A MULTIFACETED APPROACH TO ADDRESSING FEEDING INTOLERANCE IN THE PRETERM INFANT

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DISSERTATION

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ABSTRACT

In 2013, 11.4% of births in the United States occurred preterm.[1] Due to the immaturity of the gastrointestinal tract, these infants are at increased risk of feeding intolerance and necrotizing enterocolitis (NEC). NEC is the most common surgical emergency among infants and proves fatal for 25-33% those diagnosed.[2,3] Effective early detection of these conditions,[4] combined with targeted therapies to promote intestinal adaptation and weaning from parenteral nutrition (PN), represent an important opportunity to improve infant outcomes. To this end, the following studies were conducted.

1. The safety and efficacy of teduglutide, an analog of human glucagon-like peptide-2 (GLP-2) approved for use only in adults, in reducing PN requirements was assessed via a systematic review.[5] Fourteen reports met the inclusion criteria. Teduglutide reduced PN requirements vs. placebo regardless of PN dependence duration, whereas adverse event incidence was similar between groups (number needed to treat to benefit [NNTB] = 3-4; number needed to treat to harm [NNTH] = 24-187).

2. Teduglutide-stimulated intestinal adaptation, potential synergies with partial enteral nutrition (PEN), and distinct temporal markers of adaptation were investigated in a neonatal piglet model of short bowel syndrome (SBS). Teduglutide improved ($P < 0.05$) mucosal surface area (villus height: duodenum, jejunum, ileum; crypt depth: ileum, colon; proliferation: duodenum, jejunum, ileum, colon; apoptosis: jejunum, ileum, colon) and acute nutrient processing capacity (glucose: duodenum, jejunum, ileum; glutamine: duodenum, jejunum). PEN complimented and synergistically enhanced these effects. Structural adaptation preceded functional adaptation, but crypt depth was a strong indicator of adaptation, regardless of time.

3. A novel feeding intolerance and NEC risk scoring tool was implemented in the University of Illinois-affiliated Carle Foundation Hospital (CFH) level III neonatal intensive care unit (NICU). During the study period, 499 tools were completed on the 133 enrolled infants. Indices of feeding intolerance included days with emesis, abdominal distention, or gastric residuals > 50% of previous feeding volume, and NEC. Anonymous surveys (n = 42) indicated nurses' positive attitudes toward the tool (ease of use of 6.9 [SD 1.9] on 10-point scale). Estimated tool completion time was 4.2 minutes (range 1-10). Error rate (9.2%), Cronbach's alpha (0.71), the intraclass correlation coefficient (ICC; 0.99), and Fleiss' kappa (1.00) were in acceptable ranges. Gestational age at birth, hypoxia/asphyxia at birth, red blood cell (RBC) transfusion, and congenital heart disease/patent ductus arteriosis (PDA) were significantly associated with all four outcome measures. Total optimized tool score was also associated with all four outcome measures, with area under the ROC curve (AUC) and diagnostic odds ratio (OR) estimates [95% CI] of: emesis, AUC = 0.69 and OR = 1.14 [1.06, 1.23]; abdominal distention, AUC = 0.82 and OR = 1.28 [1.18, 1.41]; gastric residuals > 50% of previous feeding volume, AUC = 0.64 and OR = 1.11 [1.04, 1.20]; NEC, AUC = 0.90 and OR = 1.29 [1.12, 1.56]. Scores of infants who did and did not develop each of the four outcome measures were significantly ($P < 0.05$) different, and an "at-risk" threshold of 9 points was established.

The tool represents a clinically feasible means to discriminate infants at risk of feeding intolerance and NEC. Further refinement will improve its clinical utility and identify infants who may benefit from targeted therapies, including teduglutide and/or PEN, to promote gastrointestinal maturation and improve feeding tolerance.

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CHAPTER 1

LITERATURE REVIEW

INTRODCTION

Worldwide, approximately 15 million infants are born preterm each year.[1] The combination of gastrointestinal immaturity and high nutrient requirements for catch-up growth predispose these infants to feeding intolerance, which may lead to suboptimal nutrition and subsequent adverse outcomes including reduced brain growth, cognitive delays, and necrotizing enterocolitis (NEC).[2-4] Given the large and lasting detrimental impact of feeding intolerance on growth and development, it is crucial that this condition be detected as early as possible and treated promptly with evidence-based therapies. Targeted investigation into both treatment and prevention of feeding intolerance should effectively improve preterm infant outcomes.

PRETERM BIRTH

Preterm birth is defined as occurring prior to 37 completed weeks (259 days) gestation.[5] In 2013, 11.4% of births in the United States occurred preterm, representing a 21% increase in the overall proportion of preterm births since 1990.[6] This increase is largely attributable to higher rates of multiple gestations and advancing maternal age.[6,7] Eighty percent of preterm births occur spontaneously, and only 20% are induced as a result of maternal or fetal distress.[8]

Worldwide, the preterm birth rate is 11%, ranging from 5% in parts of Europe to 18% in parts of Africa.[1] Preterm birth is associated with one-third of all infant deaths in the United States, and direct consequences of preterm birth, including lack of feeding support, recently became the global leading cause of death of children under 5 years of age.[9,10] Survival is inversely associated with gestational age at birth, with extremely preterm infants (< 25 weeks

completed gestation) having a 50% mortality rate.[11,12] Mortality is also inversely associated with birth weight. While extremely low birth weight (< 1000 g; ELBW) infants accounted for just 0.8% of all births in the United States in 2005, they also accounted for 55% of infant deaths the same year.[12]

Fortunately, preterm infant survival rates have improved through the use of antenatal steroid and surfactant therapies.[13-18] However, these surviving infants face severe complications,[14] with rates of respiratory distress, late onset sepsis, and NEC of 93%, 36%, and 11%, respectively, in very low birth weight (< 1500 g; VLBW) infants.[19] Furthermore, cost of care is 10-fold higher in late preterm than term infants, partially due to a 4-fold increase in initial hospital length of stay.[20] Preterm infants are also twice as likely as their term counterparts to be readmitted to the hospital within the first year of life,[21] most commonly due to respiratory issues, infections, and feeding problems.[22-24]

Nutrient needs of the preterm infant

Goals for preterm infant feeding are to regain the up to 20% body weight loss experienced in the first week of life and achieve postnatal growth comparable to intrauterine rates of growth and nutrient accretion.[25,26] Enterally or parenterally fed preterm infants require 120 or 80-100 kcal/kg/day, respectively, and up to 4 g protein/kg/day.[27,28] In the event of chronic illness, enteral energy needs can increase to up to 150 kcal/kg/day.[29,30] Meeting these nutritional goals is imperative to achieve desired weight gain up to 2 times that of the term infant,[31,32] improve long-term neurological outcomes, and prevent hospital readmission.[33,34]

Implications of preterm birth for infant feeding

Parenteral nutrition (PN) administration shortly after birth minimizes weight loss, reverses protein catabolism,[35-38] reduces mortality, and improves growth and neurodevelopmental outcomes.[39-41] However, unless contraindicated, enteral nutrition (EN) is preferable to PN due its lower costs and avoidance of PN-associated complications, as well as its ability to stimulate gastrointestinal maturation and prevent intestinal atrophy. Caution must be exercised in providing EN to preterm infants since growth promotion must be balanced by concerns of feeding intolerance. Anatomical maturation of the gastrointestinal tract is largely complete by 20 weeks gestation, but functional maturation, including motility, gastric acid secretion, gastroesophageal sphincter tone, enzyme activity, and bile acid availability, may not be complete until term gestation.[42-44] Furthermore, cesarean delivery, long neonatal intensive care unit (NICU) stay, or antibiotic administration may cause aberrations in intestinal microbial colonization which predispose to feeding intolerance and NEC development.[45-50]

The signs and symptoms of feeding intolerance are numerous and span from benign fussiness and gassiness to potentially serious signs such as bilious gastric residuals, decreased gastrointestinal motility, apnea, and bradycardia. Feeding intolerance is defined as an inability to digest enteral feeds due to ineffective or uncoordinated bowel activity[51] as evidenced by (1) gastric residual volumes greater than 50% of previous feeding volume; (2) abdominal distension and/or emesis; and (3) a disruption in the feeding plan (delay, decrease or discontinuation of enteral feeds) precipitated by gastrointestinal signs.[52] The utility of this definition is limited in that it does not provide adequate information for clinicians to distinguish benign transient intolerance from early signs of potentially deadly NEC.[53] As such, individual interpretation of

signs of feeding intolerance can lead to suboptimal nutrition and delayed attainment of full enteral feeds due to fear of intolerance exacerbation.

Prevention of feeding intolerance and necrotizing enterocolitis

Feeding intolerance prevention strategies are diverse and vary substantially between institutions. However, each of the following factors should be carefully considered for preterm infant feeding.

Gastrointestinal priming

Complete absence of luminal nutrients is associated with marked intestinal mucosal atrophy, decreased intestinal size, weight, and enzyme activity, increases in intestinal permeability and bacterial translocation, a lack of hormonal response, and delayed motility maturation.[54,55] Provision of minimal EN, or hypocaloric, trophic feedings < 20 mL/kg/day,[56] reduces intestinal permeability and the risk of late-onset sepsis,[57] increases lactase activity,[58] accelerates motility pattern maturation,[55] and results in greater cumulative milk intake when feedings are advanced.[59-61] Trophic feeding does not increase the incidence of NEC, and a recent systematic review of 9 randomized controlled trials (1106 infants) determined initial feeding beyond the fourth day of life was associated with a longer time to establish full enteral feeds.[62,63] The clinical importance of a few days delay in the establishment of full enteral feeds has not been established. Thus the optimal first feed timing remains controversial.

Feeding substance

Feeding breast milk rather than formula may speed achievement of full enteral feeds, as evidenced by faster achievement of full enteral feeds in VLBW infants fed ≥ 50% human milk versus who were exclusively formula-fed.[64] Breast milk also offers protection against NEC

unmatched by commercial formulas due to its anti-inflammatory, immunomodulatory, and prebiotic characteristics as a result of inclusion of immunoglobulins, lactoferrin, oligosaccharides, and platelet-activating factor acetylhydrolase.[42,62,63,65-67] Each 10% increase in enteral intake of human milk is associated with a decrease in NEC risk by a factor of 0.8,[68] and a recent meta-analysis of randomized controlled trials demonstrated the risk of NEC to be 2.8 times higher in formula- versus donor milk-fed infants.[69] Due to these protective effects against NEC, as well as its ability to support structural and functional maturation of the gastrointestinal tract,[70] breast milk, or donor milk when the mother's milk is unavailable, should be fed rather than formula.

Energy density of feeds

In considering energy density of feeds, dilute (10 kcal/oz) versus full-strength (20 kcal/oz) formula was shown in a systematic review of 3 studies including 102 preterm or low birth weight infants to result in earlier attainment of full feeds, lower volume gastric residuals, and less abdominal distention.[71] The incidence of NEC was not reported in these studies, however, and full-strength formula has been shown to promote earlier and more persistent intestinal motility compared to $^{1}/_{3}$ and $^{2}/_{3}$ dilutions[72] as well as water.[73] The limited data in this area precludes the ability to make a universal recommendation regarding the optimal energy density of feeds for preterm infants.

Bolus versus continuous feeds

Bolus feeding mimics the usual fed-fast cycle, results in greater hormonal response than continuous infusion, and does not require an infusion pump.[74] In contrast, continuous infusion increases nutrient absorption by allowing for constant saturation of carrier proteins.[75] A systematic review of 7 trials which included 511 VLBW infants concluded there was no

difference in time to achieve full enteral feeds or regain birth weight, or in NEC rates, in infants fed via continuous drip or bolus.[76] This lack of effect of feeding mode on time to achieve full enteral feeds, combined with the small sample size, make universal recommendation regarding the most advantageous feeding mode for preterm infants impossible.

Rate of volume increase

A recent systematic review of 6 randomized controlled trials including 618 VLBW infants[63] suggests no benefit of slow enteral feeding advancement in prevention of feeding intolerance, as infants with slow (15-20 mL/kg) advancement took longer to regain birth weight and establish full enteral feedings than those receiving more rapid (30-35 mL/kg) feeding advancement, and there was no difference in NEC rate between groups. Thus, per the available data, advancement of enteral feeds at 30-35 mL/kg appears to be a well-tolerated means to promote earlier attainment of full enteral feeds and regain birth weight.

Non-nutritive suckling

While non-nutritive suckling does not increase secretion of gastrointestinal hormones, it does enhance the transition from tube to oral feeding, promote weight gain, and enhance gastrointestinal growth and maturation.[77-83] However, non-nutritive suckling does not require the level of suck-swallow-breath coordination required for safe oral feeding and thus may not be an accurate indicator of an infant's oral feeding readiness.[84]

Medications

Prokinetic agents such as erythromycin, cisapride, or domperidone may accelerate gastric emptying.[85,86] However, despite the reduction of time to full enteral feeds with prokinetic use,[87,88] severe adverse events including fatal cardiac arrhythmia have also been noted.[89] Furthermore, high variability in prokinetic efficacy between trials due to variations in agent,

dose, duration of treatment, route of administration (intravenous versus oral), and whether administered prophylactically or therapeutically, preclude their widespread use.[90,91] In addition to prokinetics, hyperosmolar medications such as multivitamins may induce mucosal injury,[92] and histamine type 2 receptor antagonists may permit bacterial overgrowth due to gastric acid suppression, both contributing to development of NEC.[93,94] Finally, prolonged antibiotic use, especially empirically, may contribute to NEC through alteration of the intestinal microbiota.[95,96] While use of the above or other medications may be necessary during the course of clinical treatment in preterm infants, each should be used judiciously and careful attention paid to potential adverse effects.

Probiotics

Probiotics have been repeatedly shown to decrease the incidence of NEC, likely due to improvement of intestinal barrier function, suppression of pathogenic bacteria, and modulation of the immune system.[97-99] Commonly used strains include Bifidobacterium and Lactobacillus, but effects of probiotics are strain-specific and should not be extrapolated to an unlike population. Use of caution in the inference of probiotic study results is particularly important in neonatal research since as compared to preterm infants, most probiotic research has been performed in comparably large/term infants. However, given the promising research in this area, one meta-analysis on the use of probiotics in prevention of NEC, including 2176 preterm VLBW infants enrolled in 11 randomized controlled trials, concluded that "withholding probiotics from high-risk neonates is now almost unethical."[100]

Individual nutrients

Supplementation of particular nutrients including arginine,[101] glutamine,[102] medium-chain triglycerides,[103] polyunsaturated fatty acids,[104] short chain fatty acids,[70] bovine

lactoferrin,[105] and prebiotics[106,107] induce gut maturation and promote enteral tolerance, but none of the above are substantiated with adequate data to fully recommend their use for prevention of feeding intolerance or NEC.

Established feeding protocols

Established feeding protocols, standard orders which take the above factors into account and include criteria for discontinuation of feedings, improve infant tolerance and outcomes.[108-113] A meta-analysis of 6 observational studies estimated reductions in NEC risk of up to 87% and 29% of infants weighing < 2500 g and < 1500 g, respectively, with the use of a feeding protocol.[114] This risk reduction was hypothesized to be due largely to heightened vigilance and increased awareness of NEC rather than specific characteristics of the feedings protocols themselves.[108,114] Unfortunately, despite best efforts to integrate the above strategies, infants may still develop severe feeding intolerance or ultimately, intestinal failure (IF).

PEDIATRIC INTESTINAL FAILURE

Etiology

IF, caused by disease, congenital defect, or surgical resection, is characterized by the inability to maintain protein, energy, fluid, electrolyte, or micronutrient balance.[115] In both adult and pediatric patients, the most common etiology of IF is an anatomical reduction in functional mass termed short bowel syndrome (SBS).[116] SBS is defined by the need for prolonged PN following bowel resection, usually for a period of at least three months.[117] NEC occurs in 1-3 per 1000 live births and up to 7.7% of all NICU admissions,[118] and is the leading cause (32%) of

pediatric SBS. Additional causes include atresia (20%), volvulus (18%), gastroschisis (17%), and aganglionosis (6%).[119]

Despite being the largest contributing cause of pediatric SBS and subsequent IF, and despite decades of research, the pathogenesis of NEC is still poorly understood, and treatment is difficult, resource-intensive, and highly complex.[120] Approximately 90% of NEC occurs in preterm, rather than term, infants,[121,122] with timing of onset inversely related to gestational age.[123] The current hypothesis regarding pathogenesis is that enteral feeding in the presence of pathogenic intestinal colonization induces an excessive inflammatory response within the immature intestinal epithelium.[124]

Early detection of NEC and development of effective prevention strategies are challenging since the initial clinical manifestations are often nonspecific, involve both systemic and gastrointestinal signs,[125] and are indistinguishable from isolated feeding intolerance.[126] Seventy percent of infants who develop NEC first experience feeding intolerance,[127] but due to its nonspecific etiology, NEC often in the advanced stages before diagnosis is made. NEC treatment accounts for 19% of all initial newborn health care costs in the United States,[128] and the average hospital stay for a neonate requiring surgical NEC treatment is 62 days and costs nearly $300,000.[129] Surgical management to resect necrotic bowel tissue is required in 44-70% of diagnosed cases,[130-132] and early surgical case mortality is nearly 50%. As such, NEC is the most common cause of death in neonates requiring gastrointestinal surgery,[133] and among the top ten causes of infant mortality in the United States.[134] Overall mortality due to NEC ranges from 15-30%, and is inversely correlated with both gestational age at birth and birth weight.[135]

For neonates, the risk of developing permanent IF following bowel resection is greatest when residual bowel length is less than 25% of the predicted length for gestational age.[136]

However, following resection, term infants with as little as 20 cm of remnant intestine plus an intact ileocecal valve, or 40 cm remnant intestine without the ileocecal valve, have successfully weaned from PN.[137-139] In infants treated with fish oil emulsion at an intestinal rehabilitation center, the probability of weaning from PN was 88% and 96% at 12 and 24 months, respectively, for infants with ≥ 50cm of small intestine. For infants with < 50 cm of small bowel, the probability weaning from PN at 12 and 24 months was 23% and 38%, respectively.[140]

Infant potential for intestinal adaptation following resection far exceeds that of adults,[141] and adaptive potential is greater in preterm than in term infants. This is due to the time course of intestinal development, in that the small intestine grows rapidly and doubles in length during the last 15 weeks of gestation,[142,143] reaching 250-300 cm at term.[144] Additional factors that determine pediatric prognosis following intestinal resection include anatomic site of resection and presence or absence of the ileocecal valve, co-morbidities, and remnant bowel functionality and adaptability.[145]

Prevalence

Estimating the prevalence of pediatric SBS is complicated by variations in diagnosis and coding, complex referral and readmission patterns, a paucity of long-term follow-up data, and a lack of population-based studies.[117,136] However, as medical treatments improve and more infants survive SBS, its prevalence is increasing worldwide.[146] The National Institute of Child Health and Development neonatal research network centers recorded a surgical SBS rate of 0.7% in very low birth weight infants born between 2002 and 2005.[147] SBS incidence was inversely correlated with birth weight in that the incidence in VLBW infants was only half that of ELBW infants (28/6,659 or 0.4% versus 61/5,657 or 1.1%, respectively). In Canada, the incidence of

SBS in a large tertiary NICU was 22.1 per 1,000 admissions (2.2%), and 24.5 per 100,000 live births (0.025%).[136] Preterm infants experienced a higher NEC incidence than their term counterparts (353.7/100,000 or 0.35% versus 3.5/100,000 or 0.0035% of live births, respectively). A study from 7 tertiary neonatal units in Italy revealed 0.1% (26/30,353) of all live births and 0.5% (26/5,088) of all patients admitted to the NICU developed IF.[148]

Prognosis

NEC is most commonly diagnosed using Bell's staging criteria,[130] which classifies infants as in the stage I suspected, stage II definite, or stage III advanced phases of NEC. Stages I and II can be treated medically, but stage III necessitates surgery. A stage II or definite diagnosis requires radiographic findings, by which time intestinal damage has already occurred. Furthermore, Bell's criteria are susceptible to inter-observer differences and do not predict the severity or course of the disease.[149]

Progress in NEC prevention is impeded by the current inability to predict which infants are at highest risk of developing the condition.[150] By the time a diagnosis of NEC is confirmed, the patient is typically prescribed *nil per os* (nothing by mouth; NPO) for 5-10 days and is administered broad-spectrum antibiotics.[151] This lack of enteral nutrients can potentiate intestinal atrophy, which in turn hinders the transition to full EN. Thus, as prevention is the key element in reducing the burden of NEC,[151] there is a need for models capable of stratifying infants according to NEC risk to favorably alter disease progression,[149] and increase primary prevention through judicious short-term feeding interruptions and provision of human milk[152,153] and/or probiotics.[100,154] In the absence of adequate prevention, treatment strategies, which are mainly

supportive, include gastric decompression, withholding feedings, and administration of antibiotics and fluids.[151]

If surgical resection is required, the resulting SBS progresses through 3 distinct stages, including an acute phase, a recovery phase, and a maintenance phase, during which bowel adaptation starts as early as 48 hours following resection.[155] The acute phase includes the period from one to approximately four weeks following resection[156] and is characterized by gastric acid hypersecretion and large fluid and electrolyte losses. During this phase, treatment with proton pump inhibitors or H_2 blockers may be necessary to prevent further damage to the intestinal epithelium if hypersecretion is severe enough to cause further malabsorption via inactivation of pancreatic enzymes or precipitation of bile acid.[145] The recovery phase lasts for several months and is characterized by gradual improvements in fluid and electrolyte balance.[156] Provision of EN during this phase should be accompanied by concurrent isoenergetic and isonitrogenous weaning from PN.[156] Treatment during the final maintenance phase of SBS focuses on compensating for any lingering malabsorption through careful diet monitoring and continued PN, if necessary.

Complications of pediatric SBS commonly include gastric hypersecretion,[157,158] bacterial overgrowth,[159] and sepsis.[160] Additional complications include prolonged hospitalization as well as growth retardation and developmental delay.[161] SBS infants have a disease-specific mortality rate nearly 5 times that of infants without SBS (20.2 versus 3.8 per 100 person-years, respectively), and compared to SBS-free controls with the same underlying condition, SBS infants experience a 3-fold increase in mortality.[117,136] SBS-related mortality ranges from 40-60%,[162,163] and is highest during the early post-operative period, after which mortality decreases until 200-350 days after surgery, until rising again upon onset of end-stage liver disease.[162]

Hepatic failure accounts for 60% of pediatric SBS mortality,[136] which highlights the need for optimal PN management as PN is the most significant risk factor for liver disease in SBS infants. Infant survival rates are highest when an integrated, multi-disciplinary management and treatment approach is used.[164]

The first year of pediatric IF treatment is estimated to cost an average of $500,000, and subsequent years approximately $300,000. The high initial costs are explained by the need for surgical resection as well as its associated complications, and the lengthy initial hospital stay as well as frequent early hospital readmissions. The five year cumulative cost of pediatric IF treatment is estimated at $1.6 million per patient.[165]

Intestinal adaptation following resection

Preclinical models

In animals, structural adaptations following intestinal resection include increased (1) proliferation in both the crypt and epithelium;[166-168] (2) crypt depth and villus height;[169,170] (3) residual intestinal mucosal mass, diameter, and length;[169,171-173] (4) intestinal DNA, RNA, and protein concentrations;[172,174] and (5) angiogenesis and subsequent blood flow.[175-177] Functional capacities, including expression of transporter proteins such as the sodium/glucose co-transporter 1 (SGLT1)[178,179] and subsequent facilitation of glucose[169] as well as lipid absorption,[180] are also increased in animals following bowel resection. In mice, rats, and pigs, the ileum displays greater adaptive potential than does the jejunum.[167,175,181,182]

Humans

Human data regarding intestinal adaptation following resection is limited for obvious reasons, particularly in infants. However, in adult patients, numerically increased enterocyte

hyperplasia and increased villus height were reported 2 years after jejunal-ileal bypass surgery,[183] and increases in colonic crypt depth and cells per crypt were reported in patients with jejunal-colonic anastomosis.[184] Furthermore, the human intestine is capable of increasing its absorptive capacity through mucosal surface area expansion and enhancement of absorptive efficiency per unit surface area.[185-188] However, the results of these studies are contradicted by others which showed no changes in cellular proliferation, crypt depth, or villus height of SBS patients compared to controls.[189-191] In neonates, increases in crypt depth and villus height have also been observed following bowel resection for NEC.[192]

Treatment of intestinal failure

Parenteral nutrition

The traditional treatment regimen for pediatric SBS includes PN,[193] which in addition to providing sufficient energy to promote growth, must also provide adequate protein to avoid catabolism. Due to their low nutrient stores and high risk for malnutrition if PN is delayed, PN should be initiated as soon as possible in infants with IF, but given the lack of direct data in the pediatric population, specific guidelines regarding PN formulation and administration are based on adult recommendations and expert opinion.[194] While PN can sustain an individual for years, it is associated with numerous complications that are especially dangerous to infants including intestinal atrophy[195,196] and sepsis from intravenous line infections.[197] The incidence of PN catheter-related infections is estimated at 11-26 infections per 1000 catheter days,[198,199] but ethanol lock therapy is efficacious in reducing infection rates.[200,201] Additionally, 40-60% of pediatric patients and up to 85% of neonates that require long-term PN develop intestinal failure-associated liver disease.[202-204]

PN fatty acid content requires careful monitoring and prescription, especially with prolonged usage. The PN n-6 fatty acid content is of particular concern since these fatty acids are precursors to many pro-inflammatory cytokines,[205-208] and contribute to the high prevalence of liver disease with prolonged PN administration. As such, several n-3 fatty acid-rich lipid preparations, including the fish oil emulsion Omegaven,[209,210] and the SMOFlipid blend of medium chain triglycerides and soybean, olive, and fish oils,[211,212] are under investigation for both parenteral and enteral use. Early enteral supplementation with Microlipid and fish oil reduces intravenous lipid requirements in preterm infants with enterostomy via increased lipid absorption[213] and intestinal RNA and protein content.[214] Recent meta-analysis of the effect of fish oil-containing lipid emulsions on prevention or reversal of PN-associated cholestasis in neonates (7 trials including 1105 infants) suggested that while effective for reversing PN-associated cholestasis, these emulsions were ineffective in prevention of the condition.[215] Despite promising results, these novel lipid formulations remain experimental and require further research before widespread use.[216]

As compared to adult IF patients which require adequate PN to maintain fluid and electrolyte levels as well as body weight and lean mass composition, pediatric IF patients are unique in that they also require additional energy and protein to support growth. Due to the complications associated with PN, the goals of pediatric IF treatment are to (1) maintain fluid and electrolyte balance; (2) maximize the functional capacity of the remnant intestine; (3) promote growth and development; and (4) minimize PN complications and ultimately eliminate the need for PN support.[119,217] Given that functional adaptations of the intestine can take up to 2 years,[155] IF does not have to be a permanent condition. Rather, complete weaning from PN to EN

can be achieved if effective strategies, including surgical, nutritional, and pharmacological approaches, are employed to maximize adaptation following resection.

Surgical management

Surgical intervention for NEC is required when necrosis extends through the bowel wall and results in perforation. As NEC is the most common cause of pediatric SBS, care must be employed during resection of necrotic tissue to conserve as much healthy bowel as possible. Following initial resection, various other surgical procedures can be utilized to maximize intestinal adaptation. The longitudinal intestinal lengthening and tailoring (LILT) procedure[218] doubles bowel length through longitudinal splitting and subsequent anastomization in series. The serial transverse enteroplasty (STEP) procedure[219] creates a lengthened, although narrower, intestinal lumen through application of surgical staples in a transverse trans-mesenteric fashion. In patients with otherwise irreversible IF and liver failure or venous access impairments, intestinal transplantation is the final surgical treatment option.

Despite increasing intestinal length and/or surface area, each of these procedures is technically challenging, and the LILT procedure is specifically recommended against in neonates.[220] In the absence of surgical intervention following initial bowel resection, an infant with 35 cm of remnant small bowel has a 50% probability of being weaned from PN.[142] Encouragingly, over 75% of children undergo some degree of spontaneous intestinal adaptation following resection with medical management alone.[138,221] For the other 25%, surgery is required if intestinal adaptation is to be achieved.

