Dowling RH. 2003. Glucagon-like peptide-2 and intestinal adaptation: an historical and clinical perspective. *J. Nutr.* 133:3703–7
183. Weih S, Nickkholgh A, Kessler M, Frongia G, Hafezi M, et al. 2013. Models of short bowel syndrome in pigs: a technical review. *Eur. Surg. Res.* 51:66–78
184. Turner JM, Wales PW, Nation PN, Wizzard P, Pendlebury C, et al. 2011. Novel neonatal piglet models of surgical short bowel syndrome with intestinal failure. *J. Pediatr. Gastroenterol. Nutr.* 52:9–16
185. Bartholome AL, Albin DM, Baker DH, Holst JJ, Tappenden KA. 2004. Supplementation of total parenteral nutrition with butyrate acutely increases structural aspects of intestinal adaptation after an 80% jejunoileal resection in neonatal pigs. *J. Parenter. Enter. Nutr.* 28:210–22; discussion pp. 22–23
186. Pereira-Fantini PM, Lapthorne S, Gahan CGM, Joyce SA, Charles J, et al. 2017. Farnesoid X receptor agonist treatment alters bile acid metabolism but exacerbates liver damage in a piglet model of short-bowel syndrome. *Cell. Mol. Gastroenterol. Hepatol.* 4:65–74
187. Pereira-Fantini PM, Lapthorne S, Joyce SA, Dellios NL, Wilson G, et al. 2014. Altered FXR signalling is associated with bile acid dysmetabolism in short bowel syndrome-associated liver disease. *J. Hepatol.* 61:1115–25
188. Lin S, Stoll B, Robinson J, Pastor JJ, Marini JC, et al. 2019. Differential action of TGR5 agonists on GLP-2 secretion and promotion of intestinal adaptation in piglet short bowel model. *Am. J. Physiol. Gastrointest. Liver Physiol.* 316:G641–52

189. Pereira-Fantini PM, Thomas SL, Wilson G, Taylor RG, Sourial M, Bines JE. 2011. Short- and long-term effects of small bowel resection: a unique histological study in a piglet model of short bowel syndrome. *Histochem. Cell Biol.* 135:195–202
190. Vegge A, Thymann T, Lund P, Stoll B, Bering SB, et al. 2013. Glucagon-like peptide-2 induces rapid digestive adaptation following intestinal resection in preterm neonates. *Am. J. Physiol. Gastrointest. Liver Physiol.* 305:G277–85
191. Naberhuis JK, Deutsch AS, Tappenden KA. 2017. Teduglutide-stimulated intestinal adaptation is complemented and synergistically enhanced by partial enteral nutrition in a neonatal piglet model of short bowel syndrome. *J. Parenter. Enter. Nutr.* 41:853–65
192. Hua Z, Turner JM, Sigalet DL, Wizzard PR, Nation PN, et al. 2013. Role of glucagon-like peptide-2 deficiency in neonatal short-bowel syndrome using neonatal pigs. *Pediatr. Res.* 73:742–49
193. Pereira-Fantini PM, Thomas SL, Taylor RG, Nagy E, Sourial M, et al. 2008. Colostrum supplementation restores insulin-like growth factor -1 levels and alters muscle morphology following massive small bowel resection. *J. Parenter. Enter. Nutr.* 32:266–75
194. Goulet O, Olieman J, Ksiazyk J, Spolidoro J, Tibboe D, et al. 2013. Neonatal short bowel syndrome as a model of intestinal failure: physiological background for enteral feeding. *Clin. Nutr.* 32:162–71
195. Aunsholt L, Jeppesen PB, Lund P, Sangild PT, Ifaoui IB, et al. 2014. Bovine colostrum to children with short bowel syndrome: a randomized, double-blind, crossover pilot study. *J. Parenter. Enter. Nutr.* 38:99–106
196. Aunsholt L, Qvist N, Sangild PT, Vegge A, Stoll B, et al. 2017. Minimal enteral nutrition to improve adaptation after intestinal resection in pigs and infants. *J. Parenter. Enter. Nutr.* 42:446–54
197. Jeppesen PB, Pertkiewicz M, Messing B, Iyer K, Seidner DL, et al. 2012. Teduglutide reduces need for parenteral support among patients with short bowel syndrome with intestinal failure. *Gastroenterology* 143:1473–81.e3
198. Carter BA, Cohran VC, Cole CR, Corkins MR, Dimmitt RA, et al. 2017. Outcomes from a 12-week, open-label, multicenter clinical trial of teduglutide in pediatric short bowel syndrome. *J. Pediatr.* 181:102–11.e5