Nutritional management

In combination with PN, hypocaloric trophic EN can be used to stimulate intestinal adaptation and promote enteral autonomy. Despite agreement that earlier initiation of feeds

promotes intestinal adaptation and minimizes PN-associated complications,[159,222] the time of initial feeding in IF remains a point of contention. Generally, enteral feedings should be started as soon as post-operative ileus resolves.[142,163] Initially, continuous, rather than bolus, feeds are preferred due to the lower risk of osmotic diarrhea[223] and increased absorption and tolerance,[75] but bolus feeds can be advantageous due to their promotion of hormonal stimulation.[224]

The volume and/or concentration of enteral feeds should be increased as adaptation progresses, but should not be advanced at a rate that causes stoma/stool output to exceed 40-50 mL/kg/d.[225] Increased output while advancing enteral feeds is typically a result of increased osmotic load, and current practice dictates aggressive advancement of enteral feeds to the point of, but not beyond, increasing stool output.[224] As EN is advanced, PN should be isoenergetically and isonitrogenously decreased while carefully monitoring patient ins and outs, including the presence of vomiting or diarrhea.[145]

In initiating neonatal EN, breast milk is associated with shorter duration of PN dependence as compared with cow's milk-based or protein hydrolysate formulas,[142] likely due to the presence of hormones and/or growth factors as well as human milk oligosaccharides. In the event where breast milk is not tolerated or is unavailable, a semi-elemental, partially hydrolyzed, or amino acid-based formula that contains medium and long chain triglycerides is typically used,[222,224] although no differences in energy intake, nitrogen balance, or intestinal permeability were observed between infants fed hydrolyzed or non-hydrolyzed protein formulas.[226]

Dietary lipids appear to be the most intestinotrophic macronutrient.[227,228] However, long chain triglycerides require micelle formation for absorption and are not well absorbed in the small intestine.[229] In contrast, medium chain triglycerides do not require micelle formation for absorption and may, therefore, enhance lipid absorption, particularly in the event of cholestasis

or bile salt malabsorption. Consequently, a 30:70 medium to long chain triglyceride ratio is recommended for SBS patients.[230] The incidence of PN-associated liver disease, as well as the NPO and PN support durations, are lower in surgical NEC patients when standardized feeding guidelines are utilized.[231] Thus, similar to prevention of feeding intolerance, use of standardized feeding guidelines may be used in conjunction with optimal lipid formations to promote enteral autonomy and decrease the incidence of PN-associated liver disease.

In addition to optimizing EN lipid content, inclusion of soluble fiber in EN may provide short-chain fatty acids (SCFAs) through anaerobic bacterial fermentation. SCFAs are an important colonic energy source,[232] and decrease fluid loss by stimulating sodium and water absorption in the colon.[233] SCFAs, and in particular butyrate,[234] prevent PN-associated mucosal atrophy[235] and enhance structural indices of intestinal adaptation[236,237] and nutrient transport[238] following resection in rats. Butyrate upregulates glucose transporter 2 (GLUT2) mRNA abundance in a Caco2-BBe model of the human intestine, providing insight into the cellular mechanism whereby this SCFA upregulates intestinal absorption.[239] The volatile nature of butyrate precludes its direct inclusion in nutritional formulations. However, provision of fermentable substrates such as short-chain fructooligosaccharides, a particularly auspicious source of soluble fiber for SBS patients due to their rapid hydrolysis,[240] provide a clinically feasible means to supply SCFAs. In a neonatal piglet model of SBS in which animals received 20% of nutrient needs enterally and the reminder parenterally,[106] supplementation of PEN with short-chain fructooligosaccharides at a level (10 g/L enteral formula) know to produce physiologically relevant butyrate concentrations within the distal intestine[241,242] augmented both structural and functional indices of intestinal adaptation. Based on these data, infants able to

tolerate even minimal enteral feeds may benefit from inclusion of soluble fiber if carefully selected and dosed to maximize butyrate production through bacterial fermentation.

Pharmacological management

Preclinical results

Preclinical results demonstrate efficacy of hepatocyte growth factor (HGF) on intestinal cell proliferation in culture,[243] and on intestinal cell repair, increased mucosal growth, and enhanced carbohydrate and protein absorption in rats.[244-246] However, no HGF clinical trials have been conducted to date. Likewise, epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) have shown promise in animal studies, but data in humans is lacking.[247-249]

Medications approved for human use

Pharmacological treatments for IF have historically focused largely on anti-secretory, anti-motility, and anti-diarrheal medications. These agents treat the symptoms of IF rather than its underlying cause, however, and are ineffective in promotion of intestinal adaptation. Furthermore, while widely used in adult IF patients, few, if any, safe and effective pharmacological treatments for pediatric SBS-associated IF exist.

In 2003, the United States Food and Drug Administration (FDA) approved recombinant human growth hormone (rHGH; somatropin [Zorbtive], EMD Serono, Rockland, MA) as a short-term treatment for adult SBS-IF patients. Animal studies demonstrate enhanced bowel growth and ion transport with rHGH treatment[250,251] and excised human intestinal tissue displays enhanced amino acid transport and intestinal protein content following growth hormone treatment.[252] FDA approval of rHGH was based on reductions in energy content and frequency of PN administration with rHGH compared to placebo.[253] When used in conjunction with rHGH, glutamine (Nutrestore, Emmaus Medical, Inc., Torrance, CA), a nonessential amino acid that is a

primary energy source for enterocytes, is also approved for short-term treatment of SBS.[254] However, despite increases in adult human sodium and protein absorption with this combination treatment,[255] the widespread use of these 2 therapies has been limited given the frequency of treatment-emergent adverse events and the attenuation of therapeutic gains following treatment discontinuation.[117] Though addition of glutamine to PN prevented atrophy and stimulated mucosal hyperplasia in animals following massive small bowel resection,[256,257] addition of glutamine to PN of ELBW infants in a large multicenter prospective study did not shorten duration of PN dependency or increase tolerance to enteral feeds, decrease sepsis or risk of NEC, or affect growth.[258] Furthermore, a recent meta-analysis (3 trials including 274 infants) concluded there was insufficient data to determine if glutamine supplementation could improve outcomes, including death or sepsis, of infants with severe gastrointestinal disease.[259]

Glucagon-like peptide-2

One promising alternative pharmacologic treatment for IF is provision of exogenous glucagon-like peptide-2 (GLP-2). GLP-2 is a 33 amino acid peptide secreted from the enteroendocrine L cells of the distal intestine in response to luminal nutrients or intestinal injury. GLP-2 is encoded carboxyterminal to glucagon-like peptide-1 (GLP-1) within the proglucagon gene, and in mammals, tissue-specific post-translational processing via prohormone convertase 1 liberates GLP-2 from GLP-1 within the intestine.[260] GLP-2 acts through a specific G-protein coupled receptor (GLP-2R), which is expressed in both the small and large intestine, as well as in the brainstem, lungs, and stomach.[261] GLP-2R is localized to enteric neurons,[262] subepithelial myofibroblasts,[261,263] and the intestinal epithelium,[264] but has not been identified in crypt cells. This localization suggests that downstream effectors such as IGF-1 may be required to elicit the intestinotropic effects observed with GLP-2 administration.[265] The GLP-2R is highly selective

for GLP-2, and exposure to GLP-1 or glucose-dependent insulinotrophic polypeptide, which are structurally related to GLP-2, cause only a weak reaction.[266]

Postprandial serum GLP-2 is decreased in patients with extensive small bowel resection,[267] and serum GLP-2 levels correlate with residual small bowel length in both adults and infants.[268] Even so, adults with resected ileum but preserved colon display plasma GLP-2 concentrations significantly elevated over those of healthy control patients.[269] Infants with any amount of remnant ileum following resection typically fare better than those whose entire ileum is resected, and GLP-2 appears to be a factor accounting for this more favorable prognosis.[270,271] Basal levels of GLP-2 secretion are high in preterm infants, suggesting that GLP-2 may also be important for inducing bowel growth during the final weeks of gestation.[272-274]

First reported to stimulate enterocyte proliferation in 1996,[275] GLP-2 is an intestinotrophic mediator capable of increasing absorptive surface area, preventing mucosal atrophy, and increasing DNA, RNA and protein concentrations in intestinal cells of animals sustained on PN.[276-278] In other preclinical models, GLP-2 enhanced nutrient and fluid absorption,[279] opposed inflammatory insults,[280,281] increased intestinal barrier function,[282] and inhibited gastric emptying and stimulated intestinal blood flow.[283-285]

In adult SBS subjects, GLP-2 increases intestinal absorption and decreases diarrhea,[286,287] and reduces fecal wet weight, energy, nitrogen, sodium, and potassium losses.[288] Additionally, both intravenous and subcutaneous GLP-2 administration increases mesenteric blood flow in healthy adult subjects.[284] Despite these promising effects in adults, only limited preclinical data is available regarding GLP-2 use in infants.

In piglets receiving 40 µg/kg/day split into 2 daily doses over a 42-day study period, GLP-2 administration had no effect on weight gain, feed intake, or behavior,[289] suggesting that

administration of exogenous GLP-2 may be safe in growing infants. Villus height, crypt depth, and crypt cell proliferation throughout the small intestine and colon were significantly increased, while the rate of apoptosis was significantly decreased throughout both the small and large intestine in GLP-2- versus vehicle control-treated animals. Overall, pharmacological levels of GLP-2 were well tolerated in these piglets, and its tropic effects appear to be confined, as desirable, to the gastrointestinal tract. However, these results are contradicted by those of another piglet study in which GLP-2 treatment significantly increased cellular proliferation, but paradoxically, also led to villus atrophy and a significant decrease in brush border enzyme activity compared to control.[290] In preterm piglets sustained on total PN following a 50% small bowel resection and jejunostomy,[291] GLP-2 administration (3.5 μg/kg/hour) increased relative wet weight, energy, and macronutrient absorption, and increased sucrase and maltase activities. Small intestinal epithelial volume, DNA and protein content, and protein synthetic rate were also significantly increased in GLP-2-treated versus control piglets. Currently there is a complete dearth of data on the use of GLP-2 for inducing intestinal adaptation in human infants as a clinical trial on the safety and dosing of GLP-2 in infants and children with IF was terminated in December 2014 due to drug stability concerns.[292]

Teduglutide

GLP-2 has clearly demonstrated therapeutic promise for IF treatment. However, the half-life of GLP-2 is extremely short due to rapid degradation by dipeptidyl peptidase IV (DPP-IV). While DPP-IV inhibition modestly potentiates the actions of native GLP-2, it does not result in a significant expansion of the mucosal epithelium.[293] Therefore, teduglutide (Gattex, NPS Pharmaceuticals, Inc., Bedminster, NJ), a DPP-IV-resistant GLP-2 analog which substitutes

glycine for alanine in the second N-terminus position, was created which extends the peptide's half-life from 7 minutes to 1.3-2 hours.[294,295]

In adult human trials, teduglutide stimulates mucosal hyperplasia, enhances fluid, macronutrient, and electrolyte absorption, increases villus height and crypt depth, and significantly reduces PN volume requirements.[296-298] In these trials, the adverse event profile of teduglutide was similar to placebo and was consistent with underlying disease states.[296-303] Based on this acceptable benefit to risk ratio, and considering there are only an estimated 3,000 SBS-IF patients in the United States eligible for treatment,[304] teduglutide was granted orphan drug designation and approved by the FDA at a dose of 0.05 mg/kg/day for treatment of PN-dependent adult patients with SBS in December 2012.[305] Teduglutide has also been approved for marketing by the European Medicines Agency under the trade name Revestive.[304]

No pediatric human trials of teduglutide have yet been completed, but a clinical trial on the pharmacodynamics and safety of teduglutide in pediatric SBS subjects is ongoing.[306] However, infants with gastroschisis show a clear positive association between circulating plasma GLP-2 concentration and enteral tolerance,[307] and infants unable to produce GLP-2 levels of at least 15 pM/L with feeds of > 40 kcal/kg died of SBS complications.[268] Further research into prophylactic or therapeutic teduglutide treatment of preterm infants is warranted given that teduglutide may accelerate intestinal maturation and prevent feeding intolerance and NEC.

Piglets with 50% small intestinal resection and jejunostomy on total PN for 7 days[308] demonstrated a dose-dependent increase in weight per length remnant intestine, and an increased intestinal protein fractional synthesis rate with teduglutide (0.2 mg/kg/d) treatment versus placebo. However, there were no differences between digestive enzyme activities or immunohistochemistry between groups. From this piglet study, it appears that while increasing

structural adaptation of the remnant intestine, teduglutide has limited effects on functional endpoints.

Synergistic therapies

Attempts to maximize intestinal adaptation following resection may be most effective if multiple strategies are employed and their synergy can be leveraged. Indeed, several treatment combinations including rHGH and glutamine,[255] partial EN (PEN) and pre and/or probiotics,[106] EN and GLP-2,[309] and GLP-2 plus epidermal growth factor,[310] have shown promising results. Taking advantage of the different modes and sites of action of these therapies will ensure the best possible patient outcomes. Furthermore, the use of a multidisciplinary intestinal rehabilitation program, including optimal EN and concurrent PN weaning as well as judicious pharmacological and surgical interventions is associated with improved enteral autonomy and survival.[311-315]

SUMMARY

Novel means by which to predict feeding intolerance and prevent its progression to NEC and IF are crucial for preterm infants to achieve desired growth rates and enteral autonomy. Greater understanding and integration of the disparate factors which impact development of feeding intolerance and NEC will contribute to their early detection. Furthermore, development of targeted, synergistic treatments for promotion of intestinal maturation in preterm infants at high risk of feeding intolerance will advance clinical practice to positively impact both short- and long-term outcomes.

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CHAPTER 2

RATIONALE AND OBJECTIVES OF RESEARCH

RATIONALE

Preterm infants are at increased risk of feeding intolerance and necrotizing enterocolitis (NEC). Despite decades of research, NEC remains a poorly understood disease responsible for nearly one-fifth of all initial newborn health care costs in the United States.[1] NEC is also the most common cause of pediatric IF,[2] which necessitates long-term parenteral nutrition (PN) unless sufficient intestinal adaptation can be induced to achieve enteral autonomy. Given the devastating effects of NEC and complications associated with long-term PN use,[3-6] it is crucial that NEC be prevented or detected as early as possible, and targeted, evidenced-based therapies be applied to infants at high risk of developing NEC. Substantial, cost-effective advancement in prevention and early detection of NEC, coupled with targeted prophylactic and therapeutic interventions such as partial enteral nutrition (PEN) and teduglutide, will improve patient outcomes through heightened awareness and standardized communication of infant NEC risk as well as individually tailored care to maximize the quality of life for infants at risk of, or who develop, feeding intolerance and NEC.

OBJECTIVES OF RESEARCH

The overall objective of this work is to reduce preterm infant morbidity and mortality caused by feeding intolerance. The central hypothesis is that accurate prediction of feeding intolerance and NEC risk can be achieved and used to identify infants who may benefit from targeted therapies to prevent these conditions. Thus, three separate studies were conducted to assess (1) the potential of teduglutide as a therapy for pediatric short bowel syndrome (SBS) via

systematic review of its safety and efficacy in PN-dependent adults; (2) the efficacy of teduglutide, alone or in combination with PEN, for promotion of intestinal adaptation in neonatal SBS; and (3) feeding intolerance and NEC risk prediction potential of a novel risk scoring tool to identify infants who may benefit from prophylactic or therapeutic teduglutide and/or PEN treatment.

The rationale for these aims is that teduglutide has demonstrated effectiveness in the treatment of adults dependent on PN but has not yet been approved for use in pediatrics. Furthermore, given the various modes and sites of actions of these therapies, pairing teduglutide with PEN may serve to maximally stimulate bowel adaptation. Finally, to date, no validated tools exist for the assessment of infant feeding intolerance and NEC risk despite wide recognition that early detection will greatly improve patient outcomes.[7,8] The following specific aims and hypotheses were investigated.

Study 1

Specific aims were to assess (1) the efficacy of teduglutide in reducing PN (parenteral nutrient and/or fluid) requirements in PN-dependent adults; and (2) the safety of teduglutide in this same population. We hypothesized that following distillation of duplicate and abstract-only publications to original results, teduglutide treatment would result in decreased PN requirements compared to placebo, and would demonstrate an acceptable safety profile. **Chapter 3** demonstrates that compared to placebo, teduglutide treatment reduces PN requirements in PN-dependent adults, regardless of PN dependence duration, and that adverse event incidence is similar between teduglutide- and placebo-treated groups. Thus, the benefits of teduglutide

treatment in this population appear to outweigh the risks, and may improve quality of life through additional days off PN.

Study 2

Specific aims were to (1) assess teduglutide-induced structural and/or functional measures of intestinal adaptation in a neonatal piglet model of SBS; (2) evaluate if the effects of teduglutide in this model are complimented or synergistically enhanced by provision of PEN; and (3) identify distinct temporal markers of adaptation stimulated by these two therapies. We hypothesized that teduglutide would enhance structural and functional adaptation of the residual small intestine via enhanced mucosal surface area and nutrient processing capacity, and these effects would be augmented by the provision of PEN. We further hypothesized that that surface area expansion would precede functional adaptation. **Chapter 4** demonstrates significant improvements in mucosal surface area and acute nutrient processing capacity with teduglutide treatment, and that these effects were complimented and synergistically enhanced by PEN in both site and timing of action. Additionally, structural markers of adaptation preceded functional markers, but crypt depth remained a strong indicator of adaptation, regardless of time. Thus, it seems the complimentary and synergistic effects of combination teduglutide and PEN enhance intestinal adaptation beyond that of either therapy alone.

Study 3

Specific aims were to (1) develop an evidence-based novel tool to assess neonatal feeding intolerance and NEC risk; (2) assess the tool's clinical utility and feasibility of nursing use; and (3) validate the tool to achieve sensitive and specific prediction of feeding intolerance and NEC

risk. We hypothesized that accurate prediction of infant feeding intolerance and NEC risk could be accomplished through comprehensive assessment of feeding practices as well as relevant infant and maternal factors, and furthermore, that the tool would be easy to use since similarly designed tools have been successful in predicting and reducing the incidence of falls in the elderly[9] and pressure ulcers in adults.[10] **Chapter 5** describes the pilot phase of this study, and demonstrates the feasibility of implementation of this tool in a neonatal intensive care unit. **Chapter 6** reinforces pilot phase data, and further, demonstrates tool error rate, consistency, discrimination, and predictive ability to be in acceptable ranges.

DISCUSSION

Chapter 7 summarizes the results of these investigations and future directions for this field of research. Emphasis is placed on the potential impact these findings may have on preterm infant outcomes, as well as possible future directions for research regarding the risk scoring tool to improve its overall clinical utility.

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CHAPTER 3

TEDUGLUTIDE FOR SAFE REDUCTION OF PARENTERAL NUTRIENT AND/OR FLUID REQUIREMENTS IN ADULTS: A SYSTEMATIC REVIEW[1]

ABSTRACT

Background: Teduglutide (Gattex, NPS Pharma, Inc., Bedminster, NJ), a recombinant analogue of human glucagon-like peptide-2 (GLP-2), is the first long-term medical therapy approved for the treatment of adults dependent on parenteral nutrition (PN). Objective: To assess the efficacy and safety of teduglutide in reducing PN (parenteral nutrient and/or fluid) requirements in PN-dependent adults. Search Methods: Studies were identified using predefined search criteria and multiple databases, including Medline and Embase. The search was completed to November 30, 2014 in the absence of date or study design restrictions. Selection Criteria: Citation inclusion criteria and methodological quality were assessed by two independent reviewers. Outcomes of interest were changes in parenteral nutrient or fluid requirements and adverse event incidence. Data Collection and Analysis: From 2693 unique citations, 76 abstracts were reviewed. Fourteen reports met the inclusion criteria, including data from 2 phase III, double-blind, placebo-controlled clinical trials and their respective extension studies. Data extraction was performed by two reviewers using a standardized form. Results: Teduglutide reduced PN requirements compared with placebo, whereas adverse event incidence was similar. Limitations: Number of subjects studied and the length of follow-up. Conclusions: Teduglutide appears to be a safe and well-tolerated means to reduce PN dependence in adults, regardless of PN dependence duration.

[1]The final, definitive version of this paper is in press in the Journal of Parenteral and Enteral Nutrition by SAGE Publications, Inc. All rights reserved. © 2015 A.S.P.E.N. Naberhuis JK, Tappenden KA. Teduglutide for safe reduction of parenteral nutrient and/or fluid requirements in adults: a systematic review. JPEN J Parenter Enteral Nutr 2015;e-pub ahead of print. doi:10.1177/0148607115582063.

CLINICAL RELEVANCY STATEMENT

Patients with intestinal failure (IF) are dependent on parenteral nutrition (PN) for nutrients and/or fluid, and prolonged PN-dependence is associated with decreased quality of life and numerous complications. Teduglutide is the first long-term pharmacologic treatment indicated for adult patients with short bowel syndrome (SBS) who are dependent on parenteral support. This systematic review demonstrates that teduglutide is efficacious for minimizing PN dependence in adults regardless of PN dependence duration, with a therapeutic gain assessed from 32.6 - 39.4% compared to placebo in reducing PN volume requirements by ≥ 20%. Furthermore, longer teduglutide treatment duration is associated with increased clinical gains, and adverse event incidence on teduglutide is similar to that observed with placebo and is consistent with underlying IF.

INTRODUCTION

Intestinal failure (IF), caused by disease, congenital defect, or surgical resection, is characterized by the inability to maintain protein, energy, fluid, electrolyte, or micronutrient balance.[1] Parenteral nutrition (PN) is often required in IF in order to maintain body weight as well as fluid, nutrient, and electrolyte balance. While life-saving, long term or permanent dependence on PN is associated with decreased quality of life[1-7] and numerous complications including catheter-related bloodstream infections and sepsis, which are the primary cause of morbidity and hospital readmission in these patients.[8] The risk of PN-related mortality rises with increasing PN-dependence duration[9] but with proper care, PN complications are rarely lethal[10,11] and the majority of deaths of patients on long-term PN are attributable to the underlying disease rather than to the administration of PN.[12]

The goal of IF treatment is to promote enteral autonomy by maximizing the functional capacity of the remnant intestine, which is capable of increasing its absorptive capacity through mucosal surface area expansion and enhancement of absorptive efficiency per unit surface area.[13-16] Capacity for this functional adaptation is maximal in the first 2 years following intestinal failure onset,[17] and if enteral autonomy is not achieved during this period, the likelihood of permanent IF and PN dependence is 95%.[18,19] However, enteral autonomy can be achieved beyond this initial 2-year period if effective long-term strategies are employed to maximize intestinal adaptation following resection.[20,21]

Adaptation of the remnant intestine can be stimulated through a variety of interventions, including both dietary and pharmacologic strategies.[22] Until recently, pharmacological treatments have focused largely on anti-secretory, anti-motility, and anti-diarrheal medications.

One promising pharmacologic intervention is the provision of exogenous glucagon-like peptide-2 (GLP-2). GLP-2 is a 33 amino acid peptide secreted from the enteroendocrine L cells of the distal intestine in response to luminal nutrients. First reported to stimulate enterocyte proliferation in 1996,[23] GLP-2 has gained widespread support as an intestinotrophic mediator capable of increasing absorptive surface area, preventing mucosal atrophy, and increasing DNA, RNA and protein concentrations in intestinal cells of animals sustained on PN.[24-26] Furthermore, GLP-2 enhances nutrient and fluid absorption,[27] increases intestinal barrier function,[28] and inhibits gastric emptying and stimulates intestinal blood flow.[29-31] In a proof of concept study, GLP-2 increased intestinal wet weight absorption and decreased diarrhea in short bowel syndrome (SBS) subjects.[32]

GLP-2 has demonstrated consistent therapeutic promise for IF treatment. However, the half-life of GLP-2 is extremely short due to rapid degradation by dipeptidyl peptidase IV. Thus

teduglutide, a GLP-2 analog which substitutes glycine for alanine in the second N-terminus position, was created which extends the half-life from 7 minutes to 1.3-2 hours.[33-35] The US Food and Drug Administration (FDA) granted teduglutide orphan drug designation in 2000, and approved it for marketing for treatment of PN-dependent adult patients with SBS in December 2012.[35] Teduglutide has also been approved for marketing in Europe under the trade name Revestive.[36]

Given the complications and decreased quality of life associated with prolonged PN-dependence, the potential for duplicate publication bias, and that extension study data is only yet available in abstract form which may change substantially or never reach publication, the objective of this systematic review is twofold: (1) to distill the available data on teduglutide safety and efficacy in reducing PN requirements to original results; and (2) to measure the impact of teduglutide via calculation of summary measures including the number needed to treat to benefit (NNTB) or harm (NNTH), the odds ratio (OR), and therapeutic gain so that treatment decisions can be evidence-based and well-informed, taking into consideration both benefits and potential harms of teduglutide treatment.

METHODS

This study was conducted according to the procedures outlined by the Cochrane Collaboration for systematic reviews[37] in order to assess the safety and efficacy of teduglutide in reducing PN requirements in PN-dependent adults. A standard protocol for study identification, inclusion, and data abstraction was developed and followed after establishment of the following study (population, intervention, comparison, and outcome [PICO]) question: "In PN-dependent adult humans, would adding teduglutide to standard intestinal rehabilitation therapies safely

result in reduced PN requirements when compared with standard intestinal rehabilitation therapies alone?" These standard rehabilitation strategies include individualized treatments based on patients' residual anatomy and SBS status and may include optimization of PN and/or conventional medications such as antisecretory agents or antidiarrheals.

Multiple databases (**Supplementary Table 3.1**), clinical trial and adverse event registries, and pharmaceutical industry databases were searched from database inception through November 30, 2014, in the absence of date or study design restrictions using the following search terms: alx-0600, gattex, gly(2)-GLP-2, (gly2)GLP-2, revestive, teduglutide. Results were restricted to English-language studies that enrolled PN-dependent adult humans, and employed teduglutide, alone or in combination with additional therapies, to investigate the efficacy and/or safety of teduglutide in reducing PN requirements. References from identified citations were cross-referenced for completeness. The outcomes of interest were changes in PN requirements and adverse event (AE) incidence. No restrictions were applied to the ways in which changes in PN requirements were expressed in study results. Hits were assessed for inclusion criteria and methodological quality by the two authors, including multiples domains of selection, performance, detection, attrition, reporting, and other biases. In the event where a risk of bias was unclear, attempts were made to clarify by contacting the senior study authors. Methodological quality of studies was graded per the Cochrane Collaboration, and discrepancies in trial bias assessments between reviewers were resolved by consensus. A data extraction form was developed and piloted jointly by the authors using a representative sample of the studies to be reviewed, after which both authors performed data extraction. Qualitative data synthesis, rather than meta-analysis, was performed due to variations in length, timing, and dosing strategies of the included trials. Summary statistics, including NNTB (NNTB = 1/[teduglutide

responder rate - placebo responder rate], rounded up to the next whole number), NNTH (NNTH = 1/[teduglutide event rate - placebo event rate], rounded up to the next whole number), OR (OR = [number of teduglutide-treated subjects experiencing event/number of event-free teduglutide treated subjects]/[number of placebo-treated subjects experiencing event/number of event-free placebo-treated subjects]), and therapeutic gain (teduglutide responder rate - placebo responder rate), were calculated as described by The Cochrane Collaboration[37] in order to directly compare the safety and clinical efficacy of teduglutide to that of placebo.

RESULTS

Included Studies

A total of 2693 citations were identified, and 1402 unique results remained after removal of duplicates. Potentially relevant citations were evaluated for inclusion after cross-referencing index terms and titles. Seventy-six abstracts were reviewed, after which the remaining 58 full-text articles and meeting abstracts were assessed for inclusion. Fourteen met the inclusion criteria (**Figure 3.1**). Reasons for article exclusion included duplicate data, review articles or articles that provided interim findings when final results were available, use of native rather than analog GLP-2, and enrollment of subjects that were not PN-dependent. Five of the included citations are full-text articles, and 9 are meeting abstracts. These citations describe three trials as well as their respective extension and sub-studies. Characteristics of included studies, including study durations, populations, and outcomes of interest, are found in **Table 3.1**.

Risk of Bias in Included Studies

All included studies had a low risk of bias in the following domains: (1) random sequence generation (selection bias); (2) incomplete outcome data (attrition bias); (3) selective reporting (reporting bias); and (4) other bias (**Figure 3.2**). Risk of allocation concealment (selection) bias in the Gilroy 2008[38] study, risks of blinding of participants and personnel (performance) and blinding of outcome assessment (detection) bias in the Jeppesen 2009 a,[39] b,[40] and c[41] studies, as well as risks of blinding of outcome assessment (detection bias) in the Jeppesen 2014a[42] and 2014b,[43] Iyer 2014,[44] and Fujioka 2014[45] studies were determined to be unclear as these domains were not specifically addressed in these citations. High risk of allocation concealment (selection) and blinding of participants and personnel (performance) bias were noted in the open-label Jeppesen 2014a,[42] 2014b,[43] Iyer 2014,[44] and Fujioka 2014[45] studies. Risk of blinding of outcome assessment (detection) bias was also high in the Gilroy 2008,[38] Compher 2011,[46] and Ukleja 2014[47] studies as the treatments were known by the outcome assessors.

Outcomes of Interest

Efficacy

Responder Rate

Table 3.2 shows the proportion of subjects classified as responders across studies, achieving ≥ 20% reduction by volume in weekly PN requirements. In Jeppesen 2011,[48] response rate at 20 and maintained at 24 weeks of treatment was higher ($P = 0.005$) in teduglutide 0.05 mg/kg/d (0.05 group) versus placebo subjects (NNTB = 3, OR = 12.63, therapeutic gain = 39.4%). Response rate of teduglutide 0.10 mg/kg/d (0.10 group) subjects did not differ ($P =$

0.17) from placebo (NNTB = 6, OR = 5.00, therapeutic gain = 18.7%) Seventeen of 25 (68.4%) 0.05 subjects and 14 of 27 (52.2%) 0.01 subjects were responders by 52 weeks of treatment.[49] As compared to week 24, by week 52, 4 of the 24 responders become non-responders, and 11 of 19 non-responders became responders. Twelve of the 18 subjects who became responders by week 24 and remained so through week 52 were treated with teduglutide 0.05, and 6 with teduglutide 0.10.[49] Of subjects receiving placebo in the initial study[48] but teduglutide in the extension study,[49] 6 of 6 (100.0%) and 2 of 7 (28.6%) responded to teduglutide 0.05 and 0.10, respectively.[38]

Similarly, in the Jeppesen 2012 (Study of Teduglutide Effectiveness in Parenteral Nutrition-Dependent SBS Subjects [STEPS]) study,[50] more ($P = 0.002$) teduglutide 0.05 versus placebo subjects were responders at week 24 (**Table 3.2**; NNTB = 4, OR = 3.89, therapeutic gain = 32.6%). In the extension study in which all subjects received teduglutide 0.05 (STEPS-2),[43,45] subjects previously treated with teduglutide 0.05, placebo, or not randomized achieved responder rates of 28 of 30 (93.3%), 16 of 29 (55.2%), and 4 of 6 (66.7%), respectively. Teduglutide response was observed regardless of subject characteristics (age, remnant anatomy, baseline PN requirements, or disease etiology).[43] Importantly, teduglutide efficacy was demonstrated in responder rate ORs of > 1 in both phase III trials.[48,50] In the Ukleja 2014 study,[47] all 6 patients (100.0%) experienced > 20% reduction in PN volume while on teduglutide.

Changes in PN Volume Requirements

Using a strict parenteral weaning algorithm which allowed for reductions in PN volumes of ≤ 10% at 4-week intervals, both the teduglutide 0.05 and teduglutide 0.10 groups in the Jeppesen 2011 trial[48] had reduced PN volume requirements compared to baseline at weeks 8, 12, 16, 20 and 24 (all $P < 0.05$). The placebo group also achieved significant reductions at weeks 12

and 24 (P = 0.02 and 0.03, respectively). At week 24, both teduglutide dose groups achieved mean PN volume requirement reductions of 2.5 L/wk, while the placebo group achieved a 0.91 L/wk reduction (P = 0.08). At week 24 the teduglutide 0.05, teduglutide 0.10, and placebo groups also achieved reductions (P = 0.001, P = 0.03, and P = 0.056, respectively) in parenteral energy intake compared to baseline, but reductions in either teduglutide-treated group did not differ (P = 0.11) from placebo. By 52 weeks of treatment,[49] the teduglutide 0.05 and teduglutide 0.10 groups decreased their PN volume requirements by 4.9 L/wk (52%) and 3.3 L/wk (26%), respectively, compared to baseline. However, 4 weeks after stopping treatment, PN requirements of both the teduglutide 0.05 and 0.10 groups increased compared to study end (from 4.0 ± 3.4 to 5.5 ± 4.4 L/wk, and 8.5 ± 5.1 to 7.9 ± 3.7 L/wk, respectively). There were no significant changes in 7-day urine outputs or oral intakes over the 52-week study period.

Subjects with increased (INC) PN requirements by 12 months after stopping teduglutide[46] had a greater (P = 0.04) PN volume reduction while on drug compared to those with stable (STABLE) or decreased (DEC) requirements at 12 months off drug (−4.7 versus −1.9 L/wk, respectively). INC had increased (P < 0.001) PN requirements at 3, 6, and 12 months off drug versus study end while STABLE/DEC requirements did not change. Furthermore, INC PN requirements were higher (P = 0.001) than STABLE/DEC (11.9 versus 5.7 L/wk) at 12 months off drug. Similar trends were observed in the subset of drug responders, in that INC had increased (P < 0.001) PN volume requirements at 3, 6, and 12 months compared to study end while STABLE/DEC PN requirements did not change, and INC requirements were greater (P = 0.003) than those of STABLE/DEC subjects at 12 months off drug.

In STEPS, [50] using a weaning algorithm which allowed for 10-30% PN volume reductions of baseline PN levels at 4-week intervals, teduglutide 0.05 and placebo subjects

achieved mean L/wk reductions in PN volume requirements of 4.4 ± 3.8 (baseline 12.9 ± 7.8) and 2.3 ± 2.7 (baseline 13.2 ± 7.4), respectively, after 24 weeks of treatment. The difference in absolute change in PN volume requirements between these groups was significant by week 8 ($P < 0.01$) and remained so through week 24 ($P < 0.001$). Similarly, the difference in percentage reduction in PN volume from baseline to week 24 between groups became significant ($P < 0.03$) at week 12 and remained significant ($P < 0.03$) through week 24. By STEPS[50]/STEPS-2[43,45] treatment, the mean PN volume requirement reduction from baseline was 7.6 (66%), 3.1 (28%), and 4.0 (39%) L/wk in the groups treated with teduglutide/teduglutide, placebo/teduglutide, and not randomized/teduglutide, respectively. By STEPS[50]/STEPS-3[44] treatment, teduglutide/teduglutide, placebo/teduglutide, and not-treated/teduglutide subjects reduced their PN requirements from baseline by 9.8 (50%), 3.3 (35%), and 5.2 (73%) L/wk, respectively. In Ukleja 2014,[47] 6 of 6 (100.0%) subjects experienced > 20% reduction in PN volume requirements from baseline requirements of 1-8 L/wk.

PN Infusion Frequency

Neither teduglutide-treated group experienced a significant reduction in the number of days per week that PN was required in the Jeppesen 2011[48] trial, but by 52 weeks of treatment,[49] 17 of 25 (68%) teduglutide 0.05 and 10 of 27 (37%) teduglutide 0.10 subjects achieved a ≥ 1 additional d/wk reduction.

In STEPS,[50] which employed a more aggressive weaning algorithm than the 2011 trial,[48] more ($P = 0.005$) teduglutide 0.05 than placebo subjects (21 of 39 [54%] versus 9 of 39 [23%], respectively) achieved ≥ 1 additional d/wk off of PN by 24 weeks of treatment. In STEPS-2,[43,45] 38 of 65 (58.5%) subjects achieved ≥ 1 additional d/wk reduction in PN requirements (by STEPS/STEPS-2 treatment: teduglutide/teduglutide, 21/30 [70.0%]; placebo/teduglutide, 14/29

[48.3%]; not treated/teduglutide, 3/6 [50.0%]), and 25 of 65 (38.5%) achieved ≥ 3 additional d/wk reduction in PN requirements. Of these 25, 18 (72%), 5 (20%), and 2 (8%) were previously treated with teduglutide, with placebo, or not randomized, respectively.[50] In STEPS-3,[44] mean weekly PN infusion was reduced by 3.0, 1.7, and 2.8 d/wk in groups with STEPS/STEPS-3 treatment of teduglutide/teduglutide, placebo/teduglutide, and not treated/teduglutide.

Complete Weaning

Three subjects completely weaned from PN by 24 weeks of treatment in the Jeppesen 2011 trial,[48] and remained off of PN 12 months later.[46] Two of these subjects received teduglutide 0.05, and 1 received teduglutide 0.10. By 52 weeks of treatment,[49] 1 additional teduglutide 0.05 subject weaned from PN.

In STEPS,[50] no subjects were completely weaned from PN by 24 weeks of treatment, but in STEPS-2,[43,45] 13 of 88 (15%) achieved independence from PN, 10 of whom received teduglutide in the original randomized controlled trial.[50] In STEPS 3,[44] 2 subjects achieved independence from PN after 126 and 130 weeks of teduglutide treatment. In the Ukleja 2014 study,[47] 4 of 6 subjects (66.7%) were able to wean from PN.

Measures of Intestinal Adaptation

In Jeppesen 2011,[48] teduglutide 0.05 subjects produced more ($P < 0.05$) urine at all time points versus baseline, despite constant oral fluid intake. Teduglutide 0.10 subjects had increased ($P = 0.04$) urine production at week 4, after which urine production returned to baseline, likely due to the reduced ($P < 0.05$) oral fluid intake at weeks 4, 8, 12, 16, 20, and 24. After 52 weeks of treatment,[49] fasting plasma citrulline, a biomarker of gut function and mucosal mass,[51] increased 68% ($P < 0.001$) in teduglutide 0.05 and 86% ($P < 0.001$) in teduglutide 0.10 subjects

compared to baseline. These levels decreased after four weeks off drug by 20% and 32%, respectively, but remained higher than at study start.

Seventy-two hour balance studies were conducted in a subset of subjects from the Jeppesen 2011 study.[48] At week 24, the pooled teduglutide groups (n = 11) demonstrated reduced fecal energy excretion[41] and wet weight[39] ($P = 0.03$ and $P = 0.01$, respectively), and in those whose dietary intake differed < 10% from baseline, intestinal absorption significantly increased ($P < 0.05$) at weeks 8 and 24.[41] Teduglutide treatment also decreased fecal sodium ($P < 0.001$) and potassium[39] ($P = 0.003$) excretion, and increased plasma citrulline from baseline to week 24 ($P = 0.001$).[40]

In STEPS,[50] oral fluid intake of placebo-treated subjects exceeded ($P < 0.05$) that of teduglutide-treated subjects at weeks 12, 20 and 24, and the reduction in fluid composite effect, defined as a summation of the increase in urine output (L/wk), reduction in PN/IV volume (L/wk), and reduction in oral fluid intake (L/wk), was greater ($P \leq 0.05$) in the teduglutide verses placebo group at all time points. After 24 weeks of treatment teduglutide, but not placebo, increased ($P < 0.001$) plasma citrulline over baseline.

Adverse Event Incidence

Table 3.3 illustrates the AE, treatment emergent-AE (TE-AE), serious AE (SAE), treatment-emergent serious AE (TE-SAE) and drop-out rates of included studies, as well as information on laboratory findings, pathology, and death. In Jeppesen 2011,[48] 79 of 83 (95.2%) subjects experienced at least one AE, most commonly abdominal pain, headache, and nausea. The most common SAE were catheter-related complications and infections, small intestinal obstruction, and fever. Compared to placebo, the AE, SAE, and drop-out rates of both the

teduglutide 0.05 (AE, OR = 1.10, NNTH = 187; SAE, OR = 1.30, NNTH = 17; drop-outs, OR = 3.10, NNTH = 10) and 0.10 groups (AE, OR = 2.07, NNTH = 32; SAE, OR = 1.15, NNTH = 32; and drop-outs, OR = 1.00, NNTH = ∞) also demonstrate the relative safety of teduglutide (**Table 3.4**). No features of small or large bowel dysplasia were found in these subjects,[52] and new secondary diagnoses, consistent with underlying disease, were found at a lower frequency in the teduglutide groups as compared to placebo (**Table 3.3**).

Similar results were observed in the extension study[49] (**Table 3.3**), in which 50 of 52 (96.2%) subjects reported at least one TE-AE, most commonly headache, nausea, abdominal pain, and nasopharyngitis. Twenty-seven of 52 (51.9%) subjects reported TE-AE related to teduglutide, most commonly gastrointestinal disturbance, injection site reactions, and stomal hypertrophy. Seven of 52 (13.5%) subjects discontinued from the study because of AE, 4 of which, all with a history of Crohn's disease, were considered treatment-related. Similarly, 27 of 52 (51.9%) subjects experienced SAE, 5 of which were considered drug-related. One teduglutide 0.05 and 1 teduglutide 0.10 subject discontinued treatment for reasons unrelated to teduglutide (1 subject had a hyperplastic colon polyp; 1 had a stroke). By 12 months off drug, 6 of 25 drug responders experienced a total of 18 complications, and 4 of 12 non-responders experienced a total of 7 complications.[46]

All subjects who received ≥ 1 dose of teduglutide were included in the STEPS[50] safety analysis. Sixty-nine of 85 (81%) subjects experienced ≥ 1 TE-AE, and similar rates of > 1 TE-AE, TE-SAE, and drop-outs were observed between the teduglutide 0.05 and placebo groups (**Table 3.3**). Compared to placebo, incidences of ≥ 1 TE-AE (OR = 1.32, NNTH = 24), TE-SAE (OR = 1.44, NNTH = 13), and drop-outs due to TE-AE (OR = 0.75, NNTH = 47) of the teduglutide 0.05 group demonstrate the safety of teduglutide (**Table 3.4**). In STEPS-2,[43,45] 84 of

88 (95.5%) subjects experienced TE-AE, most commonly abdominal pain (34%), catheter sepsis (28%), and decreased weight (25%). Fifty-six of 88 (63.6%) subjects experienced SAE, but only 10% were considered treatment-related. These serious AEs included three cases of cancer and three deaths. By STEPS[50]/STEPS-2[43,45] treatment, 7 of 37 (18.9%) teduglutide/teduglutide and 16 of 51 placebo+not treated/teduglutide subjects discontinued treatment. No major findings were reported in the laboratory values, including liver enzymes, of any included study.[42,43,45] All subjects experienced TE-AE in STEPS-3,[44] but no malignancies or death were reported. No subjects discontinued the study because of an AE. In the Ukleja 2014 study,[47] 1 (16.7%) subject experienced small bowel obstruction, 2 (33.3%) experienced stoma swelling, 1 (16.7%) discontinued treatment, and 1 (16.7%) had a teduglutide dose reduction (**Table 3.3**).

DISCUSSION

In agreement with the earlier phase II study,[53] these collective results demonstrate the efficacy of teduglutide in reducing PN requirements in PN-dependent adults. Clinical gains were augmented with increased treatment duration, and perhaps most importantly, the reductions in infusion frequency experienced by subjects in these trials may potentially have substantial implications for employment, activities, sleep, and finances,[54] as indicated by the association between reductions in PN requirements and significant ($P = 0.02$) improvements in quality of life in STEPS.[50,55] The benefits of decreasing PN requirements and allowing subjects the freedom and spontaneity that additional days or even hours free from PN offer, cannot be underestimated.[54]

AE incidence with teduglutide treatment was similar to that observed with placebo, was consistent over time and with exogenous GLP-2 administration,[56] and furthermore, the most

common AE observed were not surprising given that symptoms of abdominal pain, nausea, and vomiting also occur with anti-diarrheal treatment in SBS patients. Taken together, these data indicate that teduglutide was safe and well tolerated in these trials. However, the included randomized trials involved only 172 distinct subjects, who were by definition in stable clinical condition with relatively uncomplicated SBS. Thus, additional or more severe AEs may be observed once teduglutide is prescribed in a more complex population.

Subjects included in these phase III clinical trials and their extension studies had been dependent on PN for ≥ 12 months prior to initiation of teduglutide treatment, which suggests that teduglutide is effective even outside of the initial post-resection window where intestinal adaptation would be expected to be maximal. The close monitoring of subjects in each of these studies by their respective clinical sites lends credibility to these results, but it is also useful to examine effects of teduglutide not specifically addressed by this systematic review in order to gain a broader understanding of the effects of teduglutide treatment.

Subject body weight was significantly improved in both teduglutide dose groups compared to baseline and placebo at various time points in the Jeppesen 2011 study.[48] Encouragingly, these increases were primarily restricted to changes in lean body mass.[48,57] Total body bone mineral content also increased after 24 weeks of teduglutide treatment, but there were no significant changes in bone mineral density. By 12 months off drug,[46] median body mass index (BMI) was not different for INC versus STABLE/DEC subjects, but INC subjects did have significantly lower ($P < 0.001$) BMI at 3, 6 and 12 months relative to the first off-drug visit, while BMI of STABLE/DEC subjects did not change. Similar results were noted in the subset of drug responders in that BMI was significantly lower ($P < 0.001$) in INC subjects at 3, 6 and 12 months off drug, but did not change in STABLE/DEC subjects. In STEPS,[50] teduglutide 0.05

subjects experienced a non-significant body weight increase (1.0 ± 3.7 kg; $P = 0.10$), while placebo-treated subjects experienced a non-significant decrease (-0.6 ± 2.8 kg; $P = 0.20$) in body weight. These results demonstrate that teduglutide-stimulated increases in fluid and nutrient absorption translate to small but positive changes in body weight.

Teduglutide-induced changes in absorption efficiency and body weight can be partially explained by changes in intestinal morphology. In the Jeppesen 2011 study,[48] both teduglutide 0.05 and 0.10 subjects experienced significant absolute increases ($P = 0.01$ and 0.002, respectively) from baseline in small intestinal villus height compared to placebo. Teduglutide 0.10 subjects also had significantly increased ($P = 0.02$) mean villus surface area in the small intestine compared to placebo (teduglutide 0.05, $P = 0.08$).[52] Mean crypt depth of both the large and small intestine of the teduglutide 0.10 group was significantly greater ($P = 0.02$ and $P = 0.01$, respectively) than placebo.

Overall, these results support the use of teduglutide 0.05 in PN-dependent adults regardless of PN dependence duration. However, given that that this systematic review contains only 3 relatively small subject populations, it would be valuable to examine the effects of teduglutide in a greater number of PN-dependent subjects as well as in subjects with a wider variety of gastrointestinal disorders. The studies included in this review also did not measure endogenous GLP-2 production, which may explain some of the conflicting results in that subjects with L-cells left after resection (those with remnant ileum and/or colon) would have higher endogenous GLP-2 production and therefore may be most likely to respond to teduglutide administration. In an effort to explain drug potency, it would also be of interest to measure GLP-2 receptor expression in patients with resection since administration of exogenous GLP-2 increased ileal GLP-2 receptor expression 3-fold in rats with a 70% jejuno-ileal resection.[58]

Identification of factors that may predict patient response to teduglutide treatment would also be valuable given the annual $295,000 per patient cost associated with teduglutide treatment,[36] which is reflective of its orphan indication. Furthermore, the studies included in this review did not differentiate between subjects dependent on PN for nutrients versus those dependent on PN for fluids/electrolytes only, so it is unknown if teduglutide response varies between these groups. Additionally, longer-term follow-up studies are required to assess whether the benefits of teduglutide treatment endure following treatment discontinuation, and to directly compare the safety and efficacy of teduglutide to that of other approved therapies such as glutamine or recombinant growth hormone. Finally, while teduglutide does appear to be safe, it must be noted that close monitoring while on the drug is crucial. This is particularly true in (1) patients with cardiac issues such as congestive heart failure, due to the increased fluid absorption observed with teduglutide treatment; (2) patients with existing tumors since while teduglutide was not shown to promote growth of new tumors in the studies included here, animal studies suggest that teduglutide may enhance, rather than induce, tumor growth;[16,58] and (3) patients on additional medications since in addition to enhancing absorption of fluid and nutrients, teduglutide may also enhance absorption of other drugs, which is of particular concern for drugs such as digoxin which have narrow therapeutic indices.

In summary, teduglutide appears to be an efficacious, safe, and well-tolerated means by which to reduce PN dependency in adult patients through restoration of intestinal function. High bioavailability of subcutaneous teduglutide injections enables convenient once daily administration,[33,53,59] but careful monitoring of patients on teduglutide will be crucial to ensure safety and maximize treatment efficacy. The importance of individualized treatment cannot be overstated, and pairing teduglutide with other complementary treatments aimed at enhancing

intestinal adaptation may be beneficial. Finally, given promising results in preclinical pediatric models,[60] despite the current lack of human pediatric GLP-2 and/or teduglutide clinical trials,[61] it would also be beneficial to investigate the therapeutic effects of teduglutide in pediatric patients so the therapeutic benefits of teduglutide could be extended to this population.

FIGURES AND TABLES

Figure 3.1. Study flow chart.

Study[a]	Description	Outcomes
Jeppesen 2011[48]	**Phase III clinical trial**. SBS males and females ≥ 18 y on PN ≥ 3 d/wk for ≥ 12 mo. Randomized to teduglutide 0.05[b] (n = 35); teduglutide 0.10[b] (n = 32); or placebo (n = 16) for 6 mo.	1. PN volume 2. Responder rate[c] 3. Complete PN weaning 4. Intestinal adaptation 5. Safety
°**Jeppesen 2009 a**[39]**, b**[40]**, c**[41]	Subset of Jeppesen 2011 subjects. Teduglutide 0.05 (n = 10); teduglutide 0.10 (n = 7); or placebo (n = 4) for 6 mo.	1. Intestinal adaptation
Tappenden 2013[52]	Subset of Jeppesen 2011 subjects. Teduglutide 0.05 (n = 32); teduglutide 0.10 (n = 30); or placebo (n = 15) for 6 mo.	1. Safety
O'Keefe 2013[49] °**Gilroy 2008**[38]	Extension of Jeppesen 2011. Subjects previously on teduglutide 0.05 (n = 25) or teduglutide 0.10 (n = 27) received 7 additional mo. of same dose. Previously placebo-treated subjects randomized to teduglutide 0.05 (n = 6) or 0.10 (n = 7) for 7 mo.	1. PN infusion frequency[d] 2. Responder rate 3. Complete PN weaning 4. Safety
Compher 2011[46]	Extension of Jeppesen 2011. Subjects with stable (n = 15) or decreased (n = 7) PN requirement by 12 mo. off teduglutide compared to those with increased PN requirement (n = 15).	1. PN volume 2. Complete PN weaning 3. Safety
Jeppesen 2012[50] **(STEPS)**	**Phase III clinical trial**. SBS males and females ≥ 18 y on PN ≥ 3 d/wk for ≥ 12 mo. Previously teduglutide-treated subjects not eligible. Subjects randomized to receive teduglutide 0.05 (n = 43); or placebo (n = 43) for 6 mo.	1. PN volume 2. PN infusion frequency 3. Responder rate 4. Safety
°**Jeppesen 2014a**[42] °**Jeppesen 2014b**[43] °**Fujioka 2014**[45] **(STEPS-2)**	Open-label extension of STEPS. Treatment in STEPS/STEPS2: teduglutide/teduglutide 0.05 (n = 30); placebo/teduglutide 0.05 (n = 29); not randomized/teduglutide 0.05 (n = 6) for 18-24 mo.	1. PN volume 2. PN infusion frequency 3. Responder rate 4. Complete PN weaning 5. Safety
°**Iyer 2014**[44] **(STEPS-3)**	Open-label extension of STEPS/STEPS-2. Teduglutide 0.05 (n = 14), with STEPS/STEPS-3 treatment of teduglutide/ teduglutide (n = 5; treatment duration ≤ 42 mo); placebo/ teduglutide (n = 6; treatment duration ≤ 36 mo); not-treated/teduglutide (n = 3; treatment duration ≤ 36 mo).	1. PN volume 2. PN infusion frequency 3. Complete PN weaning 4. Safety
°**Ukleja 2014**[47]	**Retrospective chart review** of SBS patients (n = 6) following FDA approval of teduglutide. Teduglutide 0.05 administered for 1-12 mo.	1. PN volume 2. Responder rate 3. Complete PN weaning 4. Safety

a. Parent studies are in grey. Associated/extension studies are directly below each parent study.
b. mg/kg/d
c. Responder rate refers to subjects that achieved ≥ 20% volume reduction in PN requirement.
d. PN infusion frequency expressed as d/wk PN required.
°Meeting abstract.
Abbreviations: d, days; FDA, Food and Drug Administration; mo, months; PN, parenteral nutrition; SBS, short bowel syndrome; STEPS, study of teduglutide effectiveness in parenteral nutrition-dependent short-bowel syndrome subjects; wk, weeks; y, years.

Table 3.1. Characteristics of included studies.

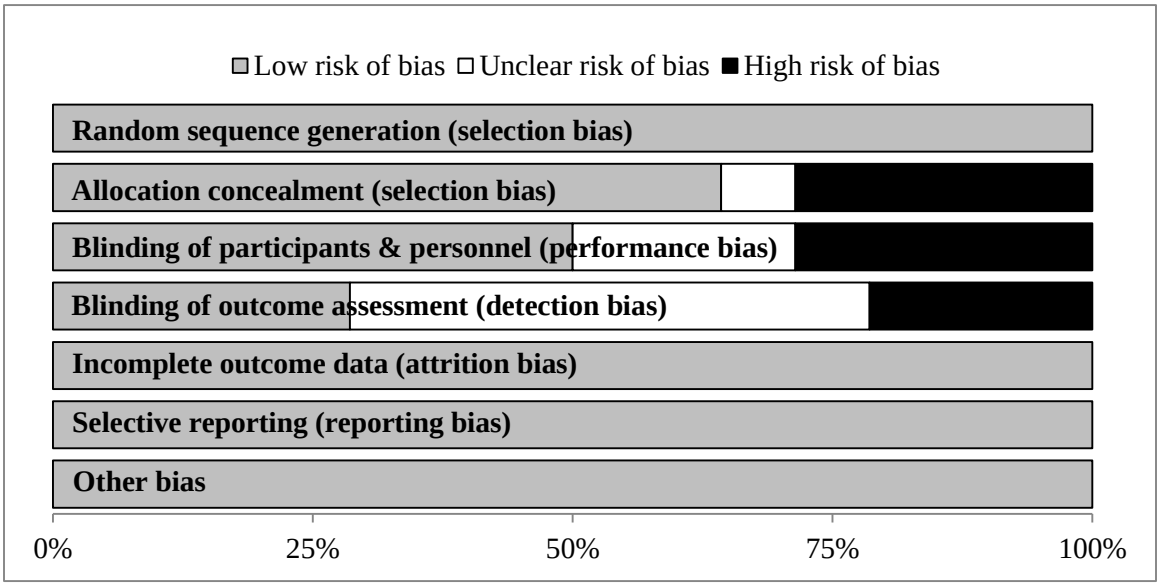

Figure 3.2. Risk of bias assessment. Results of each bias domain are presented as percentages across all included studies.