199. Guan X, Karpen HE, Stephens J, Bukowski JT, Niu S, et al. 2006. GLP-2 receptor localizes to enteric neurons and endocrine cells expressing vasoactive peptides and mediates increased blood flow. *Gastroenterology* 130:150–64
200. Guan XF, Stoll B, Lu XF, Tappenden KA, Holst JJ, et al. 2003. GLP-2-mediated up-regulation of intestinal blood flow and glucose uptake is nitric oxide-dependent in TPN-fed pigs. *Gastroenterology* 125:136–47
201. Stephens J, Stoll B, Cottrell J, Chang X, Helmrath M, Burrin DG. 2006. Glucagon-like peptide-2 acutely increases proximal small intestinal blood flow in TPN-fed neonatal pigs. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290:R283–R89
202. Burrin DG, Stoll B, Guan X, Cui L, Chang X, Hadsell D. 2007. GLP-2 rapidly activates divergent intracellular signaling pathways involved in intestinal cell survival and proliferation in neonatal pigs. *Am. J. Physiol. Endocrinol. Metab.* 292:E281–E91
203. Burrin DG, Stoll B, Guan X, Cui L, Chang X, Holst JJ. 2005. Glucagon-like peptide 2 dose-dependently activates intestinal cell survival and proliferation in neonatal pigs. *Endocrinology* 146:22–32
204. Burrin DG, Stoll B, Jiang R, Petersen Y, Elnif J, et al. 2000. GLP-2 stimulates intestinal growth in premature TPN-fed pigs by suppressing proteolysis and apoptosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279:G1249–G56
205. Cottrell JJ, Stoll B, Buddington RK, Stephens JE, Cui L, et al. 2006. Glucagon-like peptide-2 protects against TPN-induced intestinal hexose malabsorption in enterally refed pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 290:G293–300
206. Sigalet DL, de Heuvel E, Wallace L, Bulloch E, Turner J, et al. 2014. Effects of chronic glucagon-like peptide-2 therapy during weaning in neonatal pigs. *Regul. Pept.* 188:70–80
207. Amin H, Holst JJ, Hartmann B, Wallace L, Wright J, Sigalet DL. 2008. Functional ontogeny of the proglucagon-derived peptide axis in the premature human neonate. *Pediatrics* 121:e180–86

208. Barnes JL, Hartmann B, Holst JJ, Tappenden KA. 2012. Intestinal adaptation is stimulated by partial enteral nutrition supplemented with the prebiotic short-chain fructooligosaccharide in a neonatal intestinal failure piglet model. *J. Parenter. Enter. Nutr.* 36:524–37
209. Slim GM, Lansing M, Wizzard P, Nation PN, Wheeler SE, et al. 2019. Novel long-acting GLP-2 analogue, FE 203799 (apraglutide), enhances adaptation and linear intestinal growth in a neonatal piglet model of short bowel syndrome with total resection of the ileum. *J. Parenter. Enter. Nutr.* 43:891–98
210. Thymann T, Stoll B, Mecklenburg L, Burrin DG, Vegge A, et al. 2014. Acute effects of the glucagon-like peptide 2 analogue, teduglutide, on intestinal adaptation in short bowel syndrome. *J. Pediatr. Gastroenterol. Nutr.* 58:694–702
211. Suri M, Turner JM, Sigalet DL, Wizzard PR, Nation PN, et al. 2014. Exogenous glucagon-like peptide-2 improves outcomes of intestinal adaptation in a distal-intestinal resection neonatal piglet model of short bowel syndrome. *Pediatr. Res.* 76:370–77
212. Lim DW, Diane A, Muto M, Vine DF, Nation PN, et al. 2017. Differential effects on intestinal adaptation following exogenous glucagon-like peptide 2 therapy with and without enteral nutrition in neonatal short bowel syndrome [formula: see text]. *J. Parenter. Enter. Nutr.* 41:156–70
213. Naimi RM, Hvistendahl M, Enevoldsen LH, Madsen JL, Fuglsang S, et al. 2019. Glepaglutide, a novel long-acting glucagon-like peptide-2 analogue, for patients with short bowel syndrome: a randomised phase 2 trial. *Lancet Gastroenterol. Hepatol.* 4:354–63
214. Rowland KJ, Trivedi S, Lee D, Wan K, Kulkarni RN, et al. 2011. Loss of glucagon-like peptide-2-induced proliferation following intestinal epithelial insulin-like growth factor-1-receptor deletion. *Gastroenterology* 141:2166–75.e7
215. Drucker DJ, Habener JF, Holst JJ. 2017. Discovery, characterization, and clinical development of the glucagon-like peptides. *J. Clin. Investig.* 127:4217–27