Study[a]	Responder rate[b] (%) Teduglutide 0.05[c]	Teduglutide 0.10[c]	Placebo
Jeppesen 2011[48]	45.7	25.0	6.3
NNTB[d]; OR[e]; therapeutic gain[f] vs. placebo	*3; 12.63; 39.4%*	*6; 5.00; 18.7%*	-
O'Keefe 2013[49]	68.4	52.2	-
Gilroy 2008[38]	100.0	28.6	-
Jeppesen 2012[50] **(STEPS)**	62.8	-	30.2
NNTB; OR; therapeutic gain vs. placebo	*4; 3.89; 32.6%*	-	-
Jeppesen 2014b,[43] **Fujioka 2014**[45] **(STEPS-2)**	73.8	-	-
Ukleja 2014[47]	100.0	-	-

a. Parent studies are in grey. Associated/extension studies are directly below each parent study.
b. Responder rate = (number of subjects achieving ≥ 20% volume reduction in PN requirement/total number of subjects in group) x 100.
c. mg/kg/d
d. NNTB = 1/(teduglutide responder rate - placebo responder rate), rounded up to next whole number.
e. OR = (number of teduglutide-treated responders/number of teduglutide-treated non-responders)/(number placebo-treated responders/number placebo-treated non-responders).
f. Therapeutic gain = (teduglutide responder rate - placebo responder rate).
Abbreviations: NNTB, number needed to treat for an additional beneficial outcome; OR, odds ratio; STEPS, study of teduglutide effectiveness in parenteral nutrition-dependent short-bowel syndrome [SBS] subjects.

Table 3.2. Efficacy of teduglutide: Responder rate.

Study[a]	Event rate[b] (%)		
	Teduglutide 0.05[c]	Teduglutide 0.10[c]	Placebo
Jeppesen 2011[48]			
AE	94.3	96.9	93.8
SAE	37.1	34.4	31.3
Drop-out	17.1	6.3	6.3
Laboratory findings[d]		NS	
Death		0.0	
Tappenden 2013[52]			
Pathology (small & large bowel) [e]	0.0	0.0	0.0
New secondary diagnosis[f]	12.5	10.0	60.0
O'Keefe 2013[49]			
TE-AE	92.0	100.0	-
TE-AE related to teduglutide	51.9		-
SAE	51.9		-
SAE related to teduglutide	9.6		-
Drop-out	20.0	14.8	-
Pathology (colon only)	0.0	0.0	-
Laboratory findings	NS		-
Death	0.0		-
STEPS: Jeppesen 2012[50]			
≥ 1 TE-AE	83.3	-	79.1
TE-SAE	35.7	-	27.9
Drop-out due to TE-AE	4.8	-	6.9
Death	0.0	-	0.0
Laboratory findings	NS	-	NS
STEPS-2: Jeppesen 2014a,[42] **2014b**,[43] **Fujioka 2014**,[45]			
TE-AE	95.5	-	-
SAE	63.6	-	-
Drop-out	26.1	-	-
Laboratory findings	NS	-	-
STEPS-3: Iyer 2014[44]			
TE-AE	100.0	-	-
Drop-out due to AE	0.0	-	-
Malignancy, GI obstruction, death	0.0	-	-
Ukleja 2014[47]			
Complications	50.0	-	-
Teduglutide discontinuation	16.7	-	-
Teduglutide dose reduction	16.7	-	-

a. Parent studies are in grey. Associated/extension studies are directly below each parent study.
b. Event rate = (number of subjects experiencing event/total number of subjects in group) x 100. More than 1 event could occur in a single subject.
c. mg/kg/day.
d. Laboratory findings include the following values: vital signs, electrocardiogram, hemoglobin, platelets, differential white blood cells, urea, electrolytes, liver function tests, and C-reactive protein.
e. Pathology refers to dysplastic transformation, including adenomatous polyps.
f. New secondary diagnoses include colitis (ulcerative, acute non-specific, collagenous, eosinophilic, lymphocytic), Crohn's disease, non-specific increased mucosal inflammation, sarcoidosis, or villus abnormality (decreased villus height and/or volume).
Abbreviations: AE, adverse event; GI, gastrointestinal; NS, not significant; SAE, serious adverse event; STEPS, study of teduglutide effectiveness in parenteral nutrition-dependent short bowel syndrome [SBS] subjects; TE-AE, treatment-emergent adverse event; TE-SAE, treatment-emergent serious adverse event.

Table 3.3. Safety of teduglutide: Adverse event incidence.

Study	Teduglutide 0.05[a] vs. placebo		Teduglutide 0.10[a] vs. placebo	
	NNTH[b]	OR[c]	NNTH	OR
Jeppesen 2011[48]				
AE	187	1.10	32	2.07
SAE	17	1.30	32	1.15
Drop-outs	10	3.10	∞	1.00
STEPS: Jeppesen 2012[50]				
≥ 1 TE-AE	24	1.32	-	-
TE-SAE	13	1.44	-	-
Drop-outs	47	0.75	-	-

a. mg/kg/day.
b. NNTH = 1/(teduglutide event rate - placebo event rate), rounded up to next whole number.
c. OR = (number of teduglutide-treated subjects experiencing event/number of event-free teduglutide treated subjects)/(number of placebo-treated subjects experiencing event/number of event-free placebo-treated subjects).
Abbreviations: AE, adverse event; NNTH, number needed to treat for one additional harmful outcome; OR, odds ratio SAE, serious adverse event; STEPS, study of teduglutide effectiveness in parenteral nutrition-dependent short bowel syndrome [SBS] subjects; TE-AE, treatment-emergent adverse event; TE-SAE, treatment-emergent serious adverse event.

Table 3.4. Safety of teduglutide: Number needed to treat to harm and adverse event odds ratio.

General and Subject Specific Databases	
Academic OneFile	HighWire Press
AgeLine	MEDLINE
BiblioMap	National Guidelines Clearinghouse
BIOSIS Previews	National Library of Medicine
Cochrane Central Register of Controlled Trials	OTseeker
Cochrane Database of Abstracts of Reviews of Effects	Physiotherapy Evidence Database
Cumulative Index to Nursing and Allied Health	POPLINE
Database of Promoting Health Effectiveness Reviews	Scopus
Derwent Drug File	The Trials Register of Promoting Health Interventions
Embase	Turning Research Into Practice
EMCare	Web of Science
Google Scholar	
Grey Literature Databases	
Trial Registries	
AstraZeneca Clinical Trials Registry	European Medicines Agency
Bristol-Myers Squibb Clinical Trial Registry	Food and Drug Administration
CenterWatch Clinical Trials Listing Service	GlaxoSmithKline Clinical Trial Registry
Clinical Research Network Portfolio Database (UK)	International Standard Randomized Controlled Trial Number Register
Clinical Trials Gateway/National Research Register (UK)	Novartis Clinical Trial Registry
Clinicaltrialresults.org	Pfizer (Wyeth) Clinical Trial Listings
Clinicaltrials.gov	Roche Clinical Trial Protocol Registry
Community Research & Development Information Service (European Union)	South African National Clinical Trial Register
Current Controlled Trials metaRegister of Controlled Trials (including archives)	The Association of the British Pharmaceutical Industry
Eli Lilly and Company Clinical Trial Registry	World Health Organization International Clinical Trials Registry
Conference Proceedings and Dissertations	
British Library Direct Plus	King's Fund
DissOnline (Germany)	National Technical Information Service
Index to Theses in Great Britain and Ireland	New York Academy of Medicine Grey Literature Database
International Federation of Pharmaceutical Manufacturers and Associations	OAlster
International Pharmaceutical Abstracts	ProQuest Dissertations and Theses
ISI Proceedings	System for Information of Grey Literature in Europe
Regional Databases	
Index Medicus (African, Eastern Mediterranean, South-East Asian, Western Pacific)	
IndMED (India)	
Informit Medical Database (Australia)	
KoreaMed	
Latin American and Caribbean Health Sciences Literature	
Economic Databases	
Cost Effectiveness Analysis Registry (Tufts Medical Center)	European Network of Health Economic Evaluation
EconLit (American Economic Association)	National Health Service Economic Evaluation (UK)
Adverse Event Databases	
Regulatory Agency Safety Bulletins	
Current Problems in Pharmacovigilence (UK)	
Australian Adverse Drug Reactions Bulletin/Australian Register of Therapeutic Goods	
European Public Assessment Reports from the European Medicines Evaluation Agency	
Food and Drug Administration (FDA) Medwatch and Drugs@FDA	
Other Databases	
Iowa Drug Information Service	
Medicines Transparency Alliance	
Toxicology Literature Online	

Supplementary Table 3.1. Full list of databases searched.

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CHAPTER 4

TEDUGLUTIDE-STIMULATED INTESTINAL ADAPTATION IS COMPLIMENTED AND SYNERGISTICALLY ENHANCED BY PARTIAL ENTERAL NUTRITION IN A NEONATAL PIGLET MODEL OF SHORT BOWEL SYNDROME

ABSTRACT

Background: Teduglutide, a glucagon-like peptide-2 (GLP-2) analogue, is available for long-term use by parenteral nutrition- (PN) dependent adults to promote intestinal adaptation, but is not approved for use in pediatric patients. Objectives: Assess teduglutide-stimulated induced intestinal adaptation, potential synergies with partial enteral nutrition (PEN), and distinct temporal markers of adaptation in a neonatal piglet model of short bowel syndrome (SBS). Materials and methods: Neonatal piglets (48 hours old; n = 72) underwent an 80% jejuno-ileal resection and were randomized to 1 of 4 treatment groups, in a 2x2 factorial design, with total parenteral nutrition (TPN) or PEN (80% standard PN/20% standard enteral nutrition) and teduglutide (0.10 mg/kg/d) or control. Piglets received infusions for 4 hr, 48 hr, or 7 d. Results: Teduglutide improved ($P < 0.05$) mucosal surface area (villus height: duodenum, jejunum, ileum; crypt depth: ileum, colon; proliferation: duodenum, jejunum, ileum; colon; apoptosis: jejunum, ileum, colon) and acute nutrient processing capacity (glucose: duodenum, jejunum, ileum; glutamine: duodenum, jejunum). These effects were complimented and synergistically enhanced by PEN in both site and timing of action. Structural adaptations preceded functional adaptations, but crypt depth remained a strong indicator of adaptation, regardless of time. Conclusions: The combination of teduglutide and PEN enhance intestinal adaptation beyond that of either therapy alone.

CLINICAL RELEVANCY STATEMENT

Patients dependent on parenteral nutrition (PN) are most likely to achieve enteral autonomy if strategic post-operative therapies aimed at maximizing intestinal adaptation are employed. Teduglutide has been shown to effectively stimulate intestinal adaptation and promote weaning from PN in both preclinical and adult human trials, but is not approved for use in the pediatric population. The data presented here demonstrate that teduglutide increases structural, and transiently increases functional, measures of intestinal adaptation in a neonatal piglet model of short bowel syndrome (SBS), and furthermore, these adaptations are complimented and synergistically augmented by provision of partial enteral nutrition (PEN).

INTRODUCTION

Short bowel syndrome (SBS) is a malabsorptive state occurring as a result of reduced functional bowel length.[1,2] In infants, SBS results in inadequate intestinal surface area for digestion and absorption of sufficient enteral nutrients to support growth and development.[1] SBS is one of the most lethal conditions in infancy and childhood,[2] and accounts for 1.4% of all deaths of children under 4 years of age.[3] Necrotizing enterocolitis (NEC) is the principal cause of pediatric SBS, responsible for 32% of cases.[4] Preterm infants, which comprised 11.4% of births in the United States in 2013,[5] are especially vulnerable to developing NEC due to the immaturity of the gastrointestinal tract. SBS is particularly devastating in this population since infants who require parenteral nutrition (PN) secondary to intestinal failure following bowel resection require adequate nutrients not only to maintain fluid and electrolyte levels, body weight, and lean mass composition as adults do, but also require additional energy and protein to support growth.[6]

PN should be initiated as soon as possible in infants with SBS to reduce the risk of malnutrition precipitated by low nutrient stores.[7] However, prolonged PN usage is associated with numerous complications including intestinal atrophy,[8,9] liver damage,[10] and sepsis from intravenous line infections.[2] Consequently, in addition to promoting growth and development in pediatric patients,[11] one of the primary goals of SBS treatment is to maximize the functional capacity of the remnant intestine and ultimately eliminate the need for PN support.[4]

Provision of partial enteral nutrition (PEN) maintains intestinal structural and functional integrity[12,13] and augments bowel adaptation following resection.[6,14-18] Luminal nutrients are the primary stimulus for this adaptation,[19,20] as well as for the release of humoral factors including glucagon-like peptide-2 (GLP-2). GLP-2 is important for inducing bowel growth during the final weeks of gestation,[21-23] and serum GLP-2 levels correlate with residual small bowel length in both adults and infants.[24] Provision of exogenous GLP-2 induces numerous indices of intestinal adaptation in preclinical models,[25-37] including improved enteral tolerance and a reduction in the number of days per week PN is required.[37] Furthermore, while GLP-2 and PEN synergistically stimulate intestinal adaptation in adult rat models of SBS,[38,39] the efficacy of this combination therapy has yet to be investigated in a pediatric model of SBS.

GLP-2 has clearly demonstrated therapeutic promise for SBS treatment, but the half-life of GLP-2 is extremely short due to rapid degradation by dipeptidyl peptidase IV (DPP-IV). Therefore, teduglutide (Gattex, NPS Pharmaceuticals, Inc., Bedminster, NJ), a DPP-IV-resistant GLP-2 analog, was created which extends the peptide's half-life from 7 minutes to 1.3-2 hours.[40-42] Based on reductions in PN requirements as well as an adverse event profile similar to placebo and consistent with underlying disease states, teduglutide has been approved for long-term treatment of adults with SBS intestinal failure in both the United States and Europe, but no

human pediatric GLP-2 and/or teduglutide safety and efficacy studies have yet been completed.[18] Teduglutide treatment in TPN-fed piglets with 50% small bowel resection improved structural, but not functional, measures of adaptation.[43]

Given the immense human suffering and healthcare burden associated with pediatric SBS, development of novel synergistic medical nutrition therapies aimed at intestinal rehabilitation is critical. The objective of this work was to explore the efficacy of teduglutide, alone or in combination with PEN, for enhancing intestinal adaptation in a well-characterized[14,44-47] neonatal piglet model of SBS. Our focus was on three particular questions:

1. Does teduglutide induce structural and/or functional measures of intestinal adaptation in a neonatal piglet model of SBS?

2. Are the effects of teduglutide in this model complimented or synergistically enhanced by the provision of PEN?

3. Can distinct temporal markers of adaptation stimulated by these two therapies be identified?

We hypothesized that teduglutide would enhance structural and functional adaptation of the residual small intestine via enhanced mucosal surface area and nutrient processing capacity, and that these effects would be augmented by the provision of PEN. Furthermore, we hypothesized that surface area expansion would precede functional adaptation.

METHODS

Experimental design

Neonatal piglets (n = 72; Duroc x Landrace cross) were obtained from a University of Illinois Urbana-Champaign swine producer within 48 hours of birth and underwent placement of a jugular catheter and an 80% proximal jejuno-ileal resection, as previously described.[47] Littermate piglets were randomly assigned to 1 of 4 treatment groups:

1. 100% PN with vehicle control (TPN-);

2. 80% PN and 20% enteral nutrition with vehicle control (PEN-);

3. 100% PN with teduglutide (0.10 mg/kg/d; TPN+), or;

4. 80% PN and 20% enteral nutrition with teduglutide (0.10 mg/kg/d; PEN+).

Within each treatment group, animals were further randomized to receive infusions for various time points following surgery to allow for examination of acute (4- and 48-hour) and chronic (7-day) adaptations. All animal procedures were approved by the Illinois Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign.

Animal care and housing

Vital signs and activity levels were monitored in each piglet during recovery from surgery. Buprenex analgesic (0.01 mg/kg; Reckitt Benckiser Pharmaceuticals, Richmond, VA) and Naxcel broad spectrum antibiotic (3.0 mg/kg; Pharmacia & UpJohn Company, Inc., Kalamazoo, MI) were provided intravenously immediately after surgery and for the following 48 hours to minimize pain and decrease postoperative infection. Piglets were individually housed in metabolic cages as previously described.[47] A full clinical assessment, including the following

criteria, was performed each morning: body temperature, weight gain, activity level, healing of surgical site, and absence of edema and guarded posture. A partial clinical assessment was performed each evening to reevaluate piglet condition.

Nutrient solutions and administration

Nutrition was provided to piglets in amounts necessary to meet daily requirements of 253 kcal/kg/d and 12.8 g protein/kg/d, as determined by the National Research Council.[48] PN was formulated and compounded daily, as previously described,[47] and was continuously infused via a Flo-Gard 6200 volumetric infusion pump (Travenol Laboratories, Deerfield IL). The PN solution provided 253 kcal/kg/d and 12.8 g amino acids/kg/d to TPN piglets, and 202 kcal/kg/d and 10.24 g amino acids/kg/d to PEN piglets. Infused volumes were quantified and recorded daily.

Polymeric pig milk replacer formula (Animix LLC, Juneau, WI) was freshly reconstituted each morning to a concentration of 2.7 kcal/mL. Piglets receiving PEN were provided 20% of their daily nutritional needs via oral gavage delivered in 2 separate boluses, with 60 mL delivered in the morning, and the residual volume provided in the evening. PN infusion was stopped 2 hours before euthanasia, at which time PEN piglets received 60 mL of formula.

Teduglutide composition and administration

Teduglutide ([Gly2]GLP-2, Gattex, NPS Pharmaceuticals, Bedminster, NJ) was aseptically diluted into vehicle buffer (35 mM dibasic sodium phosphate, 50 mM D-histidine, 3% wt/vol D-mannitol) and administered intravenously every 12 hours for a total dose of 0.10

mg/kg/d. Vehicle control animals received a weight-calculated equivalent volume of vehicle buffer.

Sample collection

Piglets were euthanized via intravenous delivery of 0.39 g/mL sodium pentobarbital (Fatal Plus; Vortech Pharmaceuticals, Ltd., Chicago, IL). Blood samples were collected via cardiac puncture as previously described,[47] and plasma was stored at -80°C until further use. The gastrointestinal tract was quickly excised, and samples for assessment of both structural and functional adaptations were processed as previously described.[47] The weight of the visceral organs (heart, liver, kidneys, pancreas, stomach, spleen) and eviscerated carcass were also recorded.

Intestinal tissue composition

DNA and protein concentrations of all intestinal segments were determined by the Hoechst[49] and Bradford[50] methods, respectively, as previously described.[14]

Gross histomorphology

Intestinal length, weight, and mucosal dry weight were quantified and recorded. Intestinal segment lengths were normalized to body weight (cm/kg) and intestinal wet weights were assessed per unit length (g/cm). Mucosal and non-mucosal dry weights were normalized to unit sample length (mg/cm) and expressed as a percentage of mucosa to total mucosal weight. Percentage mucosa was calculated as (mg mucosa) / (mg mucosa + mg non-mucosa) x 100.

Morphometric analysis of mucosal architecture was completed as previously described.[47] Sections were visualized at 5x magnification on a Zeiss Axioskop (Model 40, Zeiss, Thornwood, NY) with an AxioCam MRc5 and analyzed using the AxioVision software package (Version 4.5, Zeiss). Villus height and crypt depth were measured in 8-10 intact, well-oriented villi and crypts within each sample.

Epithelial cell proliferation

Epithelial cell proliferation was assessed by immunohistochemical staining for proliferating cell nuclear antigen (PCNA) as previously described.[14] Antigen retrieval was performed by placing slides in a 95°C citrate buffer (10 mM citric acid, 0.05% Tween-20, pH 6.0; Sigma-Aldrich) bath for 10 minutes for small intestinal segments, and 15 minutes for colon segments. Endogenous peroxidase was quenched for 10 minutes with a 3.0% peroxide solution, and samples were incubated with 2% normal horse serum (NHS) for 20 minutes to prevent nonspecific binding. Primary PCNA antibody was diluted 1:1000 in 1% NHS phosphate buffered saline. Nanozoomer Slide Scanner Digital Pathology System and NDP View imaging software were used to capture images at 20× magnification. Within each sample, PCNA-positive cells in 8-10 intact, well-oriented crypts were counted using ImageJ (National Institutes of Health, Bethesda, MD).

Epithelial cell apoptosis

DNA fragmentation was measured immunohistochemically as previously described[14] with the ApopTag Plus Peroxidase *In Situ* Apoptosis Detection Kit (terminal deoxynucleotidyl transferase dUTP nick end labeling [TUNEL] assay; Millipore) to assess jejunal, ileal, and

colonic epithelial cell apoptosis. Nanozoomer Slide Scanner Digital Pathology System and NDP View imaging software were used to capture images at 20× magnification. TUNEL-positive cells in 8-10 intact, well-oriented crypts of each sample were counted using ImageJ (National Institutes of Health, Bethesda, MD).

Epithelial cell differentiation

As previously described,[14] real-time reverse transcription polymerase chain reaction was used to quantify jejunal, ileal, and colonic Cdx2 mRNA abundance, a marker of intestinal cellular differentiation. Cdx2 and endogenous 18S controls were measured in separate wells with a TaqMan ABI 9700. Samples were quantified using the ABI Sequence Detection System software and a pooled cDNA standard curve, and were normalized to 18S expression.

Mucosal ion and nutrient transport

Mucosal ion and nutrient transport were measured using modified Ussing chambers (Physiologic Instruments, San Diego, CA) as described previously.[14] Dual-channel voltage/current clamps (VCC MC2, Physiologic Instruments, San Diego, CA) with a computer interface allowed for real-time data acquisition and analysis (Acquire & Analyze, Physiologic Instruments).

Statistics

Statistical analyses were performed using the SAS (Version 9.4, SAS Institute Inc., Cary, NC) mixed model procedure. A log, square-root, or reciprocal transformation of the residuals was performed if data were determined to be non-normal by the univariate procedure.

Comparisons included (1) drug (teduglutide versus vehicle; pooling route of nutrient administration [PEN and TPN]), and interactions with time (4 hours versus 48 hours vs 7 days); and (2) route of nutrient administration (PEN versus TPN) and interactions with drug (teduglutide versus vehicle) and/or time (4 hours versus 48 hours vs 7 days). Means were separated using the least significant difference when a main effect of drug or route of nutrient administration existed in the absence of significant interactions. Litter was included as a random effect. Each intestinal segment was analyzed separately from the others, and differences within time points were assessed individually. Statistical significance was defined as $P < 0.05$.

Principal component analysis (PCA) was completed on data derived from all treatment groups at 4 hours (PCA 1; n = 24), 48 hours (PCA 2; n = 23), and 7 days (PCA 3; n = 24), as well as on the entire pooled group (PCA 4; n = 71) as outlined by Jolliffe.[51] All PCAs initially included 81 variables. The primary objective was to investigate the temporal sequence of teduglutide- and/or PEN-mediated intestinal adaptation following massive small bowel resection, and to identify distinct markers of adaptation over time. In PCAs 1-3, 80% of the total variance was accounted for by 10 factors; therefore, 10 factors were retained in the final analysis. For PCA 4, 18 factors were retained in order to explain 80% of the variance. Variables that did not load on any factor retained (correlation coefficient between variables and factors $|r| \leq 0.5$) were excluded from the final analyses. When several variables were significantly correlated ($|r| > 0.6$, $P < 0.05$) within a group of similar variables, only the variables with the highest factor loadings were retained for the final analyses. PCA was performed using JMP (Version 11; SAS Institute, Cary, NC).

RESULTS

Growth and nutrition support

A total of 72 surgeries were performed with 71 piglets completing the study. One piglet died of surgical complications. Piglet body weight did not differ between treatments at any point during the study. All piglets received adequate nutrients via TPN or PEN to provide satisfactory growth and meet nutritional needs, and all groups gained weight over time following initial post-surgical weight loss at 4 hours. Daily weight gain was not affected by treatment, and weight of the heart, kidney, and pancreas, normalized to body weight (g/kg), did not differ among treatments at any time (data not shown). Stomach weight was not different between groups at 4 hours, but decreased (4 hours = 7.55 ± 0.46, 48 hours = 7.34 ± 0.75, and 7 days = 5.83 ± 0.23 g/kg body weight; $P = 0.03$) over time in TPN vehicle control (TPN-) animals. Spleen weight increased over time (4 hours = 1.73 ± 0.27, 48 hours = 1.89 ± 0.22, 7 days = 3.07 ± 0.37 g/kg body weight; $P < 0.001$), regardless of treatment. Liver weight increased (4 hours = 37.77 ± 2.45, 48 hours = 42.76 ± 3.96, 7 days = 33.57 ± 1.53 g/kg body weight; $P < 0.001$) from 4 to 48 hours, but returned to 4 hour values by 7 days, regardless of treatment.

Question 1: Does teduglutide induce structural and/or functional measures of intestinal adaptation in a neonatal piglet model of SBS?

Structural adaptations

Gross intestinal morphology

Teduglutide increased colonic wet weight (teduglutide = 0.401 ± 0.058 versus vehicle = 0.274 ± 0.020 g/cm; $P = 0.02$) and ileal mucosal mass (teduglutide = 55.8 ± 3.20 versus vehicle

= 47.2 ± 2.69% mucosa; $P = 0.04$), with a trend for increased duodenal length ($P = 0.056$) regardless of route of nutrient administration or time. Teduglutide did not affect wet weight, mucosal mass, or length of any other intestinal segments (data not shown).

Intestinal tissue composition

Intestinal DNA concentration (μg DNA/mg mucosa), a marker of cellularity used to assess compositional changes associated with growth, was not affected by teduglutide administration in any intestinal segment (data not shown). Teduglutide administration also did not affect protein concentration or protein/DNA concentration of any intestinal segment (data not shown).

Crypt-villus architecture

Teduglutide increased villus height by 20% in the duodenum ($P = 0.005$) and jejunum ($P = 0.002$), and by 12% in the ileum ($P = 0.03$), regardless of time or route of nutrient administration (**Figure 4.1**). Similarly, teduglutide increased ileal ($P < 0.001$) and colonic ($P = 0.006$) crypt depth versus vehicle, regardless of time or route of nutrition administration (**Figure 4.2**).

Epithelial cell turnover

Epithelial cell proliferation was increased by teduglutide in all intestinal segments, regardless of route of nutrient administration or time (duodenum, jejunum, ileum all $P < 0.001$; colon, $P = 0.008$; **Figure 4.3A)**. Teduglutide treatment also decreased apoptosis (TUNEL-positive cells/crypt) in the jejunum ($P < 0.001$), ileum ($P < 0.001$), and colon ($P = 0.007$) regardless of time or route of nutrient administration (**Figure 4.3B**).

Mucosal cell differentiation

Cdx2, a marker of cellular differentiation, was quantified in jejunal, ileal, and colonic mucosa to examine changes in mucosal cell maturation. Teduglutide increased (teduglutide = 0.534 ± 0.026 versus vehicle = 0.473 ± 0.027; $P = 0.04$) ileal Cdx2 mRNA abundance regardless of time and route of nutrient administration, but did not affect Cdx2 mRNA abundance of any other segment (data not shown).

Functional adaptations

Mucosal ion transport

Basal short circuit current ($\mu A/cm^2$), transmucosal resistance ($\Omega*cm^2$), and potential difference (V) were assessed using modified Ussing chambers to evaluate active, passive, and total ion transport, respectively. Transmucosal resistance was transiently numerically decreased in the duodenum ($P = 0.07$) of teduglutide- versus vehicle-treated animals, regardless of route of nutrient administration. Teduglutide treatment did not affect transmucosal resistance of any other intestinal segment, or basal short circuit current or potential difference of any intestinal segment (data not shown).

Mucosal nutrient transport

Nutrient transport was assessed by measuring deflections in short circuit current induced by addition of nutritive substrates to the mucosal chamber medium. Addition of 10 mM D-glucose was used to assess intestinal monosaccharide transport via the sodium/glucose co-transporter 1 (SGLT1). Teduglutide treatment resulted in rapid, reversible increases in duodenal ($P = 0.01$), jejunal ($P = 0.03$) and ileal ($P = 0.04$) glucose transport (**Table 4.1**) regardless of route of nutrient administration. Glutamine transport, important as a source of fuel for the small

intestine, was assessed via addition of 10 mM L-glutamine to the mucosal chamber. Teduglutide acutely increased duodenal ($P = 0.002$) and jejunal ($P < 0.001$) glutamine transport regardless of route of nutrient administration (**Figure 4.4**). Peptide transport via peptide transporter 1 (PepT1) was measured by addition of 10 mM glycyl-sarcosine. Teduglutide increased (teduglutide = 0.465 ± 0.368 versus vehicle = -3.265 ± 1.18 μA/cm^2; $P = 0.04$) ileal peptide transport at 7 days regardless of route of nutrient support, with a trend ($P = 0.06$) for decreased duodenal peptide transport with teduglutide versus vehicle treatment, regardless of time or route of nutrient administration. Jejunal peptide transport was not affected by teduglutide. Neural and immune-based chloride secretion were assessed by sequential addition of 10 mM serotonin (5-HT) and 10 mM carbmylchloride (CCH), respectively. Teduglutide did not affect 5-HT- or CCH-mediated secretion of any intestinal segment (data not shown).

Question 2: Are the effects of teduglutide in this model complimented or synergistically enhanced by the provision of PEN?

Structural adaptations

Gross intestinal morphology

Duodenal and jejunal length were greater in PEN versus TPN animals at day 7 (duodenum, PEN = 8.91 ± 0.21 versus TPN = 7.11 ± 0.33 cm/kg, $P = 0.04$; jejunum, PEN = 7.35 ± 0.46 versus TPN = 5.54 ± 0.44 cm/kg, $P = 0.04$), complementing the action of teduglutide in the duodenum. Animals that received both teduglutide and PEN showed trends for greatest duodenal ($P = 0.051$) and colonic ($P = 0.13$) lengths. Route of nutrient administration did not affect wet weight or mucosal mass of any intestinal segment (data not shown).

Intestinal tissue composition

While teduglutide did not affect DNA concentration of any intestinal segment, colonic DNA concentration was greater (PEN = 2.00 ± 0.27 versus TPN = 1.67 ± 0.24 μg DNA/mg; *P* = 0.03) in PEN versus TPN animals regardless of drug or time. PEN animals also maintained ileal DNA concentration over 48 hours, while that of TPN animals decreased (PEN animals, 4 hours = 3.05 ± 0.25 and 48 hours = 3.35 ± 0.19 μg DNA/mg; TPN animals, 4 hours = 3.47 ± 0.25 and 48 hours = 2.67 ± 0.23 μg DNA/mg; P = 0.002) from 4 to 48 hours. PEN did not affect protein or protein/DNA concentrations of any intestinal segment (data not shown).

Crypt-villus architecture

Jejunal (*P* = 0.001) and ileal (*P* = 0.01) villus height were greater in PEN versus TPN animals regardless of drug or time, and villus height was numerically greatest in all small intestinal segments and at all time points in PEN+ animals (**Table 4.2, Figure 4.5**). Ileal crypt depth was greater (*P* = 0.002) in PEN versus TPN animals regardless of drug or time, and crypt depth was numerically greatest in the 7 day PEN+ group in the jejunum, ileum, and colon (**Table 4.3**).

Epithelial cell turnover

While teduglutide increased proliferation in both the small intestine and colon, PEN increased proliferation in the small intestine only (duodenum, jejunum, ileum all P < 0.001; colon, *P* = 0.74), regardless of drug or time (**Table 4.4**). Ileal proliferation was greatest in the PEN+ group at all time points (4 hours, *P* = 0.01; 48 hours, *P* = 0.09; 7 days, *P* = 0.09; **Figure 4.6**). Similar to teduglutide administration, PEN decreased apoptosis in both the jejunum (PEN = 1.81 ± 0.04 versus TPN = 2.15 ± 0.05 TUNEL-positive cells/crypt; *P* < 0.001) and ileum (PEN = 1.86 ± 0.12 versus TPN = 2.18 ± 0.12 TUNEL-positive cells/crypt; *P* = 0.009) regardless of drug

or time (**Figure 4.7**). PEN did not affect colonic apoptosis (data not shown). A significant route*drug interaction was noted in that PEN decreased (PEN- = 1.93 ± 0.06 versus TPN- = 2.42 ± 0.07 TUNEL-positive cells/villi; $P = 0.01$) jejunal apoptosis in vehicle control animals, but the PEN-induced decrease (PEN+ = 1.70 ± 0.06 versus TPN+ = 1.87 ± 0.06 TUNEL-positive cells/villi; $P = 0.055$) in jejunal apoptosis of teduglutide-treated animals was not significant.

Mucosal cell differentiation

PEN treatment did not affect Cdx2 mRNA abundance in any intestinal segment (data not shown).

Functional adaptations

Mucosal ion transport

Ileal transmucosal resistance of PEN animals was transiently increased ($P = 0.01$) at 48 hours (**Figure 4.8**). PEN did not affect jejunal or colonic transmucosal resistance (data not shown). While teduglutide did not affect potential difference, duodenal potential difference of PEN animals was greater (PEN = 3.40 ± 0.26 versus TPN = 2.38 ± 0.27 mV; $P = 0.007$) than that of TPN animals regardless of time or drug, indicative of increased resistance to charged ions.

Mucosal nutrient transport

Converse to the increases in duodenal, jejunal, and ileal glucose transport observed with teduglutide treatment, PEN administration decreased (PEN = 22.25 ± 11.3 versus TPN = 41.2 ± 24.0 $\mu A/cm^2$; $P = 0.049$) duodenal glucose transport. PEN acutely increased (PEN = 11.3 ± 4.87 versus TPN = 4.9 ± 2.40 $\mu A/cm^2$; $P = 0.047$) colonic glutamine transport at 4 hours, complementing the duodenal and jejunal effects of teduglutide, but PepT1 activity was unaffected by route of nutrient administration (data not shown). Finally, while teduglutide did not affect CCH-mediated secretory response of any intestinal segment, PEN increased (PEN =

39.7 ± 9.5 versus TPN = 15.6 ± 10.4 μA/cm^2; P = 0.001) duodenal CCH-mediated secretion versus TPN regardless of time or drug. A route*drug interaction was noted in that ileal 5-HT-mediated secretion was decreased in TPN animals that received teduglutide (TPN+), and in PEN animals that received vehicle (PEN-), as compared to animals that received TPN and vehicle (TPN-; TPN- = 3.29 ± 1.0, TPN+ = 1.78 ± 1.0, and PEN- = 1.36 ± 1.0 μA/cm^2; P = 0.03).

Question 3: Can distinct temporal markers of adaptation stimulated by these two therapies be identified?

PCA 1

PCA of data from 4-hour piglets was optimized by removing from the analysis the variables that did not load on any factor retained. Redundant variables were also removed as described in the methods. For example, duodenal DNA and protein/DNA concentrations were highly correlated (r = 0.84), and duodenal protein/DNA concentration was used for the final analysis. The final analysis included 24 pigs and 43 variables. Ten retained factors accounted for 79.7% of the total variance. The first four factors are shown in **Table 4.5**, and the remaining factors each explained less than 8.0% of the total variance. Crypt depth of all intestinal segments accounted for the greatest percent of the total variance at 4 hours. The second and third factors were mainly associated with body and individual organ weights. Thus, it appears that teduglutide and/or PEN impacted structural indices to a greater extent than functional indices at 4 hours.

PCA 2

PCA of data from 48-hour piglets was optimized in the same manner as PCA 1. The final analysis included 23 pigs and 52 variables. Ten retained factors accounted for 79.7% of the total

variance. The first three factors are shown in **Table 4.6**. The remaining factors each explained less than 8.0% of the total variance. Structural and functional characteristics of the duodenum, as well as ileal ion and nutrient transport, accounted for the greatest portion of total variance at 48 hours. The second and third factors were mainly associated with jejunal structural and functional characteristics as well as measures of growth. By 48 hours, functional measures of adaptation begin to compliment structural measures.

PCA 3

Following optimization, final PCA of data from 7-day piglets included 24 pigs and 48 variables. Ten retained factors accounted for 80.7% of the total variance. The first four factors are shown in **Table 4.7**. The remaining factors each explained less than 8.0% of the total variance. Crypt depth and PCNA of the small intestine and colonic weight and functional capacity accounted for the greatest percent of the total variance compared with all other factors at 7 days. The second and third factors were mainly associated with small intestinal segment length and ion and nutrient transport. Similar to 48 hours, both structural and functional indices of adaptation appear to be of importance at this chronic time point.

PCA 4

PCA of data from all piglets included 71 pigs and 37 variables after optimization. Eighteen retained factors accounted for 82.1% of the total variance. The first four factors are shown in **Table 4.8**, and the remaining factors each explained less than 5.0% of the total variance. Crypt depth and PCNA in the small intestine accounted for the greatest percent of the total variance in the pooled piglet group. The second, third and fourth factors were mainly

associated with small intestinal segment length and nutrient transport within the jejunum and ileum. Small intestine structural indices and proximal gut ion and nutrient transport accounted for the greatest portion of total variance compared with all other factors in the pooled group of piglets. These variables are indicative of differences in intestinal adaptation between groups, and support the roles of teduglutide and/or PEN in improving intestinal structural and functional indices in this neonatal piglet model of SBS.

DISCUSSION

The objectives of this study were three-fold: (1) to investigate the role of teduglutide in inducing structural and/or functional measures of adaptation in a neonatal piglet model of SBS; (2) to assess complimentary or synergistic effects of combination teduglutide and PEN; and (3) to identify distinct temporal markers of adaptation stimulated by these two therapies. This neonatal piglet model was chosen due to anatomic and physiologic similarities between neonatal piglets and human infants, and because neonatal piglets are a well-characterized model of the PN-fed infant[19,47] that display full clinical intestinal failure symptoms following massive small bowel resection.[46] Nutrition support adequacy of this model was confirmed in that body weight did not differ between treatment groups at any time point, and all piglets gained appropriate weight over time, regardless of treatment.

The interventions utilized in this study were selected due to their efficacy in reducing PN dependence in adult patients, as well as their clinical relevancy to the pediatric population. Though human pediatric teduglutide trials have yet to be completed, one trial of PN-dependent children 1-17 years of age is estimated to be completed in March 2015,[52] and another is currently enrolling patients with SBS of any age to evaluate the long-term safety profile of teduglutide.[53]

Neonatal piglets with 50% small intestinal resection fed TPN for 7 days[43] demonstrated a teduglutide-induced dose-dependent increase in weight per length remnant intestine, as well as increased intestinal protein fractional synthesis rate with the highest (0.2 mg/kg/d) dose versus placebo. However, there were no differences in digestive enzyme activity between groups, indicating that while increasing structural adaptation of the remnant intestine, teduglutide had limited effects on functional endpoints. Given the limited effects of teduglutide on functional indices of adaptation in this model, we investigated complementary and/or synergistic effects induced by combination teduglutide and PEN treatment in stimulation of intestinal adaptation.

In the current study, beneficial effects of teduglutide and PEN administration were similar to those observed in other preclinical trials and adult human studies,[25,26,54-59] and demonstrate complementary, synergistic roles for teduglutide and PEN in treatment of pediatric SBS. Teduglutide and PEN were complementary in anatomical site of action (for example, teduglutide treatment resulted in acute increases in duodenal and jejunal glutamine transport, while PEN acutely increased glutamine transport within the colon), as well as in both structural and functional measures (for example, teduglutide increased colonic wet weight, proliferation, and crypt depth as well as decreased colonic apoptosis, while PEN stimulated an increase in colonic glutamine transport). Furthermore, the greatest clinical gains were observed in the PEN+ group for (1) duodenal and colonic segment length; (2) villus height of all small intestinal segments; (3) crypt depth of the jejunum, ileum, and colon; and (4) ileal proliferation. Coupling these two therapies represents an opportunity to augment intestinal adaptation beyond that of either therapy alone, and potentially accelerate enteral autonomy.

An additional important finding from this study was the establishment of crypt depth, through PCA, as a strong indicator of neonatal intestinal adaptation following resection

regardless of time. As we hypothesized, structural indices preceded functional indices of adaptation, and assessment of crypt depth was identified to be a potential reliable, single measure to assess overall intestinal adaptation in pediatric SBS. Opportunities to assess crypt depth of human patients are limited, but in the event of need for further resection or biopsy, attainment of a sample for assessment of crypt depth may be possible.

Two piglet studies[60,61] have reported a lack of effect of GLP-2 administration on prevention of NEC onset. However, these studies were limited in use of GLP-2 rather than teduglutide and a subjective clinical scoring system. Thus, despite these two studies, there is sufficient preclinical data reported here and elsewhere,[43,62] to support establishment of pediatric clinical trials of teduglutide for treatment, and potentially prevention, of SBS. In planning trials of this vulnerable population, appropriate dosing must be carefully evaluated given that teduglutide is eliminated primarily through glomerular filtration,[63] which may be at various stages of maturation in infants, particularly those born preterm. Furthermore, changes in percentage of total body water may also affect absorption and distribution of teduglutide.[62] Teduglutide did not affect body weight in the current study, but body composition was not assessed and the possibility of teduglutide-induced alterations in body composition cannot be precluded. However, teduglutide treatment significantly improved body weight of PN-dependent adult human subjects versus placebo, and these increases were primarily restricted to changes in lean body mass.[64,65] Thus, in addition to stimulation of intestinal adaptation, teduglutide may also serve to improve protein accretion in the rapidly growing infants. However, due to a paucity of data regarding the pharmacokinetics of teduglutide in pediatric patients, care must be utilized to ensure the minimum dose capable of stimulating adaptation is used.

The data presented here illustrate an important opportunity to improve the care of infants with SBS and increase the efficacy of current treatments to promote enteral autonomy further. This work also directly addresses two of the six research areas recently identified by an American Society for Nutrition working group "whose advancement will have the greatest projected impact on the future health and well-being of global populations."[66] Clearly, teduglutide represents an immense opportunity not only for treatment of pediatric SBS, but also for potential prophylactic acceleration of gut maturation in preterm infants.

FIGURES AND TABLES

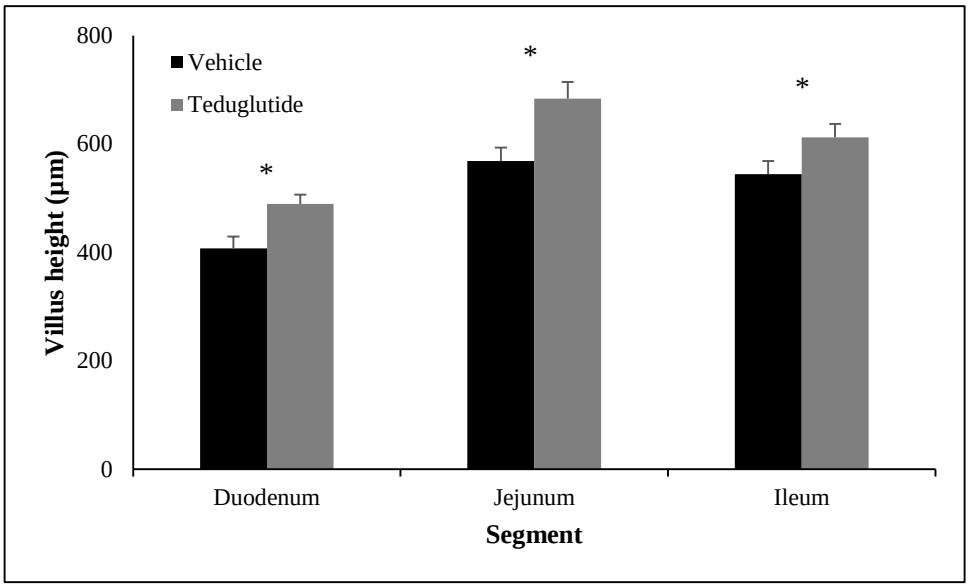


Figure 4.1. Villus height of small intestinal segments of animals treated with teduglutide or vehicle.
Data are expressed as mean (pooled by drug) ± SEM.
*$P < 0.05$ within segment teduglutide versus vehicle.

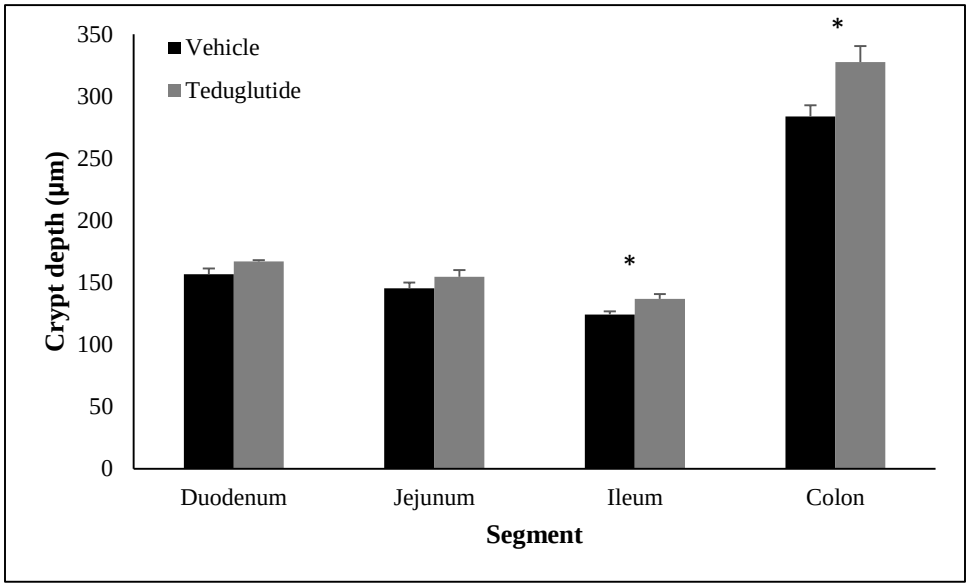


Figure 4.2. Crypt depth of all intestinal segments of animals treated with teduglutide or vehicle. Data are expressed as mean (pooled by drug) ± SEM.
*$P < 0.05$ within segment teduglutide versus vehicle.

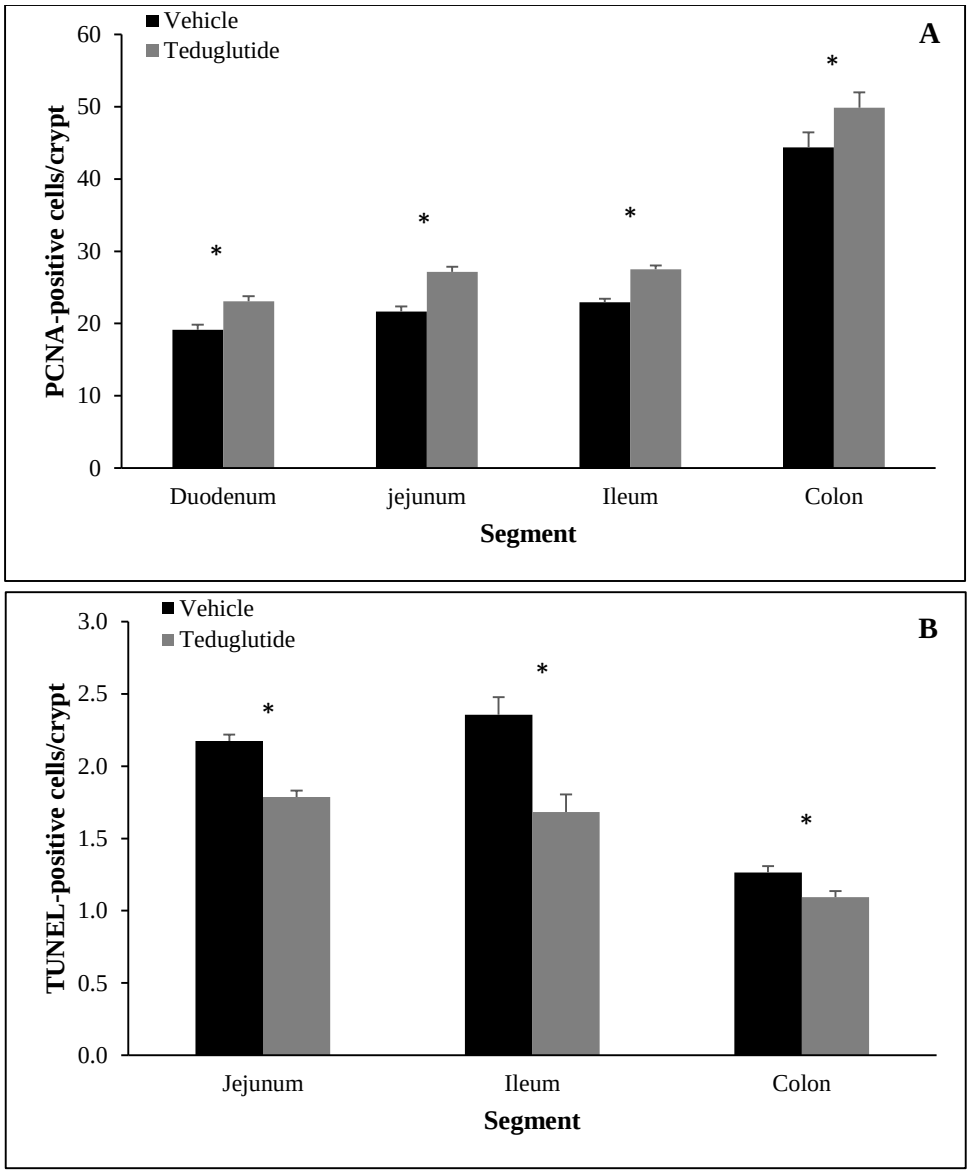


Figure 4.3 Epithelial cell (A) proliferation and (B) apoptosis of animals treated with teduglutide or vehicle.
Data are expressed as mean (pooled by drug) ± SEM.
*****$P < 0.05$ within segment teduglutide versus vehicle.
Abbreviations: PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling**.**

	Vehicle	Teduglutide
Duodenum (μA/cm^2)		
4 hour	22.1 ± 17.0	64.7 ± 31.1*
48 hour	41.5 ± 28.4	12.5 ± 5.9
7 day	26.6 ± 10.6	22.6 ± 7.7
Jejunum (μA/cm^2)		
4 hour	29.6 ± 10.7	47.9 ± 13.0*
48 hour	11.0 ± 15.8	19.2 ± 5.0
7 day	43.5 ± 10.9	23.0 ± 7.2
Ileum (μA/cm^2)		
4 hour	13.6 ± 4.9	26.5 ± 7.9*
48 hour	9.7 ± 5.0	5.2 ± 2.2
7 day	16.2 ± 5.9	14.3 ± 4.5

Table 4.1. Glucose transport within the small intestine of animals treated with teduglutide or vehicle.
Data are expressed as mean (pooled by drug) ± SEM.
*$P < 0.05$ within segment and time, teduglutide versus vehicle.

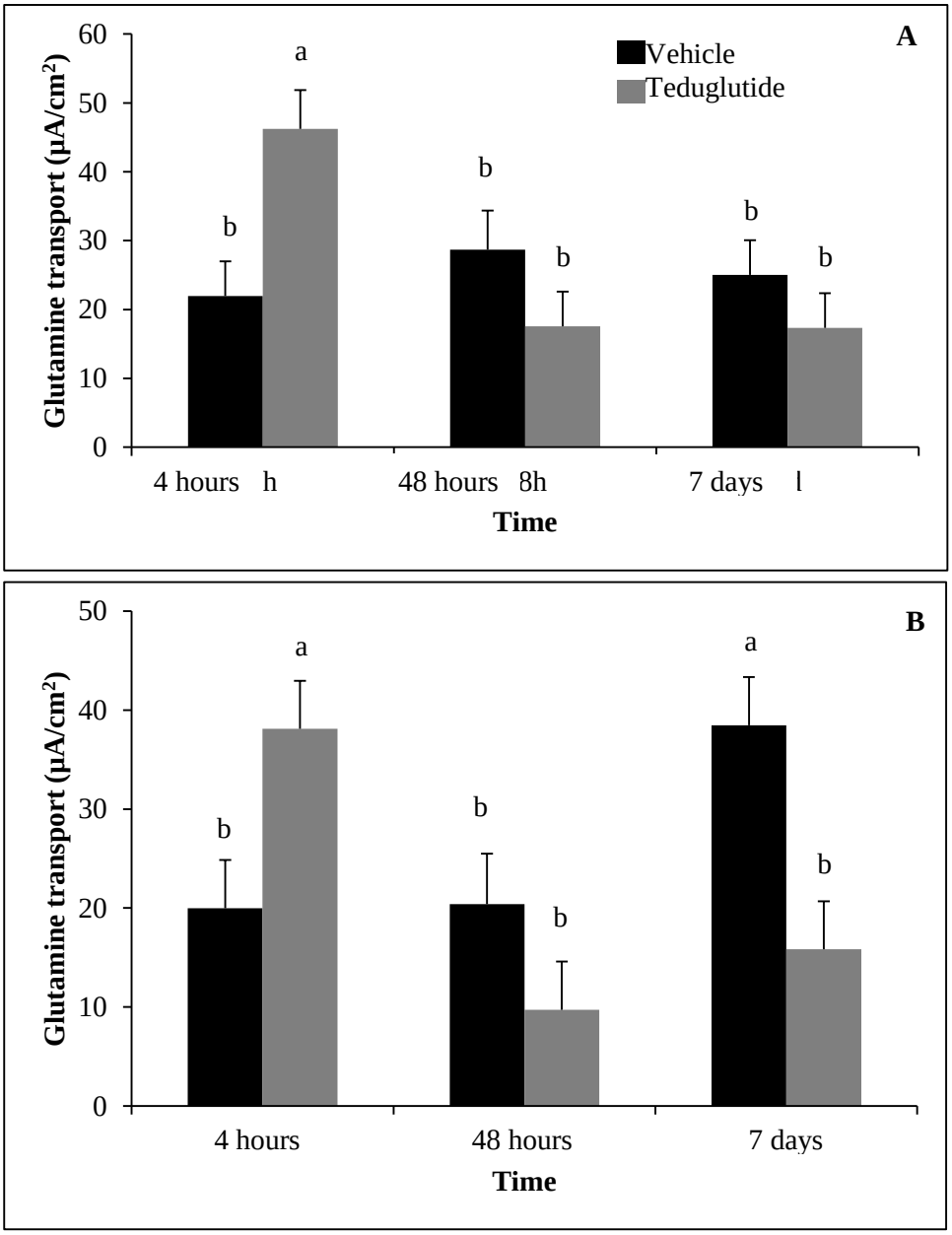


Figure 4.4. Glutamine transport within the (A) duodenum and (B) jejunum of animals treated with teduglutide or vehicle.
Data are expressed as mean (pooled by drug) ± SEM.
Different letters over bars indicate a significant ($P < 0.05$) within-segment difference.

	Vehicle		Teduglutide	
	TPN	**PEN**	**TPN**	**PEN**
Duodenum (µm)				
4 hours	476 ± 32.5	391 ± 42.1	442 ± 18.4	500 ± 48.7
48 hours	409 ± 66.9	447 ± 55.0	487 ± 59.4	515 ± 46.5
7 days	313 ± 29.8	405 ± 81.2	487 ± 38.1	507 ± 44.7
Mean[1]	*407.1 ± 22.4*		*489 ± 16.8**	
Jejunum (µm)^				
4 hours	593 ± 45.1	605 ± 65.1	644 ± 87.2	670 ± 58.0
48 hours	601 ± 38.8	705 ± 42.1	733 ± 42.0	787 ± 70.5
7 days	388 ± 42.1	527 ± 50.6	456 ± 65.5	783 ± 51.1
Mean[1]	*568 ± 25.2*		*683 ± 30.6**	
Ileum (µm)^				
4 hours	470 ± 41.4	479 ± 30.4	520 ± 53.0	545 ± 46.0
48 hours	492 ± 38.4	663 ± 54.9	574 ± 34.4	745 ± 87.5
7 days	518 ± 62.9	636 ± 74.8	651 ± 31.6	660 ± 76.1
Mean[1]	*544 ± 24.2*		*612 ± 24.9**	

Table 4.2. Villus height within small intestine.
Data are expressed as mean ± SEM.
[1]Pooled by drug.
*$P < 0.05$ within-segment, teduglutide versus vehicle.
^$P < 0.05$ within segment, pooled by route of nutrient administration; partial enteral nutrition (PEN) versus total parenteral nutrition (TPN).