216. Lim DW, Levesque CL, Vine DF, Muto M, Koepke JR, et al. 2017. Synergy of glucagon-like peptide-2 and epidermal growth factor coadministration on intestinal adaptation in neonatal pigs with short bowel syndrome. *Am. J. Physiol. Gastrointest. Liver Physiol.* 312:G390–G404
217. Jain AK, Stoll B, Burrin DG, Holst JJ, Moore DD. 2012. Enteral bile acid treatment improves parenteral nutrition-related liver disease and intestinal mucosal atrophy in neonatal pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 302:G218–24
218. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. 2006. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. *Pediatrics* 117:1253–61
219. Ziegler EE, Thureen PJ, Carlson SJ. 2002. Aggressive nutrition of the very low birthweight infant. *Clin. Perinatol.* 29:225–44
220. Lee WS, Sokol RJ. 2015. Intestinal microbiota, lipids, and the pathogenesis of intestinal failure-associated liver disease. *J. Pediatr.* 167:519–26
221. Zambrano E, El Hennawy M, Ehrenkranz RA, Zelterman D, Reyes-Mugica M. 2004. Total parenteral nutrition induced liver pathology: an autopsy series of 24 newborn cases. *Pediatr. Dev. Pathol.* 7:425–32
222. Duro D, Mitchell PD, Kalish LA, Martin C, McCarthy M, et al. 2011. Risk factors for parenteral nutrition-associated liver disease following surgical therapy for necrotizing enterocolitis: a Glaser Pediatric Research Network Study [corrected]. *J. Pediatr. Gastroenterol. Nutr.* 52:595–600
223. Stoll B, Horst DA, Cui L, Chang X, Ellis KJ, et al. 2010. Chronic parenteral nutrition induces hepatic inflammation, steatosis, and insulin resistance in neonatal pigs. *J. Nutr.* 140:2193–200
224. Stoll B, Puiman PJ, Cui L, Chang X, Benight NM, et al. 2012. Continuous parenteral and enteral nutrition induces metabolic dysfunction in neonatal pigs. *J. Parenter. Enter. Nutr.* 36:538–50
225. Turner JM, Josephson J, Field CJ, Wizzard PR, Ball RO, et al. 2015. Liver disease, systemic inflammation, and growth using a mixed parenteral lipid emulsion, containing soybean oil, fish oil, and medium chain triglycerides, compared with soybean oil in parenteral nutrition-fed neonatal pigs. *J. Parenter. Enter. Nutr.* 40:973–81
226. Muto M, Lim D, Soukvilay A, Field C, Wizzard PR, et al. 2017. Supplemental parenteral vitamin E into conventional soybean lipid emulsion does not prevent parenteral nutrition-associated liver disease in full-term neonatal pigs. *J. Parenter. Enter. Nutr.* 41:575–82

227. Ng K, Stoll B, Chacko S, Saenz de Pipaon M, Lauridsen C, et al. 2016. Vitamin E in new-generation lipid emulsions protects against parenteral nutrition-associated liver disease in parenteral nutrition-fed preterm pigs. *J. Parenter. Enter. Nutr.* 40:656–71
228. Vlaardingerbroek H, Ng K, Stoll B, Benight N, Chacko S, et al. 2014. New generation lipid emulsions prevent PNALD in chronic parenterally fed preterm pigs. *J. Lipid Res.* 55:466–77
229. Guthrie G, Kulkarni M, Vlaardingerbroek H, Stoll B, Ng K, et al. 2016. Multi-omic profiles of hepatic metabolism in TPN-fed preterm pigs administered new generation lipid emulsions. *J. Lipid Res.* 57:1696–711
230. Lavoie JC, Chessex P. 2019. Parenteral nutrition and oxidant stress in the newborn: a narrative review. *Free Radic. Biol. Med.* 142:155–67
231. El Kasmi KC, Anderson AL, Devereaux MW, Vue PM, Zhang W, et al. 2013. Phytosterols promote liver injury and Kupffer cell activation in parenteral nutrition-associated liver disease. *Sci. Transl. Med.* 5:206ra137
232. Koelfat KVK, Schaap FG, Hodin C, Visschers RGJ, Svavarsson BI, et al. 2017. Parenteral nutrition dysregulates bile salt homeostasis in a rat model of parenteral nutrition-associated liver disease. *Clin. Nutr.* 36:1403–10
233. Zhan L, Yang I, Kong B, Shen J, Gorczyca L, et al. 2016. Dysregulation of bile acid homeostasis in parenteral nutrition mouse model. *Am. J. Physiol. Gastrointest. Liver Physiol.* 310:G93–G102