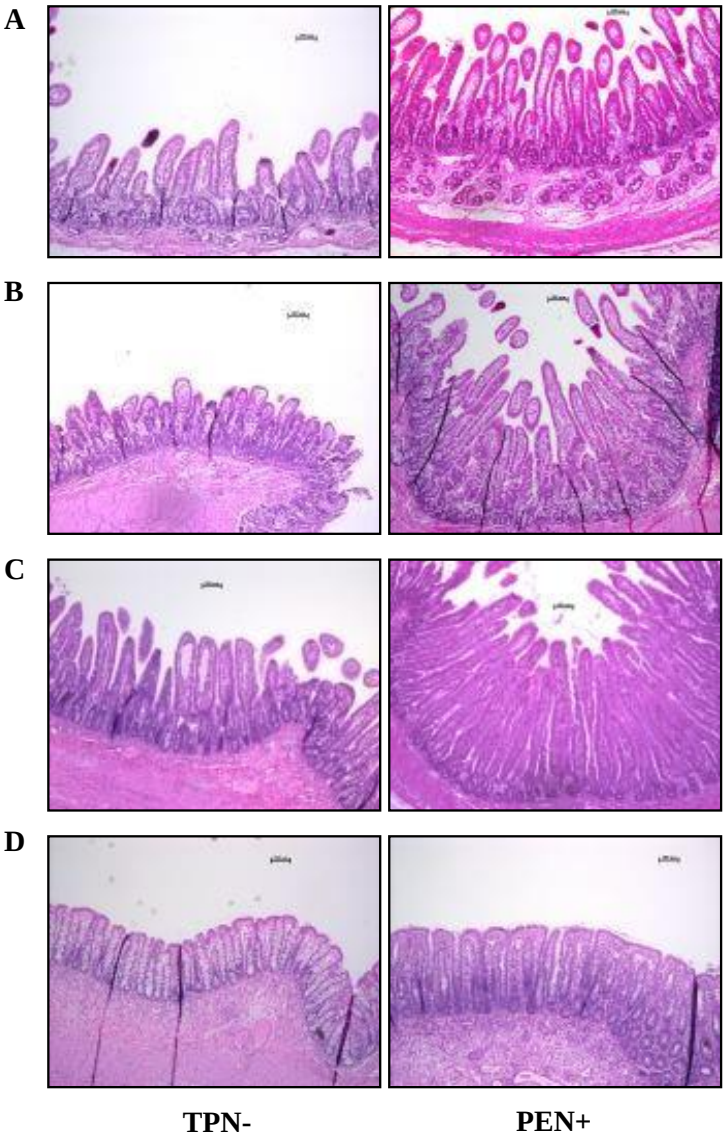


Figure 4.5. Mucosal architecture of the (A) duodenum; (B) jejunum; (C) ileum; (D) colon after 7 days of treatment.
Representative cross-sectional images of 5µm intestinal tissues with hematoxylin and eosin stain at 5× magnification. Scale bars indicate 100 µm.
Columns: left, tissue from piglets receiving TPN and vehicle control (TPN-); right, tissue from piglets receiving PEN and teduglutide (PEN+).

	Vehicle		Teduglutide	
	TPN	**PEN**	**TPN**	**PEN**
Duodenum (µm)				
4 hours	137 ± 9.12	154 ± 6.90	149 ± 11.3	143 ± 9.06
48 hours	154 ± 15.5	156 ± 14.0	161 ± 20.7	157 ± 12.9
7 days	168 ± 11.1	171 ± 8.64	194 ± 10.2	194 ± 10.1
***Mean*[1]**	*157 ± 4.62*		*167 ± 5.94*	
Jejunum (µm)				
4 hours	131 ± 10.0	129 ± 11.1	130 ± 12.2	134 ± 7.42
48 hours	137 ± 10.5	162 ± 16.0	163 ± 17.7	150 ± 10.7
7 days	155 ± 9.34	156 ± 7.84	169 ± 8.81	186 ± 4.81
***Mean*[1]**	*145.38 ± 4.82*		*155 ± 5.47*	
Ileum (µm)^				
4 hours	120 ± 5.57	117 ± 5.62	119 ± 4.64	123 ± 7.91
48 hours	113 ± 3.86	131 ± 3.15	132 ± 5.64	140 ± 7.93
7 days	123 ± 5.13	140 ± 9.83	138 ± 2.81	169 ± 10.2
***Mean*[1]**	*124.26 ± 2.75*		*137 ± 3.84**	
Colon (µm)				
4 hours	278 ± 22.2	274 ± 33.7	294 ± 42.3	333 ± 32.7
48 hours	287 ± 18.2	301 ± 26.3	328 ± 38.1	321 ± 23.5
7 days	264 ± 7.96	299 ± 24.4	339 ± 30.9	347 ± 30.0
***Mean*[1]**	*284 ± 8.99*		*328 ± 12.8**	

Table 4.3. Crypt depth of all intestinal segments.
Data are expressed as means ± SEM.
[1]Pooled by drug.
*$P < 0.05$ within-segment, teduglutide versus vehicle.
^$P < 0.05$ within segment, pooled by route of nutrient administration; partial enteral nutrition (PEN) versus total parenteral nutrition (TPN).

	TPN	PEN	P-value
Duodenum	19.1 ± 0.69	23.1 ± 0.70	< 0.001
Jejunum	22.5 ± 0.73	26.3 ± 0.72	< 0.001
Ileum	23.9 ± 0.52	26.5 ± 0.52	< 0.001
Colon	46.8 ± 2.07	47.5 ± 2.08	0.7369

Table 4.4. Proliferation within all intestinal segments assessed by proliferating cell nuclear antigen- (PCNA) positive cells per crypt.
Data pooled by route of nutrient administration and expressed as mean ± SEM.
Abbreviations: PEN, partial enteral nutrition; TPN, total parenteral nutrition.

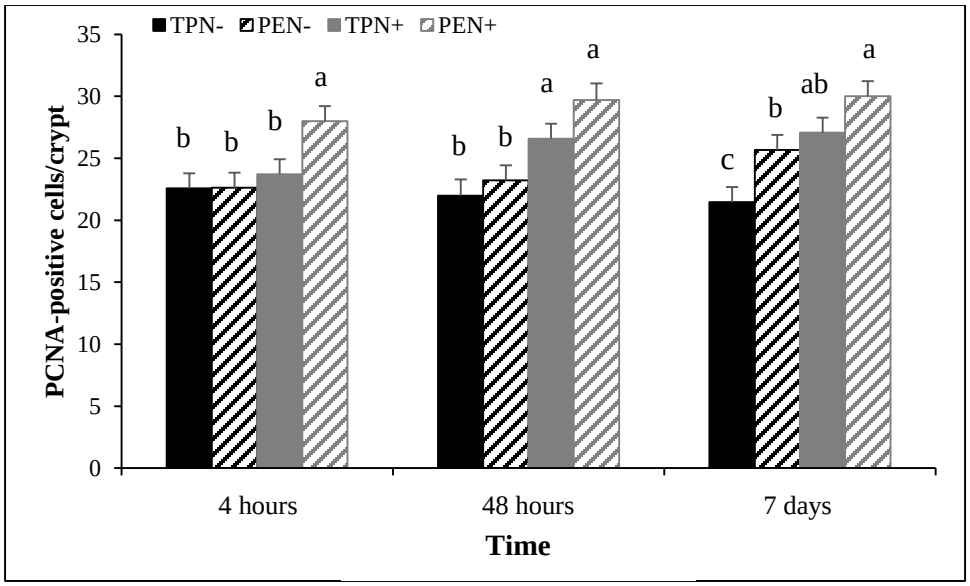
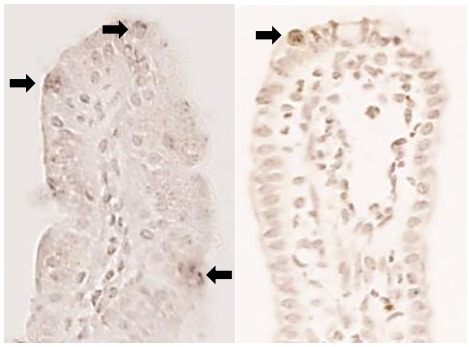


Figure 4.6. Ileal proliferation assessed by proliferating cell nuclear antigen- (PCNA) positive cells per crypt.
Data are expressed as means ± SEM.
Different letters over bars indicate a significant ($P < 0.05$) within-time difference.
Abbreviations: PEN-, animals receiving partial enteral nutrition and vehicle control; PEN+, animals receiving partial enteral nutrition and teduglutide; TPN-, animals receiving total parenteral nutrition and vehicle control TPN+, animals receiving total parenteral nutrition and teduglutide.



TPN- **PEN+**

Figure 4.7. Representative slide of ileal epithelial cell apoptosis at 7 days as measured by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining for DNA fragmentation.
Magnification of 20× with DAB stain. Black arrows indicate apoptotic cells.
Abbreviations: TPN-, total parenteral nutrition vehicle control; PEN+ partial enteral nutrition plus teduglutide.

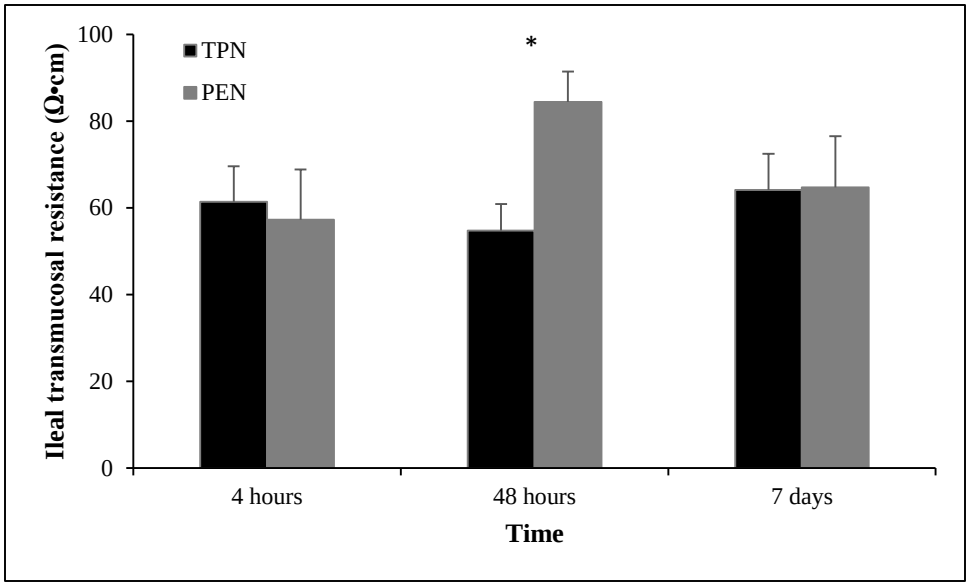


Figure 4.8. Ileal transmucosal resistance of total parenteral nutrition- (TPN) and partial enteral nutrition- (PEN) fed animals.
Data are expressed as means ± SEM.
*$P < 0.05$ within time, PEN versus TPN.

Factor	1	2	3	4
Variance Explained (%)	11.5	10.5	9.1	8.7
Cumulative (%)	11.5	22.0	31.1	39.7
Variable Loading[1]				
Duodenum crypt depth	0.76			
Jejunum crypt depth	0.86			
Ileum crypt depth	0.77			
Colon crypt depth	0.70			
Duodenum PCNA	0.66			
Duodenum protein/DNA	0.55			
Colon conductance	0.52			
Colon CCH	0.57			
Heart weight		0.72		
Spleen weight		0.83		
Pancreas weight		0.62		
Ileum Cdx2		0.58		
Duodenum glucose transport		0.81		
Duodenum short circuit current		0.65		
Final body weight			-0.71	
Ileum length			0.66	
Kidney weight			0.72	
Jejunum villus height			0.76	
Jejunum transmucosal resistance			0.70	
Ileum mucosal mass				0.70
Jejunum PCNA				0.54
Ileum PCNA				0.74
Duodenum CCH				0.60
Jejunum glucose transport				0.66
Jejunum glutamine transport				0.60

[1]Only correlations with $|r| \geq 0.5$ are indicated.
Abbreviations: CCH, carbachol-mediated chloride secretion; PCNA, proliferating cell nuclear antigen.

Table 4.5. Major factors obtained by principal component analysis using varimax rotation with Kaiser normalization of 43 variables characterizing the gut structure and function of 4 hour pigs.

Factor	1	2	3	4
Variance Explained (%)	16.0	9.8	9.4	7.2
Cumulative (%)	16.0	25.7	35.1	42.3
Variable Loading[1]				
Duodenum weight	0.74			
Duodenum length	-0.65			
Ileum DNA	-0.77			
Duodenum mucosal mass	-0.58			
Ileum crypt depth	-0.69			
Duodenum PCNA	-0.63			
Jejunum PCNA	-0.83			
Jejunum TUNEL	0.63			
Colon Cdx2	0.61			
Duodenum glutamine transport	0.73			
Duodenum glycyl-sarcosine transport	0.85			
Duodenum 5-HT	0.55			
Jejunum glycyl-sarcosine transport	0.75			
Ileum glucose transport	0.54			
Ileum glutamine transport	0.75			
Ileum 5-HT	0.69			
Colon short circuit current	-0.50			
Duodenum glucose transport	0.60	0.57		
Jejunum weight		-0.58		
Jejunum length		0.68		
Duodenum conductance		0.80		
Jejunum conductance		0.88		
Jejunum CCH		0.71		
Daily weight gain			0.85	
Final body weight			0.81	
Colon length			-0.81	
Heart weight			-0.79	
Spleen weight			-0.55	
Jejunum Cdx2			0.68	
Duodenum DNA				-0.54
Duodenum crypt depth				0.62
Colon PCNA				0.75
Colon 5HT				0.82
Jejunum crypt depth				0.58

[1]Only correlations with $|r| \geq 0.5$ are indicated.

Abbreviations: 5-HT, serotonin-mediated chloride secretion; CCH, carbachol-mediated chloride secretion; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

Table 4.6. Major factors obtained by principal component analysis using varimax rotation with Kaiser normalization of 52 variables characterizing the gut structure and function of 48 hour pigs.

Factor	1	2	3	4
Variance Explained (%)	11.6	10.6	9.4	8.4
Cumulative (%)	11.6	22.2	31.7	40.0
Variable Loading[1]				
Daily weight gain	0.53			
Colon weight	0.56			
Duodenum villus height	0.76			
Duodenum crypt depth	0.78			
Jejunum crypt depth	0.86			
Colon crypt depth	0.71			
Duodenum PCNA	0.72			
Ileum PCNA	0.76			
Colon conductance	0.62			
Colon CCH	0.54			
Ileum TUNEL	-0.59			
Duodenum length		0.73		
Jejunum length		0.72		
Ileum length		0.85		
Colon length		0.78		
Liver weight		-0.82		
Jejunum mucosal mass		0.56		
Ileum villus height		0.62		
Duodenum glucose transport			0.74	
Jejunum conductance			0.62	
Jejunum glutamine transport			0.72	
Jejunum glycyl-sarcosine transport			0.87	
Ileum glutamine transport			0.62	
Ileum glycyl-sarcosine transport			0.83	
Ileum 5-HT				0.86
Ileum CCH				0.89
Colon glucose transport				0.92
Colon glutamine transport				0.62
Jejunum DNA				-0.53
Colon 5-HT				0.64

[1]Only correlations with $|r| \geq 0.5$ are indicated.

Abbreviations: 5-HT, serotonin-mediated chloride secretion; CCH, carbachol-mediated chloride secretion; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

Table 4.7. Major factors obtained by principal component analysis using varimax rotation with Kaiser normalization of 48 variables characterizing the gut structure and function of 7 day pigs.

Factor	1	2	3	4
Variance Explained (%)	8.7	7.6	5.5	5.0
Cumulative (%)	8.7	16.3	21.8	26.8
Variable Loading[1]				
Duodenum crypt depth	0.53			
Ileum crypt depth	0.71			
Duodenum PCNA	0.74			
Jejunum PCNA	0.81			
Ileum PCNA	0.75			
Ileum TUNEL	-0.74			
Final weight		-0.67		
Duodenum length		0.76		
Jejunum length		0.74		
Ileum length		0.79		
Duodenum mucosal mass		0.55		
Jejunum conductance			0.85	
Jejunum glutamine transport			0.81	
Ileum 5-HT				0.88
Ileum CCH				0.91

[1]Only correlations with $|r| \geq 0.5$ are indicated.
Abbreviations: 5-HT, serotonin-mediated chloride secretion; CCH, carbachol-mediated chloride secretion; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

Table 4.8. Major factors obtained by principal component analysis using varimax rotation with Kaiser normalization of 37 variables characterizing the gut structure and function of all pooled piglets.

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CHAPTER 5

A NOVEL NEONATAL FEEDING INTOLERANCE AND NECROTIZING ENTEROCOLITIS RISK SCORING TOOL IS EASY TO USE AND VALUED BY NURSING STAFF

ABSTRACT

Background: Preterm infants are at increased risk of developing feeding intolerance and necrotizing enterocolitis (NEC). Comprehensive, targeted nursing assessments can evaluate the risk for and identify early signs of these conditions in an effort to prevent their destructive sequela. Purpose: Develop an easy to use scoring tool valued by nurses to predict infant feeding intolerance and necrotizing enterocolitis risk. Methods: A novel risk scoring nursing tool was implemented in the University of Illinois-affiliated Carle Foundation Hospital (CFH) 48-bed level III neonatal intensive care unit (NICU). Data was collected from all preterm infants with parental consent during the initial 6 month study period. Scoring accuracy, ease of use, and nurses' attitudes toward the tool were assessed at the study site and by evaluators at a national neonatal nursing conference. Results: Fourteen nurses scored 166 tools on 62 infants. Sixteen tools (9.6%) contained errors. Mean study site tool ease of use was 8.1 (SD 2.2) on a 10-point scale. Ninety percent of conference evaluators agreed/strongly agreed that the tool addressed important knowledge gaps. Implications for Practice: The tool is easy to use and valued by nurses. Widespread implementation is expected to be a clinically feasible means to improve infant clinical outcomes for minimal time and financial cost. Implications for Research: Tool validation and refinement based on nursing feedback will improve its broad applicability and predictive utility.

WHAT THIS STUDY ADDS

- Identifies clinical factors nursing staff can evaluate relevant to feeding intolerance and NEC.
- Establishes the acceptability and feasibility of implementation of a novel nursing tool to assess neonatal feeding intolerance and NEC risk.
- Provides clear next steps for tool improvement and validation.

INTRODUCTION

In 2013, 11.4% of births in the United States occurred preterm.[1] Due to the immaturity of the gastrointestinal tract, these infants are at increased risk of developing feeding intolerance and necrotizing enterocolitis (NEC). NEC is the most common surgical emergency among infants and proves fatal for 25-33% of infants diagnosed with the disease.[2,3] NEC is also the second leading cause of morbidity in preterm infants, including both short- and long-term gastrointestinal complications as well as impaired neurodevelopment.[4] Total annual cost of care for infants with NEC in the United States is between $500 million and $1 billion.[5] The multi-factorial, fulminant nature of NEC makes medical and/or surgical management difficult, so efforts aimed at prevention and early detection, rather than development and application of new treatments following full disease onset, will be most effective in reducing infant morbidity and mortality.[5] Furthermore, because NEC is difficult to diagnose, nursing assessments provide assistance in identifying early signs of feeding intolerance and NEC.[6] Consistency in nursing assessments and early interventions could lead to better clinical outcomes for these vulnerable patients.

Although the substantial potential for sensitive and specific NEC risk assessment and early detection to improve patient outcomes has been broadly recognized,[5,7,8] no approach to date

has effectively addressed this issue. Numerous previous investigations have attempted to develop prediction models based on individual clinical findings such as gestational age at birth,[9] birth weight,[10] feeding practices,[11,12] or antibiotic administration,[13] and a comprehensive NEC risk score was developed in 1985,[14] but later demonstrated to lack validity.[7] A new integrated risk tool built through expert consensus and statistical modeling is currently undergoing direct clinical testing,[15-17] but this recent effort has not assessed the crucial aspect of feasibility of nursing implementation. Furthermore, as retrospective data may be miscoded, of low quality, or altogether missing,[16] the predictive ability of risk scoring tools should be prospectively validated.[18] Given the limitations of previous work in this area, the overall goal of this work is to develop a prospectively validated, evidence-based, simple to use bedside tool valued by nurses to predict infant feeding intolerance and NEC risk. The objective of the pilot phase presented here is to assess tool ease of use and nurses' attitudes toward the tool since a tool that is difficult to use or interpret will be of little value in a busy neonatal intensive care unit (NICU). Based on extensive literature review of factors pertinent to NEC development,[19,20] as well as the success of similarly designed nursing tools,[21,22] we hypothesized that nurses will value and find the tool easy to use.

METHODS

Construction of the feeding intolerance and necrotizing enterocolitis risk scoring tool

Multiple databases, including Medline and Embase, were searched using predefined search criteria to identify factors relevant to development of feeding intolerance and NEC in

preterm infants. Five categories of germane variables were identified, including gestational age at birth, birth weight, infant feeding substance, postnatal infant factors, and perinatal maternal factors. These five categories of variables were populated with multiple risk factors, each assigned a numeric point value ranging from 1-3. Point values were assigned based on the level of evidence available to support inclusion in the tool. The numeric score generated upon tool completion, ranging from 1-44, reflects the sum of points from each risk factor. A score of 1-5 places infants in the low risk category, 6-8 in the moderate risk category, and 9 or more in the high risk category. The tool (**Table 5.1**) was modeled after existing nursing tools[21,22] in an effort to enhance familiarity and ensure its wide acceptance. It was designed to be utilized at admission and weekly until discharge.

Tool pilot and formal testing

A paper-based version of the tool was pilot tested as part of a nursing protocol improvement project in the University of Illinois-affiliated Carle Foundation Hospital (CFH) 48-bed level III NICU, which provides complete care for infants born ≥ 22 weeks gestation. The tool was utilized by both day and night shift bedside nurses with 2-20 years of nursing experience. Following pilot testing, an electronic version of the tool was integrated into the electronic medical records (EMR) system. Preterm infants (gestational age at birth < 37 weeks) admitted during the initial 6 month study period with parental consent were followed throughout their NICU hospitalization. Data was collected from the EMR by nursing and protocol staff. Feasibility of tool use was evaluated by scoring accuracy of all tools completed during the initial study period. Anonymous, electronically-administered surveys were conducted at CFH and following tool presentation at the 2013 National Association of Neonatal Nurses (NANN)

conference to assess tool ease of use and nurses' attitudes toward the tool. The study was approved by the CFH and University of Illinois Institutional Review Boards (IRB).

RESULTS

Pilot testing

A total of 188 paper-based tools, scoring 72 infants, were completed during the pilot study. Infant risk category (low, moderate, or high) was accurately determined in 94.7% of the completed tools, but errors in total point value determinations were frequent. Thus, the electronic, EMR-integrated version of the tool was created which includes drop-down menus, check boxes, and automatic score totaling.

Formal Testing

Study subjects

Sixty-three infants were enrolled in the initial 6 month study period, including 9 pairs of twins and 2 sets of triplets. Gestational age at birth ranged from 22 weeks and 6 days to 36 weeks and 5 days. Twelve (19.0%) infants were normal birth weight, 25 (39.7%) low birth weight, 19 (30.2%) very low birth weight, and 7 (11.1%) extremely low birth weight. Thirty-nine (61.9%) were male. Fourteen nurses scored a total of 166 tools in the initial 6 month study period. Excluding tools scored by the protocol nurse (124 tools on 57 infants), nurses completed an average of 3.32 tools on 2.77 infants. Thirteen (7.8%) tools classified the infant as low risk, 25 (15.1%) as moderate risk, and 128 (77.1%) as high risk.

Tool implementation at Carle Foundation Hospital

Sixteen errors were made in the 166 scored tools, but as the number of tools scored by a particular nurse increased, the proportion of tools containing an error decreased. CHF survey respondents (n = 28) had < 1 to > 15 years of nursing experience, and the highest degree of education attained ranged from an associate degree to a master of science or master of science in nursing (**Table 5.2**). The mean ease of use ranking on a scale from 1 (very hard) to 10 (very easy), was 8.1 (SD 2.2). Twenty-one (75.0%) nurses ranked the ease of use as 8 or higher (**Table 5.2**). All 28 (100%) respondents replied "yes" when asked if the tool raised their awareness of factors contributing to feeding intolerance and NEC development. All 28 (100%) also responded "yes" when asked if they believe the tool accurately identifies babies at risk of developing feeding intolerance or NEC. When asked whether knowing a baby's risk (low, moderate, or high) provides better information to care for the baby, 20 (71.4%) nurses stated that it does. One (3.6%) said that it sometimes does, and commented that the care an infant receives is determined primarily by the provider on duty. Seven (25.0%) nurses responded negatively, of which 3 noted that that NICU nurses are highly vigilant and monitor babies closely regardless of the tool's risk determination.

NANN evaluator assessment of tool

More than 80% of surveyed NANN evaluators (**Table 5.3**) work in neonatal care full-time, and 71% have been in neonatal care ≥ 11 years and are certified in a specialty. Seventy-nine percent practice in a level III NICU, and 31% are advanced practice nurses, having post-graduate nursing education. Mean response when asked, on a scale of 1 (strongly disagree) to 5 (strongly agree), whether the tool addresses important gaps in knowledge in the field (n = 109) was 4.45 (SD 0.75; **Table 5.3**). Ninety-eight (89.9%) evaluators agreed or strongly agreed.

Eighty-eight (53.0%) of 166 evaluators thought the session provided information that would change the clinical care they provide, and 15 respondents commented that they would like to implement the tool at their institution. When asked if they would attempt to utilize session information to implement changes in their competence, performance, and/or patients' outcomes (n = 146), 88 (60.3%) evaluators indicated that they would, with 27 commenting the information would be used for staff education at their institution. Twelve evaluators commented that the tool did not include any new information or practices. Two evaluators stated that the tool may be useful for new nurses, but not advanced practice nurses or those with many years of experience, and 3 noted that the tool requires validation.

DISCUSSION

Accurately representing the factors that contribute to feeding intolerance and NEC on a comprehensive, practical nursing tool has the potential to dramatically improve infant outcomes through earlier, individually-tailored treatment. However in order to be effective, the tool must be perceived as valuable by nurses, and be as simple, consistent, and objective as possible since a tool that is difficult to use or interpret will be of little value in a busy NICU. Previous and other ongoing studies have not addressed this facet of tool development, and this is a strength of the novel tool presented here.

Both the CFH and NANN nursing surveys demonstrate the tool's importance to the field of neonatal nursing, nurses' positive attitudes toward, and willingness to utilize, the scoring tool. These results are tremendously encouraging in that nurses are well-versed in the use of the EMR system, and the potential to positively impact infant clinical outcomes through tool use is substantial compared to the minimal nursing effort required to complete the tool. However, the

results presented here also highlight the opportunity for further research regarding tool improvement. Three of 5 CFH nurses who ranked the tool's ease of use as ≤ 5 had an associate's degree as their highest level of education. While this low ease of use ranking may simply be due to a lesser degree of familiarity with the tool, it also suggests the possibility that tool training should be tailored to education level. This possibility is reinforced by the comment regarding insufficient training noted by the nurse who ranked the tool's ease of use as a 2. Additionally, 5 nurses commented that finding perinatal maternal factors can be difficult, so further training on this issue is currently being implemented.