234. Feng Y, Demehri FR, Xiao W, Tsai YH, Jones JC, et al. 2017. Interdependency of EGF and GLP-2 signaling in attenuating mucosal atrophy in a mouse model of parenteral nutrition. *Cell. Mol. Gastroenterol. Hepatol.* 3:447–68
235. Gura KM, Duggan CP, Collier SB, Jennings RW, Folkman J, et al. 2006. Reversal of parenteral nutrition-associated liver disease in two infants with short bowel syndrome using parenteral fish oil: implications for future management. *Pediatrics* 118:e197–e201
236. Nandivada P, Fell GL, Gura KM, Puder M. 2016. Lipid emulsions in the treatment and prevention of parenteral nutrition-associated liver disease in infants and children. *Am. J. Clin. Nutr.* 103:629S–34S
237. Diamond IR, Grant RC, Pencharz PB, de Silva N, Feldman BM, et al. 2016. Preventing the progression of intestinal failure-associated liver disease in infants using a composite lipid emulsion. *J. Parenter. Enter. Nutr.* 41:866–77
238. Repa A, Binder C, Thanhaeuser M, Kreissl A, Pablik E, et al. 2018. A mixed lipid emulsion for prevention of parenteral nutrition associated cholestasis in extremely low birth weight infants: a randomized clinical trial. *J. Pediatr.* 194:87–93.e1
239. Molina TL, Stoll B, Mohammad M, Mohila CA, Call L, et al. 2019. New generation lipid emulsions increase brain DHA and improve body composition, but not short-term neurodevelopment in parenterally-fed preterm pigs. *Brain Behav. Immun.* In press
240. Clayton PT, Bowron A, Mills KA, Massoud A, Casteels M, Milla PJ. 1993. Phytosterolemia in children with parenteral nutrition-associated cholestatic liver disease. *Gastroenterology* 105:1806–13
241. Carter BA, Taylor OA, Prendergast DR, Zimmerman TL, Von Furstenberg R, et al. 2007. Stigmasterol, a soy lipid-derived phytosterol, is an antagonist of the bile acid nuclear receptor FXR. *Pediatr. Res.* 62:301–6
242. Guthrie G, Tackett B, Stoll B, Martin C, Olutoye O, Burrin DG. 2018. Phytosterols synergize with endotoxin to augment inflammation in Kupffer cells but alone have limited direct effect on hepatocytes. *J. Parenter. Enter. Nutr.* 42:37–48
243. El Kasmi KC, Vue PM, Anderson AL, Devereaux MW, Ghosh S, et al. 2018. Macrophage-derived IL-1β/NF-κB signaling mediates parenteral nutrition-associated cholestasis. *Nat. Commun.* 9:1393
244. Burrin DG, Ng K, Stoll B, Saenz De Pipaon M. 2014. Impact of new-generation lipid emulsions on cellular mechanisms of parenteral nutrition-associated liver disease. *Adv. Nutr.* 5:82–91
245. Teitelbaum DH, Tracy TF Jr., Aouthmany MM, Llanos A, Brown MB, et al. 2005. Use of cholecystokinin-octapeptide for the prevention of parenteral nutrition-associated cholestasis. *Pediatrics* 115:1332–40
246. Degirolamo C, Sabba C, Moschetta A. 2016. Therapeutic potential of the endocrine fibroblast growth factors FGF19, FGF21 and FGF23. *Nat. Rev. Drug Discov.* 15:51–69

247. Wright TJ, Ladher R, McWhirter J, Murre C, Schoenwolf GC, Mansour SL. 2004. Mouse FGF15 is the ortholog of human and chick FGF19, but is not uniquely required for otic induction. *Dev. Biol.* 269:264–75
248. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, et al. 2005. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab.* 2:217–25
249. Mudaliar S, Henry RR, Sanyal AJ, Morrow L, Marschall HU, et al. 2013. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology* 145:574–82
250. Sánchez-Infantes D, Gallego-Escuredo JM, Díaz M, Aragonés G, Sebastiani G, et al. 2015. Circulating FGF19 and FGF21 surge in early infancy from infra- to supra-adult concentrations. *Int. J. Obes.* 39:742–46
251. Memon N, Griffin IJ, Lee CW, Herdt A, Weinberger BI, et al. 2018. Developmental regulation of the gut-liver (FGF19-CYP7A1) axis in neonates. *J. Matern. Fetal Neonatal Med.* In press
252. Owen BM, Mangelsdorf DJ, Kliewer SA. 2015. Tissue-specific actions of the metabolic hormones FGF15/19 and FGF21. *Trends Endocrinol. Metab.* 26:22–29