In addition to tailored/further training, the tool is currently undergoing validation of included risk factors, and point values assigned to those factors. Assessment of multiple measures of consistency and reliability, as well as construct validity, sensitivity, specificity, and positive and negative predictive values is ongoing. Optimal risk category cut off ranges, which were initially assigned arbitrarily, are also being optimized to better correlate with infant outcomes. Tool implementation is demonstrated here to be feasible at a single institution, but future work following validation may involve implementation at additional institutions, and potential inclusion of an institutional NEC risk factor.[15]

These ongoing and future steps will be crucial in ensuring a broadly applicable risk scoring tool that can maximally improve infant outcomes for minimal time and financial cost. Validation and further development of this tool has potentially wide-reaching implications for practice including the ability to (1) provide NICU doctors and nurses with a practical, objective means by which to assess infant risk of feeding intolerance and NEC, (2) better know when, and for which infants, to institute preventative measures at the earliest possible time so that the destructive sequela of NEC can be avoided, (3) save resources through targeted personalized

medicine, and (4) ultimately utilize this tool as a validated screening device for future research focused on development and implementation of new feeding intolerance and NEC interventions or to validate potential biomarkers of NEC.

SUMMARY OF RECOMMENDATIONS FOR PRACTICE AND RESEARCH

What we know

- Comprehensive nursing assessments have the potential to dramatically improve infant outcomes through early identification of signs of feeding intolerance and NEC.
- Previous and other ongoing efforts to develop NEC risk scoring tools have not assessed the crucial aspect of feasibility of nursing implementation.
- Nurses are willing to use and have positive attitudes toward the tool presented here.

What needs to be studied

- Optimization of training regarding tool use.
- Validation of factors included in the tool and point values assigned to those factors in order to maximize tool sensitivity and specificity.

What we can do today

- Become familiar with the clinical characteristics of feeding intolerance and NEC, and encourage vigilance of its signs among all neonatal nurses.
- Improve tool ease of use based on the nursing feedback presented here.

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FIGURES AND TABLES

Points	Variable Category and Risk Factors
	Gestational Age at Birth (select one)
1	32 - 36 6/7 weeks (preterm)
2	28 - 31 6/7 weeks (very preterm)
3	< 28 weeks (extremely preterm)
	Birth Weight (select one)
0	≥ 2500 g
1	1500 - 2499 g (low birth weight)
2	1000 - 1499 g (very low birth weight)
3	< 1000 g (extremely low birth weight)
	Feeding Substance (select all that apply)
0	Mother's own milk
1	Donor breast milk
1	Bovine human milk fortifier
3	Bovine-based formula
	Postnatal Infant Factors (select all that apply)
1	Red blood cell transfusion
1	Congenital heart disease or patent ductus arteriosis
2	Polycythemia (hematocrit > 60)
2	Respiratory distress (> 24 hours assisted ventilation)
3	Hypoxia/asphyxia at birth
3	Sepsis
3	Antibiotics for ≥ 5 days
3	Intrauterine growth restriction or small for gestational age
	Perinatal Maternal Factors (select all that apply)
1	Cigarette use during pregnancy
2	Placenta abruption
2	Clinical chorioamnionitis
2	Illicit drug use during pregnancy
2	Preterm premature rupture of membranes
2	Prolonged rupture of membranes (≥ 18 hours)
3	Incomplete or no antenatal glucocorticoid therapy
3	Absent or reversed end diastolic flow to infant

Table 5.1. Feeding intolerance and necrotizing enterocolitis risk scoring tool.[10,23-26] Scoring risk ranges: 1-5, low; 6-8, moderate; ≥ 9, high.

CFH respondent demographics (n = 28)	Response n	%
Years of nursing experience		
0-5	5	17.9
6-10	11	39.3
11-15	4	14.3
> 15	8	28.6
Highest degree of education completed		
Associate	7	25.0
BS or BSN	16	57.1
MS or MSN	2	7.1
Nursing school diploma	3	10.7

CFH survey question	Response n	%	Comments (n)
Rank the ease of use of the tool.			
1 (very difficult)	0	0	
2	1	3.6	Received insufficient training on tool use (1)
3	1	3.6	
4	0	0	
5	3	10.7	Can be difficult to find maternal factors (2)
6	0	0	
7	2	7.1	
8	6	21.4	Can be difficult to find maternal factors (2)
9	6	21.4	Can be difficult to find maternal factors (1)
10 (very easy)	9	32.1	
Mean (SD)	*8.1 (2.2)*		
Has the tool raised your awareness of risk factors that contribute to feeding intolerance and NEC?			
Yes	28	100	
No	0	0	
Do you believe the tool accurately identifies babies at risk of developing feeding intolerance or NEC?			
Yes	28	100	
No	0	0	
Does knowing a baby's risk provide you with better information to care for the baby?			
Yes	20	71.4	
No	7	25.0	Monitor infants closely regardless of score (3)
Sometimes	1	3.6	Infant care dependent on provider (1)

Table 5.2. Carle Foundation Hospital (CFH) survey respondent demographics and results. Abbreviations: BS, bachelor of science; BSN, bachelor of science in nursing; MS, master of science; MSN, master of science in nursing; SD, standard deviation.

NANN Evaluator Demographics	%
Work full time in neonatal care	80
Work in level III NICU	79
In neonatal care ≥ 11 years and certified in a specialty	71
Advanced practice (post-graduate education)	31

	Response		
NANN Survey Question	**n**	**%**	**Comments (n)**
The session addressed important gaps in knowledge in the field. (n = 109)			
1 (strongly disagree)	0	0	
2	3	2.6	
3	8	7.3	
4	35	32.1	
5 (strongly agree)	63	57.8	
Mean (SD)	*4.45 (0.75)*		
Did the session provide information that will change the clinical care you provide? (n = 166)			
Yes	88	53.0	Like to implement tool at home institution (15) Think tool is helpful (14)
No	78	47.0	
Will you attempt to address these changes in order to implement changes in your competence, performance, and/or patients' outcomes? (n = 146)			
Yes	88	60.3	Use tool for staff education (27) Aid critical thinking (1) Guide own further research (1)
No	58	39.7	No new information/practices (12) Useful for novice, but not experienced or advanced practice nurse (2) Tool requires validation (3)

Table 5.3. National Association of Neonatal Nurses (NANN) evaluator demographics and survey results. Abbreviations: NICU, neonatal intensive care unit; SD, standard deviation.

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CHAPTER 6

DISCRIMINATION OF FEEDING INTOLERANCE AND NECROTIZING ENTEROCOLITIS RISK IN THE PRETERM INFANT IS POSSIBLE USING A NOVEL RISK SCORING TOOL

ABSTRACT

The etiologies of feeding intolerance and necrotizing enterocolitis (NEC) are complex, and accurately representing the variables that contribute to these conditions on a single, practical risk scoring tool has the potential to dramatically improve infant outcomes through earlier, individually-tailored treatment. Preliminary results (Chapter 5) demonstrate nurses' positive attitudes toward a novel neonatal feeding intolerance and risk scoring tool as well as general ease of use. The objective of this work was to simplify the tool, reevaluate its clinical utility, and assess its accuracy, consistency, inter-rater reliability, and validity. Methods: Anonymous, electronically-administered surveys were used to assess study site nurses' attitudes toward the tool, as well as its estimated completion time. Tool error rate and consistency (Cronbach's alpha) were calculated, and inter-rater reliability was assessed by the intraclass correlation coefficient (ICC) and Fleiss' kappa. Risk factors significant to the development of feeding intolerance (days with emesis, abdominal distention, or gastric residuals > 50% of previous feeding volume) and NEC were identified through chi-square testing. Tool discrimination for each of the four outcomes was evaluated using a receiver operating characteristic (ROC) curve. Construct validity of each variable category included on the tool was assessed using Pearson's correlation coefficient and independent t-test. The tool was also compared to another published NEC risk scoring tool,[1] GutCheck^{NEC}, using Pearson correlation coefficients and independent t-test. Following these assessments, the tool was optimized and its predictive and construct validity reassessed. Results: Mean ease of use on a scale from 1 (very difficult) to 10 (very easy) was 6.9

(SD 1.9). Mean time to complete the tool was 4.2 minutes (range: 1-10 minutes). Error rate (9.2%), Cronbach's alpha (0.71), ICC (0.99), and Fleiss' kappa (1.00) were in acceptable ranges. Gestational age at birth, hypoxia/asphyxia at birth, red blood cell (RBC) transfusion, and congenital heart disease/patent ductus arteriosis (PDA) were significantly associated with all four outcome measures. Total optimized tool score was also associated with all 4 outcome measures (area under the ROC curve (AUC) and diagnostic odds ratio (OR) estimates [95% CI]: emesis, AUC = 0.69 and OR = 1.14 [1.06, 1.23]; abdominal distention, AUC = 0.82 and OR = 1.28 [1.18, 1.41]; gastric residuals > 50% previous feeding volume, AUC = 0.64 and OR = 1.11 [1.04, 1.20]; NEC, AUC = 0.90 and OR = 1.29 [1.12, 1.56]). Pearson correlation coefficient for the optimized tool and GutCheck^{NEC} was 0.82 ($P < 0.001$), and similar correlation coefficients were demonstrated for the two tools for each of the four outcome measures. Scores of infants who did and did not develop each of the outcome measures were significantly different using both the optimized tool and GutCheck^{NEC}. Conclusions: The tool represents a clinically feasible means to discriminate infants at risk of feeding intolerance and NEC. Further refinement will improve its clinical utility and allow for implementation at additional institutions.

INTRODUCTION

Necrotizing enterocolitis (NEC) is a progressive disease in which general feeding intolerance and other nonspecific signs may present in advance of gastrointestinal signs.[2,3] These signs are not limited to, but commonly include, intrauterine growth restriction (IUGR) or low birth weight,[4-6] prolonged empiric antibiotic administration,[7,8] maternal cocaine use,[9,10] chorioamnionitis,[11,12] formula feeding,[13,14] red blood cell (RBC) transfusion,[15,16] and prematurity.[17] Strong nursing assessment skills and an integrated understanding of how

combination of these individual risk factors contribute to NEC may allow for heightened vigilance and early NEC detection. Early detection may in turn prevent infant mortality as evidenced by a cohort study in which infants who died of NEC were diagnosed an average of 3 day-of-life days later than those who survived.[3] Nurses are instrumental in detecting and communicating early signs of NEC,[18] and a standardized means by which to assess and communicate this risk may ensure application of timely, targeted interventions.

Early detection of NEC is complicated not only by its multifactorial nature, but also because information germane to its development is often found in multiple, disparate places within the electronic medical record (EMR), making integration of this information difficult. Use of a composite risk score may facilitate meaningful assimilation of this information by the clinician[19] in much the same was as an Apgar score has been used for decades as a concise index of early neonatal clinical condition.

The first NEC risk prediction score was developed 20 years ago based on a retrospective cohort of 29 infants in a single center.[20] However, using a single center case-control design, this method later resulted in scores of NEC-free infants that were higher (indicative of increased risk) than those of infants that ultimately developed NEC.[20,21] More recently, a new risk index (GutCheck^{NEC}) was developed based on evidence synthesis, expert consensus, and statistical modeling.[1] Though prospective clinical testing of GutCheck^{NEC} is underway, it is limited in that its validation was completed via retrospective database analysis, and because it has not yet addressed the crucial factor of nursing acceptance and feasibility of use.

The overall goal of this work is to develop a prospectively validated, evidence-based, simple to use bedside nursing tool to predict preterm infant feeding intolerance and NEC risk. Pilot results (Chapter 5) identified clinical factors nursing staff could evaluate relevant to feeding

intolerance and NEC development, and established the feasibility of implementation of this novel nursing tool. The pilot study also provided clear next steps for tool improvement and validation, which are addressed here.

METHODS

Tool development, subjects, and study site

A novel neonatal feeding intolerance and NEC risk scoring tool was developed and implemented in the University of Illinois-affiliated Carle Foundation Hospital (CFH) 48-bed level III neonatal intensive care unit (NICU) as previously described (Chapter 5). The study was approved by the CFH and University of Illinois Institutional Review Boards. Following this pilot study, infant enrollment and nursing tool use continued as previously described, and the following changes were made to the tool.

Nil per os (NPO) and total parenteral nutrition (TPN) were added to the feeding category for 1 point each. These options previously did not appear within the tool, leading to confusion of how to categorize feeding of infants that were receiving TPN or were NPO. Additionally, the RBC transfusion risk factor was assigned 2 points rather than 1, as recommended by the study site neonatologists and as evidenced in the recent literature.[15] The tool was also relocated from its own flow sheet within the EMR to the Vitals flow sheet. This sheet is frequently used, reducing the need to toggle back and forth to additional flow sheets or areas within the chart. An attempt was also made to split the tool into "static" and "dynamic" factors so that static factors which remain constant over time (for example, birth weight and gestational age at birth) could be carried forward and automatically populated each time the tool was completed for a given infant.

However, this was impossible due to limitations in EMR coding and organization. Additional training on tool use was also provided to nurses as part of a mandatory EMR upgrade information session.

Outcome measures

Feeding intolerance was defined as an inability to digest enteral feedings due to ineffective or uncoordinated bowel activity[22] as evidenced by (1) abdominal distension and/or emesis, (2) gastric residual volumes greater than 50% of previous feeding volume, or (3) a disruption in the feeding plan.[23] Using this definition, outcome measures included the number of days with emesis, abdominal distention, and gastric residuals > 50% of previous feeding volume. Number of days, rather than volume of emesis or degree of abdominal distention was used since individual nursing assessments of staged photos of an infant's abdomen and emesis amounts vary widely.[24] All enrolled infants experienced at least one disruption in feeding plan (ICD-9 779.31 and 787.3).[25] Thus, this was not utilized as an outcome measure. Diagnosis of NEC (ICD-9 557.0, 777.50, 777.51, 777.52, or 777.53)[25] was also used as an outcome measure.

Feasibility of nursing use

As follow-up to the initial nursing survey conducted during the pilot study (Chapter 5), a second electronically-administered anonymous survey of study site nurses to re-assess tool feasibility of use was conducted. The survey was approved by both the CFH and University of Illinois Institutional Review Boards, and responses were voluntary.

Tool error rate and scoring consistency

Tool error rate was calculated, and scoring consistency of variable categories (gestational age at birth, birth weight, feeding, maternal factors, and infant factors) included on the tool were assessed using Cronbach's alpha.[26]

Inter-rater reliability

Inter-rater reliability of both total tool score and risk category determination (high, moderate, or low) was evaluated with the intraclass correlation coefficient (ICC) and Fleiss' kappa, respectively.[27] A subset of 147 tools completed by 38 nurses was used to calculate the ICC and Fleiss' kappa to allow for examination of equivalence of ratings obtained by distinct raters of given infant on a given day, since infant scores may change over time.

Identification of risk factors significant to feeding intolerance and NEC

Individual risk factors included in the tool significant to the development of each of the four outcomes measures were identified by chi-square testing. Factors common to the development of all four outcome measures were identified as underlying common factors relevant to the development of feeding intolerance and NEC.

Predictive validity

Logistic regression was conducted for each of the four outcome measures against total tool score. Tool discrimination was tested using receiver operating characteristic (ROC) curves for each of the four outcome measures using the median value of each outcome as respective cut-

points. The diagnostic odds ratio (OR) for each of the four outcome measures was also determined.

Construct validity and comparison with GutCheck^{NEC}

Construct validity was evaluated by independent t-test to determine whether a difference existed between mean scores of infants who did and did not ultimately develop each of the four outcome measures. As GutCheck^{NEC} contains overlapping, but also unique risk factors, GutCheck^{NEC} scores were calculated for all infants enrolled in this study. Independent t-test was similarly used to determine whether a difference existed between mean GutCheck^{NEC} scores of enrolled infants who did and did not ultimately develop each of the four outcome measures. Median values of each outcome were used as respective cut-points. Overall correlation between the two tools, as well as the correlation of the total score generated with each tool to each of the four outcome measures, was assessed with the Pearson correlation coefficient.

Tool optimization

The tool was optimized by removing factors which were not significant to the development of at least two of the three feeding intolerance outcomes, or to the development of NEC. Additional factors suggested in the literature to be significant to feeding intolerance and/or NEC development were also evaluated. This included gender, race, cesarean section versus vaginal delivery, singleton versus multiple gestation, outborn versus inborn, Apgar scores at one and five minutes, metabolic acidosis, hypotension treated with inotropic medication, placental abnormality, and maternal hypertension. If a significant correlation ($P < 0.05$) existed between pairs of factors assessing similar constructs, only the factor significant to the development of a

greater number of the four outcomes was retained. Factors were assigned 1 point for each of the four outcome measures they were significant to the development of. The tool was reassessed for predictive and construct validity as described above. Validation was also utilized to determine a single "at risk" threshold, simplifying the risk category determination to either "at risk" or "not at risk." All statistical procedures were completed using SAS (Version 9.4; SAS Institute, Cary, NC).

RESULTS

Tool development and study subjects

The modified tool is shown in **Table 6.1**. From August 2013 to December 2014, 49 nurses scored 499 tools on the 133 enrolled infants. Gestational age at birth of enrolled infants ranged from 22 weeks and 6 days to 36 weeks and 6 days, and included extremely low, very low, low, and normal birth weight infants (**Figure 6.1**). Eighteen 18 pairs of twins, 2 sets of triplets, and 89 singleton infants were enrolled. Seventy-six (57.1%) were male.

Feasibility of nursing use

Of 42 nurses who responded to the survey, 60.5% had ≥ 6 years of nursing experience. 73.8% held a Bachelor of Science or Bachelor of Science in Nursing, 23.8% an Associate degree, and 2.4% a nursing school diploma. Survey results are shown in **Table 6.2**. Briefly, 83.3% of nurses surveyed had used the tool at least six times. Ease of use ranking on a scale of 1 (very difficult) to 10 (very easy) was 6.9 (SD 1.9), and mean time to complete the tool was estimated to be 4.2 minutes (range: 1-10 minutes). When asked if the tool raised had raised his

or her awareness of risk factors that contribute to feeding intolerance and NEC, 97.6% of nurses responded that it had. Similarly, 81.0% of nurses said the tool provides them with better information to care for an infant, and 85.7% stated they would be willing to use it at least daily for an infant under their care. However, 76.2% said the tool could be improved to make it easier to use, with comments most frequently related to the difficulty in finding maternal information within the EMR.

Tool error rate and scoring consistency

Forty-six tools (9.2%) contained errors, with 50 total errors yielding an item selection error rate of 0.35%. Three errors were made in the gestational age at birth category, 4 in the birth weight category, 0 in the feeding category, 20 on infant factors, and 23 on maternal factors. Thirty-three of 49 (67.3%) nurses made no errors, and error rate was inversely related to the number of tools a given nurse scored ($R^2 = 0.53$, $P = 0.01$).

Cronbach's alpha for variable categories included in the tool was 0.71. Removing the feeding category would increase Cronbach's alpha to 0.82 and removing the infant factor category would slightly increase alpha to 0.73, while removal of any of the other variable categories would decrease alpha (**Table 6.3**).

Inter-rater reliability

The ICC was 0.99, indicating excellent consistency between nurses. Fleiss' kappa was 1.00, indicating that risk categorization determination (high, moderate, or low) was consistent even when total score disagreement was present.

Identification of significant risk factors

The following risk factors were identified by chi-square testing to be significant ($P < 0.05$) to the development of all four outcome measures: gestational age at birth, hypoxia/asphyxia at birth, RBC transfusion, and congenital heart disease/patent ductus arteriosis (**Table 6.4**). In addition, birth weight, feeding, and respiratory distress were significant to the number of days with emesis. Birth weight, incomplete or no antenatal steroids, respiratory distress, antibiotics ≥ 5 days, and sepsis were significant to days with abdominal distention. Birth weight was significant to number of days with gastric residuals > 50% of previous feeding volume, and maternal cigarette use, antibiotics ≥ 5 days, and sepsis were significant to NEC development.

Predictive validity

Area under the ROC curve (AUC) as well as diagnostic OR estimates and 95% CI for the number of days with emesis (AUC = 0.71; OR = 1.14 [1.07, 1.23]), abdominal distention (AUC = 0.81; OR = 1.27 [1.17, 1.40]), or gastric residuals > 50% of previous feeding volume (AUC = 0.63; OR = 1.09 [1.03, 1.16]) and NEC (AUC = 0.94; OR = 1.45 [1.20, 1.91]), demonstrated total tool score to be associated with all 4 of the outcomes measures. (**Figure 6.2**).

Construct validity and comparison with GutCheck^{NEC}

Pearson correlation coefficient for the two tools was 0.80 ($P < 0.001$), indicating strong correlation between the two scores of a given infant. The tools demonstrated similar correlation coefficients to one another for each of the four outcome measures (**Table 6.5**). Furthermore, independent t-test revealed significant differences in scores of infants who did and did not

develop each of the four outcome measures when both the tool presented here and the GutCheck^{NEC} tool were used (**Table 6.6**).

Tool optimization

The following factors were not significantly associated with at least two of the three feeding intolerance outcomes, or with NEC, and were thus removed from the tool: feeding, polycythemia, IUGR or small for gestational age, placental abruption, clinical chorioamnionitis, maternal illicit drug use during pregnancy, preterm premature rupture of membranes, prolonged rupture of membranes, and absent or reversed end diastolic flow. Based on chi-square assessment of additional factors from the literature (**Table 6.7**), and following elimination of redundant variables, Apgar score at one minute and cesarean section delivery were added to the tool. The following pairs of factors were significantly correlated: 1) gestational age at birth and birth weight ($P < 0.001$); 2) respiratory distress and hypoxia ($P < 0.001$); 3) RBC transfusion and hypotension treated with inotropic medication ($P < 0.01$); and 4) sepsis and antibiotics ≥ 5 days ($P = 0.001$). The final version of the tool is show in **Table 6.8**. This optimized tool is greatly simplified, containing only 9 factors, with possible point totals ranging from 2 to 24.

In reassessing predictive validity, AUC as well as diagnostic OR estimates and 95% CI for the number of days with emesis (AUC = 0.69; OR = 1.14 [1.06, 1.23]), abdominal distention (AUC = 0.82; OR = 1.28 [1.18, 1.41]), or gastric residuals > 50% of previous feeding volume (AUC = 0.64; OR = 1.11 [1.04, 1.20]) and NEC (AUC = 0.90; OR = 1.29 [1.12, 1.56]), again demonstrated total tool score to be associated with all 4 of the outcomes measures. (**Figure 6.3**).

Pearson correlation coefficient for the optimized tool and GutCheck^{NEC} improved slightly to 0.82 ($P < 0.001$), again indicating strong correlation between the two scores of a given infant.

Correlation coefficients for optimized total tool score and each of the three feeding intolerance outcomes improved versus the un-optimized tool (emesis, 0.51; abdominal distention, 0.61; gastric residuals > 50% previous feeding volume, 0.49), while that of NEC decreased slightly to 0.35. Independent t-test again revealed significant differences in optimized tool score of infants who did and did not develop each of the four outcome measures (**Figure 6.4**). This allowed for identification of a single "at risk" threshold of ≥ 9 points, simplifying the risk categorization determination.

DISCUSSION

Results presented here demonstrate the clinical utility and predictive validity of this novel feeding intolerance and NEC risk scoring tool. In reinforcement of the pilot study results, the tool was assessed as easy to use and valued by nurses. The error rate remained similar to that in the pilot study, but with continued use, this error rate may decrease given that the study protocol nurse had an error rate of 7.1%. Cronbach's alpha indicated the five categories of variables included in the tool all measure the same construct with reasonable clinical accuracy, and inter-rater reliability was demonstrated to be excellent. Gestational age at birth, hypoxia/asphyxia at birth, RBC transfusion, and congenital heart disease/patent ductus arteriosis were identified as significant to the development of all four risk factors. The AUC, or probability that a randomly chosen infant who developed a given outcome scored higher than a randomly chosen infant who did not develop the given outcome,[28] was most discriminatory for abdominal distention and NEC. The diagnostic OR (and 95% CI) was > 1 for all 4 outcome measures, and scores of infants who did and did not develop each of the 4 outcome measures were significantly different from one another using both the optimized tool and GutCheck^{NEC}. The tool was demonstrated to be

similar to GutCheck^{NEC}, despite inclusion of overlapping, but also unique, risk factors. Finally, given that maternal factors were the greatest source of error and nurse confusion prior to optimization, it is likely that the ease of use of the tool will be increased, and its estimated completion time decreased, though this optimization.

In considering similarities and differences between the two tools, it is important to note that GutCheck^{NEC} was designed to be predictive only of NEC, not of other indices of feeding intolerance. Furthermore, GutCheck^{NEC} was developed via modeling only for use in very low birth weight (VLBW) infants, and the feasibility of nursing use of GutCheck^{NEC} has yet to be evaluated. It is also unclear at what point in an infant's clinical care GutCheck^{NEC} is designed to be used. Inclusion of a feeding risk factor which requires information on what the infant is fed on both days 7 and 14 of life indicate that GutCheck^{NEC} is to be used after the 14th day of life. Waiting until the 14th day of life to assess risk NEC risk is justified as NEC is often not diagnosed until beyond day 14 of life.[29] However, the tool presented here is designed to assess risk of both feeding intolerance and NEC, and is thus intended to be used at NICU admission and weekly until discharge since early and often evaluation of risk will be crucial for prevention of disease progression. Here, 85.7% of surveyed nurses stated they would be willing to use the tool at least daily for an infant under their care, indicating that weekly scoring would be supported.

Unit NEC rate carries the most weight in the summed GutCheck^{NEC} score, and can potentially be used as a proxy to represent multiple institutional practices that impact NEC risk.[1] The study site NEC rate was 2.0 ± 0.1% among VLBW infants in 2014, which may be reflective of strict use of standardized feeding protocols and prioritization of human milk feeding. While this low study site NEC rate is a boon for infants, caution must be exercised in interpreting tool NEC predictive validity as only 6 cases of NEC presented in enrolled infants. However, the

diagnostic OR is independent of outcome prevalence, so reasonable confidence in the ability of the tool to predict NEC risk is warranted.

Following presentation of the tool at the 2013 National Association of Neonatal Nurses (NANN) conference, multiple attendees expressed interest in implementing the tool at their home institutions (Chapter 5). However, before doing so, it is important to consider how the tool's predictive validity might differ if used in an institution that does not use standardized feeding protocols, has a NEC rate very different from the study site, or utilizes probiotics. It must also be noted that tool performance (for example, AUC) may change when the tool is applied in different clinical situations or populations, and that the most robust validation of the tool would be achieved through a pooled analysis of prospective studies at multiple study sites.[30] Such an analysis may also serve to further refine the factors included in the tool. In its current form, the tool may be used to raise nursing awareness of the factors that contribute to preterm infant feeding intolerance and NEC risk. Additional refinement should further improve its clinical utility, ensure broad applicability, and justify individualized infant care according to risk categorization.

FIGURES AND TABLES

Points	Variable Category and Risk Factors
	Gestational Age at Birth (select one)
1	32 - 36 6/7 weeks
2	28 - 31 6/7 weeks
3	< 28 weeks
	Birth Weight (select one)
0	≥ 2500 g
1	1500 - 2499 g (low birth weight)
2	1000 - 1499 g (very low birth weight)
3	< 1000 g (extremely low birth weight)
	Feeding Substance (select all that apply)
0	Mother's own milk
1	Donor breast milk; *nil per os* (NPO); total parenteral nutrition (TPN)
2	Bovine human milk fortifier
3	Bovine-based formula
	Infant Risk Factors (select all that apply)
1	Congenital heart disease or patent ductus arteriosis
2	Red blood cell transfusion
2	Polycythemia (hematocrit > 60)
2	Respiratory distress (> 24 hours assisted ventilation)
3	Hypoxia/asphyxia at birth
3	Sepsis
3	Antibiotics for ≥ 5 days
3	Intrauterine growth restriction or small for gestational age
	Maternal Factors (select all that apply)
1	Cigarette use during pregnancy
2	Placenta abruption
2	Clinical chorioamnionitis
2	Illicit drug use during pregnancy
2	Preterm premature rupture of membranes
2	Prolonged rupture of membranes (≥ 18 hours)
3	Incomplete or no antenatal glucocorticoid therapy
3	Absent or reversed end diastolic flow to infant

Table 6.1. Revised feeding intolerance and necrotizing enterocolitis risk scoring tool.(Chapter 5) Scoring risk ranges: 1-5, low; 6-8, moderate; ≥ 9, high.

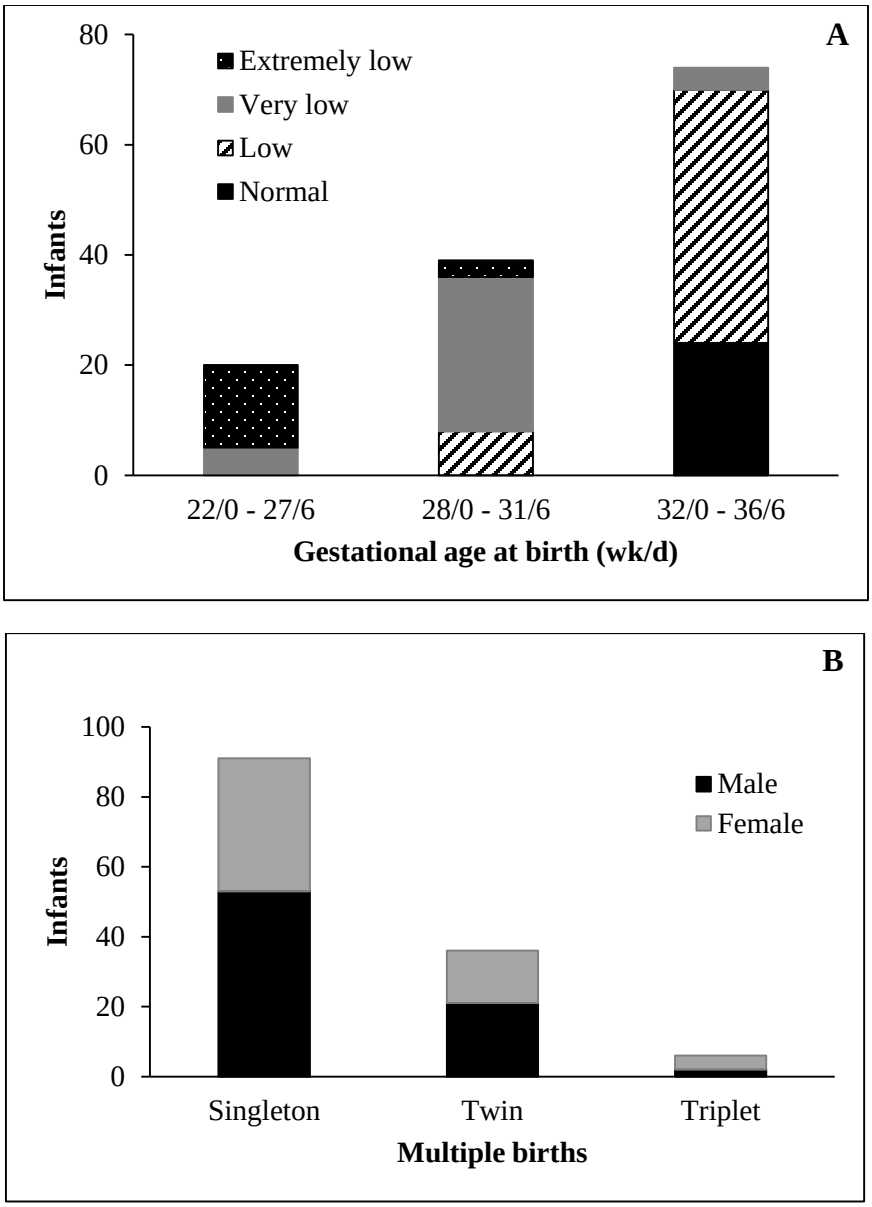

Figure 6.1. Infant demographics. A) Birth weight and gestational age at birth of enrolled infants. Birth weight categories: extremely low, < 1000 g; very low, < 1500 g; low, < 2500 g; normal, ≥ 2500 g. B) Gender and single versus multiple gestation of enrolled infants.

Survey Question (n = 42)	Response n	%	Comments (n)
Estimate the number of times you have used the tool.			
1-5	7	16.7	
6-10	12	28.6	
11-15	6	14.3	
> 15	17	40.5	
Rank the ease of use of the tool.			
1 (very difficult)	0	0	
2	0	0	
3	2	4.8	Difficult to identify/correct previous errors (1)
4	4	9.5	Info not always available or is difficult to find (2)
5	3	7.1	Can be difficult to find maternal factors (1)
6	7	16.7	Can be difficult to find historical information (1) Require more formal tool education (1)
7	8	19	Can be difficult to find maternal factors (1)
8	11	26.2	Can be difficult/take time to find maternal factors (2)
9	3	7.1	Can be difficult to find historical information (2)
10 (very easy)	4	9.5	Quick, effective (1)
Mean (SD)	*6.9 (1.9)*		
Estimate the number of minutes the tool requires to complete, on average.			
Mean (range)	4.2 (1-10)		
Has the tool raised your awareness of risk factors that contribute to feeding intolerance and NEC?			
Yes	41	97.6	
No	1	2.4	
Does the tool address important gaps in knowledge in the area of feeding intolerance within the NICU?			
Yes	39	92.9	
No	3	7.1	
Does knowing a baby's risk provide you with better information to care for the baby?			
Yes	34	81.0	
No	8	19.0	Care dependent on provider (1) Already on high alert for signs of NEC regardless of score (3)
How often would you be willing to utilize the tool for an infant under your care?			
Every shift	25	59.5	Increases awareness for potential complications (1)
Daily	11	26.2	
Weekly	5	11.9	Less frequent OK for infants on regular feedings (1)
Monthly	1	2.4	
Was the training you received regarding tool use adequate?			
Yes	34	81.0	
No	8	19.0	Need clarification on a particular item (6) Was unable to attend trainings (1)
Could improvements be made to the tool to make it easier to use?			
Yes	32	76.2	Mother's information not consistently available in infant's chart (5) Need clarification on particular item (1) Location of tool in chart (1) Need more practice using the tool (1)
No	10	23.8	Easy and comprehensive (1)

Table 6.2. Study site survey results. Abbreviations: NEC, necrotizing enterocolitis.

Deleted variable category	Cronbach's alpha
Gestational age at birth	0.62
Birth weight	0.62
Feeding	0.82
Infant factors	0.73
Maternal factors	0.63

Table 6.3. Scoring consistency of tool variable categories as assessed by Cronbach's alpha.

Variable included in tool	Outcomes			
	Emesis[1]	Abdominal distention[1]	Gastric residuals[1,2]	NEC[3]
*Gestational age at birth	**< 0.001**	**< 0.001**	**0.01**	**0.03**
Birth weight	**< 0.001**	**< 0.001**	**< 0.001**	0.14
Feeding	**0.03**	0.48	0.09	0.75
Infant risk factors				
*Congenital heart disease/patent ductus arteriosis	**0.01**	**< 0.001**	**< 0.001**	**0.01**
*Red blood cell transfusion	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Polycythemia (hematocrit > 60)	0.996	0.21	0.83	0.5
Respiratory distress (> 24hr assisted ventilation)	**< 0.001**	**0.001**	0.09	0.09
*Hypoxia/asphyxia at birth	**0.04**	**< 0.001**	**0.04**	**0.002**
Sepsis	0.69	**< 0.001**	0.33	**< 0.001**
Antibiotics ≥ 5 days	0.07	**< 0.001**	0.051	**< 0.001**
IUGR or small for gestational age	0.25	0.83	0.41	0.45
Maternal factors				
Cigarette use during pregnancy	0.58	0.29	0.27	**0.008**
Placenta abruption	0.57	0.62	0.98	0.38
Clinical chorioamnionitis	0.16	0.21	0.72	0.32
Illicit drug use during pregnancy	0.61	0.19	0.68	0.38
Preterm premature rupture of membranes	**0.0497**	0.19	0.58	0.99
Prolonged rupture of membranes (≥ 18 hr)	0.6	0.12	0.54	0.72
Incomplete/no antenatal glucocorticoid therapy	**0.005**	**0.03**	0.1	0.64
Absent or reversed end diastolic flow	0.93	0.98	0.42	0.62

Table 6.4. Chi-square test of association between risk factors and outcome measures.
*Significant to the development of all 4 outcome measures.
[1]Number of days during neonatal intensive care unit stay the outcome occurred.
[2]Gastric residuals > 50% of previous feeding volume.
[3]Diagnosis of necrotizing enterocolitis (NEC) per ICD-9 codes 557.0, 777.50, 777.51, 777.52, or 777.53.
Abbreviations: hr, hours; IUGR, intrauterine growth restriction; NEC, necrotizing enterocolitis.

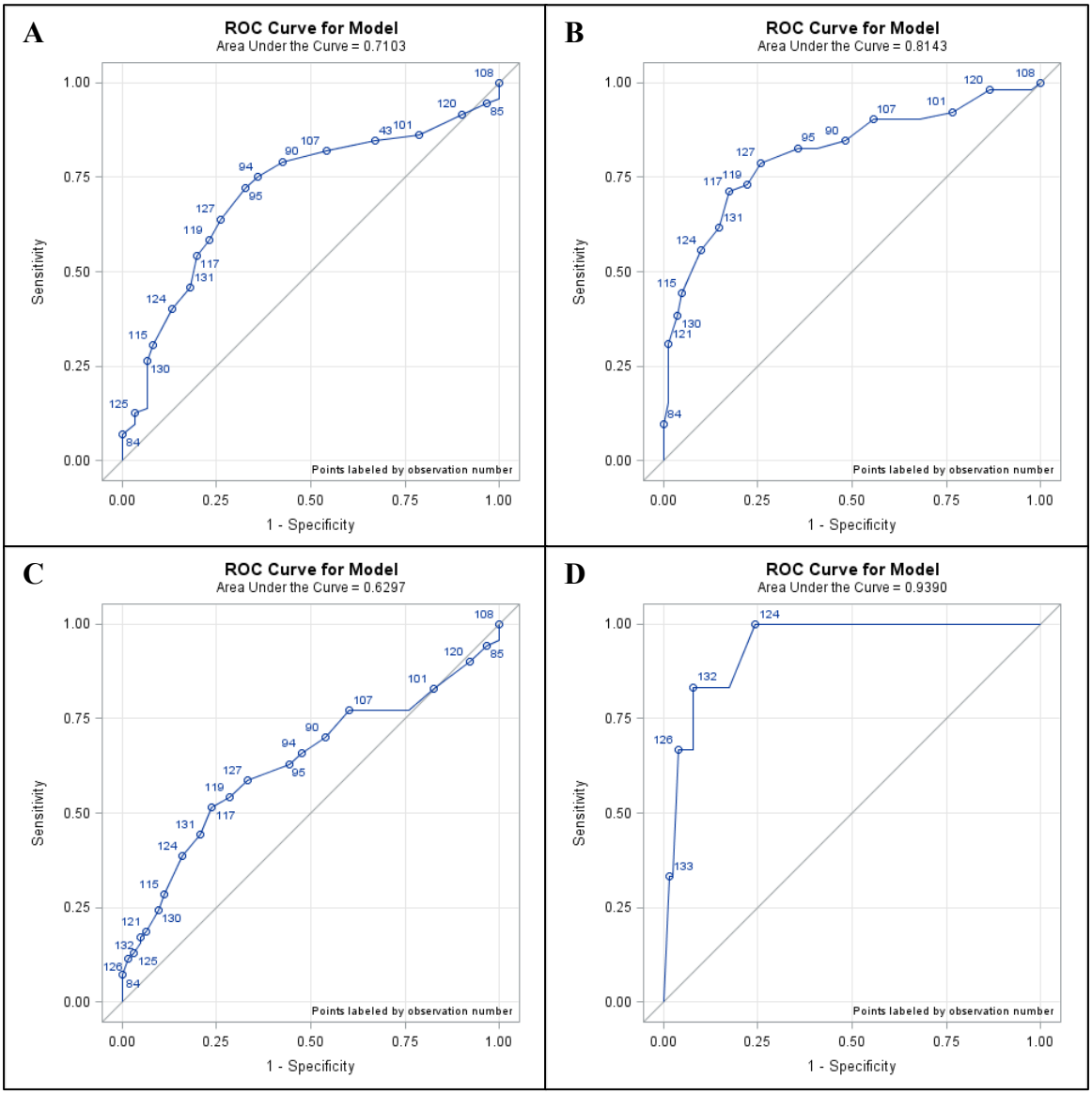

Figure 6.2. Assessment of tool discrimination using receiver operating characteristic (ROC) curves for (A) emesis; (B) abdominal distention; (C) gastric residuals > 50% previous feeding volume; and (D) necrotizing enterocolitis (NEC) per ICD-9 codes 557.0, 777.50, 777.51, 777.52, or 777.53.

Outcome	Tool	GutCheck^{NEC}
Emesis days	0.46	0.49
Abdominal distention days	0.57	0.65
Gastric residual days[1]	0.41	0.39
NEC[2]	0.38	0.40

Table 6.5. Pearson correlation coefficient of total tool and GutCheck^{NEC} scores with each of the four outcome measures.
[1]Gastric residuals > 50% of feeding volume.
[2]Diagnosis of necrotizing enterocolitis (NEC) per ICD-9 codes 557.0, 777.50, 777.51, 777.52, or 777.53.

		Developed outcome			Did not develop outcome			P-value
		Mean	95% CL	SD	Mean	95% CL	SD	
Emesis[1]	Tool	13.69	12.23, 15.16	6.24	9.39	8.13, 10.66	4.95	**<0.001**
	GutCheck^{NEC}	17.04	15.15, 18.93	8.04	12.16	10.68, 13.65	5.81	**<0.001**
Abdominal distention[1]	Tool	15.85	14.17, 17.53	6.03	9.07	8.11, 10.04	4.38	**<0.001**
	GutCheck^{NEC}	19.56	17.13, 21.99	8.72	11.75	10.77, 12.74	4.45	**<0.001**
Gastric residuals[1,2]	Tool	13.11	11.52, 14.71	6.67	10.17	8.94, 11.41	4.89	**0.005**
	GutCheck^{NEC}	16.54	14.42, 18.67	8.91	12.87	11.64, 14.10	4.88	**0.004**
NEC[3]	Tool	22.33	18.31, 26.35	3.83	11.22	10.22, 12.22	5.68	**<0.001**
	GutCheck^{NEC}	28.67	20.91, 36.43	7.39	14.15	12.95, 15.35	6.85	**0.005**

Table 6.6. Comparison of scores of infants who did and did not develop feeding intolerance and NEC outcomes using an independent t-test of both the tool and GutCheck^{NEC}.
[1]Number of days during neonatal intensive care unit stay the outcome occurred.
[2]Gastric residuals > 50% of feeding volume.
[3]Diagnosis of necrotizing enterocolitis (NEC) per ICD-9 codes 557.0, 777.50, 777.51, 777.52, or 777.53.
Abbreviations: CL, confidence limit; NEC, necrotizing enterocolitis; SD, standard deviation.

Variable	Outcome			
	Emesis[1]	**Abdominal distention[1]**	**Gastric residuals[1,2]**	**NEC[3]**
Gender (male vs. female)	0.27	0.92	0.34	0.66
*Delivery mode (vaginal vs. cesarean section)	**0.02**	**0.01**	**0.07**	0.97
Ethnicity	0.39	0.20	0.10	0.76
^Race (Black or Hispanic vs. all other races)	0.97	0.21	0.77	0.06
Multiple gestation (singleton vs. multiple)	0.11	0.39	0.47	0.08
^Birth site (outborn vs. inborn)	0.53	0.81	0.73	0.23
*Apgar at 1 minute < 5	**0.04**	**0.006**	0.44	0.95
Apgar at 5 minutes < 7	0.07	0.28	**0.03**	0.70
Maternal hypertension during pregnancy	0.81	0.85	0.48	0.34
Placental abnormality	0.68	0.48	0.87	0.37
^Culture-proven infection since day 3 of life	0.69	**< 0.001**	0.33	**< 0.001**
^Hypotension treated with inotropic medication	0.39	**0.002**	0.23	**0.02**
^Metabolic acidosis	**0.047**	**< 0.001**	0.13	**0.01**

Table 6.7. Assessment of additional factors for inclusion in the scoring tool.
*Included in optimized tool.
^Included in GutCheck^{NEC}.
[1]Number of days during neonatal intensive care unit stay the outcome occurred.
[2]Gastric residuals > 50% of previous feeding volume.
[3]Diagnosis of necrotizing enterocolitis (NEC) per ICD-9 codes 557.0, 777.50, 777.51, 777.52, or 777.53.

Points	Variable Category and Risk Factors
	Gestational Age at Birth (select one)
2	32 - 36 6/7 weeks
3	28 - 31 6/7 weeks
4	< 28 weeks
	Infant Risk Factors (select all that apply)
2	Apgar at 1 minute < 5
2	Antibiotics for ≥ 5 days
4	Congenital heart disease or patent ductus arteriosis
4	Red blood cell transfusion
4	Hypoxia/asphyxia at birth
	Maternal Factors (select all that apply)
1	Cigarette use during pregnancy
2	Incomplete or no antenatal glucocorticoid therapy
3	Delivered via cesarean section

Table 6.8. Optimized tool. An infant is determined to be at risk of feeding intolerance and/or NEC when the summed point total is ≥ 9.

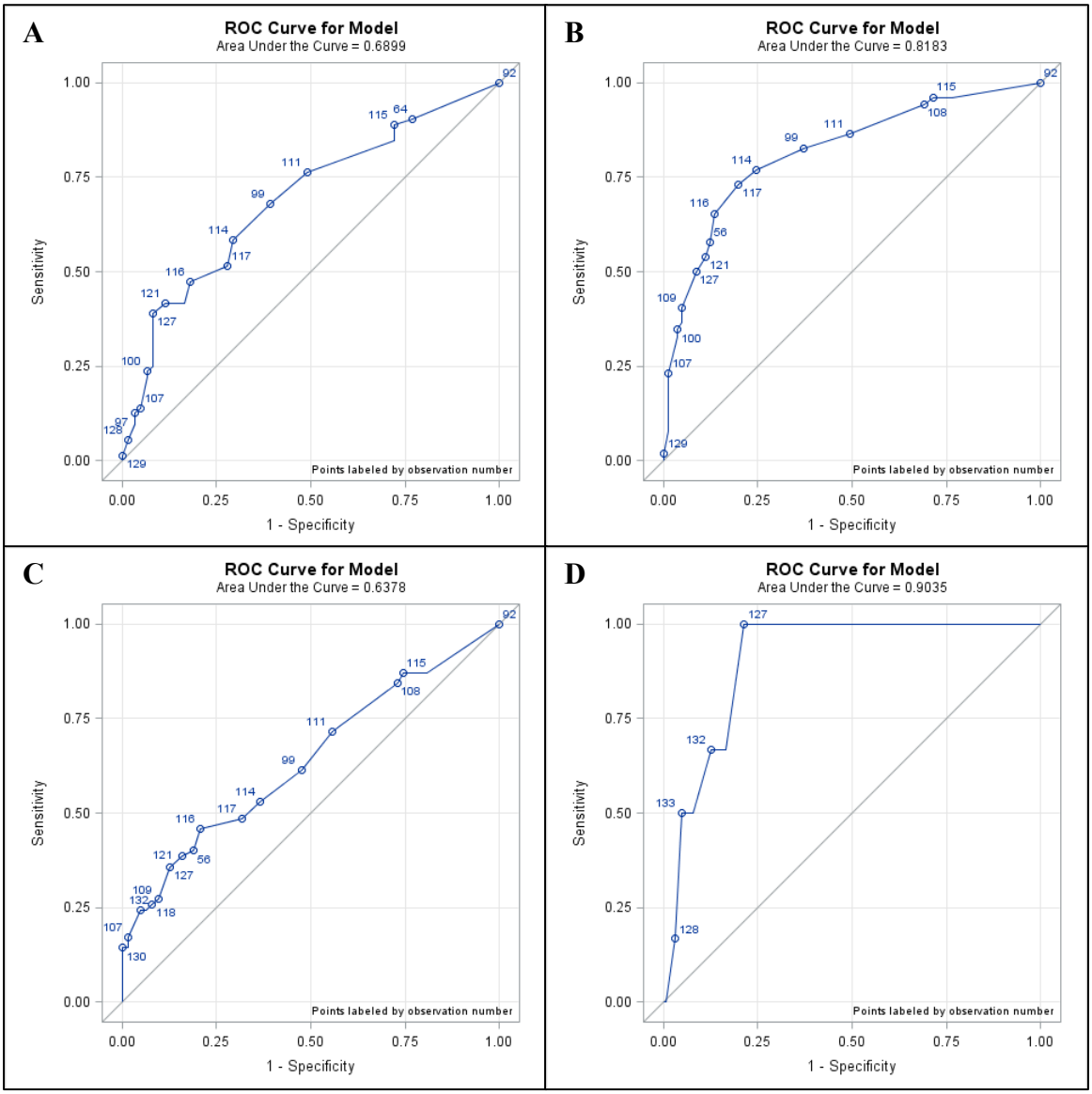

Figure 6.3. Assessment of optimized tool discrimination using receiver operating characteristic (ROC) curves for (A) emesis; (B) abdominal distention; (C) gastric residuals > 50% previous feeding volume; and (D) necrotizing enterocolitis (NEC) per ICD-9 codes 557.0, 777.50, 777.51, 777.52, or 777.53.

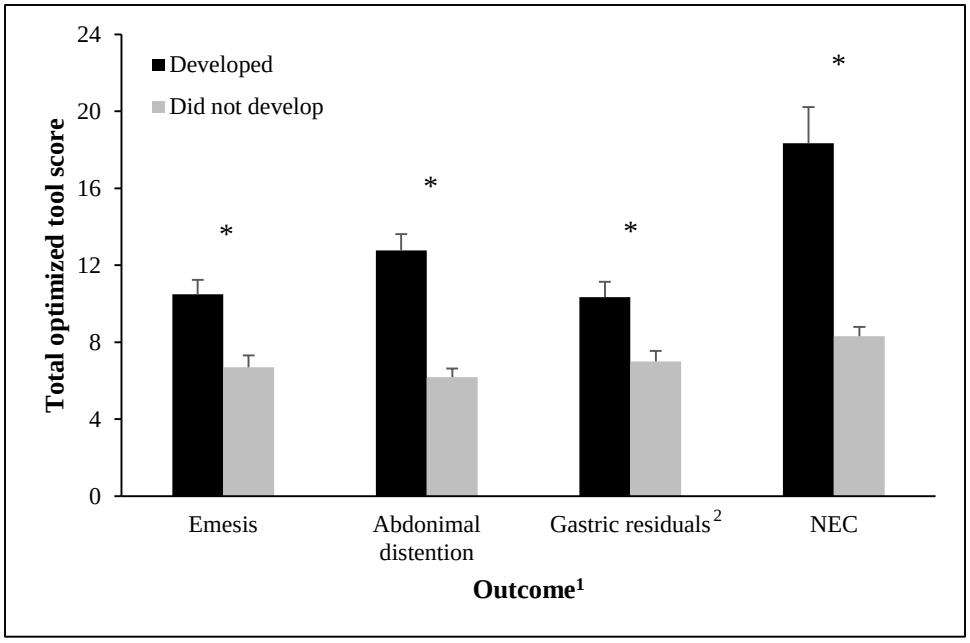

Figure 6.4. Comparison of scores of infants who did and did not develop feeding intolerance and NEC outcomes using an independent t-test.
Data displayed as mean ± SEM.
*Significant ($P < 0.05$) difference between mean total optimized tool score in infants who did and did not develop a given outcome.
[1]Number of days during neonatal intensive care unit stay the outcome occurred.
[2]Gastric residuals > 50% of previous feeding volume.
Abbreviations: NEC, necrotizing enterocolitis.

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CHAPTER 7

SUMMARY AND FUTURE DIRECTIONS

SUMMARY

Due to the immaturity of the gastrointestinal tract, preterm infants are at increased risk of feeding intolerance and NEC. If these conditions cannot be prevented and intestinal failure ensues, an infant may require long-term parenteral nutrition (PN) in order to support growth as well as maintain hydration and micronutrient status.[1] Despite the life-saving nature of PN, it is also associated with numerous complications that are especially dangerous to infants.[2-4] Thus, in order to prevent dependence on PN as well promote weaning when PN is required, the goal of treatment for these patients is to stimulate intestinal adaptation and achieve enteral autonomy.[5] To achieve this goal, it is crucial that the intestine undergo structural and functional adaptations to increase digestive and absorptive capabilities to a level which will be able to support infant growth.

Teduglutide reduces PN requirements in PN-dependent adults but is not approved for use in infants. Partial enteral nutrition (PEN) has been repeatedly demonstrated in both animal and human trials to prevent mucosal atrophy and promote intestinal adaptation. Furthermore, there is wide agreement that prediction and prevention of feeding intolerance and NEC will be the most efficacious means to improve infant outcomes related to feeding.[6,7]

Given the above, the specific aims of this research were to (1) assess the efficacy and safety of teduglutide in reducing PN (parenteral nutrient and/or fluid) requirements in PN-dependent adults; (2) assess the efficacy of teduglutide and/or PEN in inducing intestinal adaptation in a neonatal piglet model of short bowel syndrome (SBS); and (3) develop and

validate a novel preterm infant feeding intolerance and NEC risk scoring tool to identify infants who may benefit from prophylactic or therapeutic teduglutide and/or PEN treatment.

Hypothesis 1

In a systematic review of the literature, teduglutide treatment would decrease PN requirements of PN-dependent adults compared to placebo and demonstrate an acceptable safety profile.

Both efficacy (**Table 3.2**) and safety (**Table 3.3**) of teduglutide were demonstrated in the 14 studies included in the systematic review, with number needed to treat to benefit (NNTB) ranging from 3 to 4, and the number needed to treat to harm (NNTH) ranging from 24 to 187. Thus, far more patients would need to be treated with teduglutide to cause harm to one additional patient than would need to be treated for one additional patient to benefit from treatment. This review was important to perform since duplicate publication, particularly with multiple meeting abstracts, appeared to artificially inflate the effects of teduglutide.

The distillation of data to original results presented here clearly describes both the benefits and risks of teduglutide treatment in adults, and provides preliminary data for consideration of its use in the pediatric population. Teduglutide is now available for prescription outside of clinical trials, but is mandated to participate in the United States Food and Drug Administration's (FDA) Risk Evaluation & Mitigation Strategies (REMS) program. Rationale for requirement of REMS participation stems from the risks of gastrointestinal obstruction, biliary and pancreatic disorders, and acceleration of neoplastic or colon polyp growth associated with teduglutide use. Long-term data on the safety of teduglutide in adults has only yet been carried out for approximately three years, and caution must be exercised when considering

prescription of teduglutide in the more vulnerable pediatric population. Regardless of patient age, both benefits and risks of teduglutide treatment must be carefully considered on an individual patient basis before beginning treatment.

Hypothesis 2

Teduglutide will enhance structural and functional adaptation of the residual small intestine via enhanced mucosal surface area and nutrient processing capacity in a neonatal piglet model of SBS, and these effects would be augmented by the provision of PEN. Furthermore, mucosal surface area expansion will precede functional adaptation.

In agreement with a previous piglet study,[8] teduglutide enhanced structural, and transiently increased functional, measures of intestinal adaptation. Indices of intestinal adaptation stimulated by teduglutide and/or PEN were similar to those observed in preclinical GLP-2 and adult human teduglutide studies.[9-14] Furthermore, complimentary roles for teduglutide and PEN were demonstrated in anatomical site and timing of action and structural versus functional measures of adaptation. Synergistic effects included, most notably, villus height of all intestinal segments and crypt depth of the jejunum, ileum, and colon.

The lack of lasting effect of teduglutide on functional indices of adaptation in this study and others[8] may be due to the dosing strategies used in either piglet study, thus further work on optimal pediatric dosing needs to be completed. Additionally, as noted above, data on long-term safety of teduglutide in adults may also serve to inform optimal pediatric dosing, once that data is available. Nonetheless, coupling teduglutide and PEN therapies represents an opportunity to augment intestinal adaptation in neonatal SBS beyond that of either therapy alone, and potentially accelerate enteral autonomy in this population.

Hypothesis 3

Accurate prediction of infant feeding intolerance and NEC risk can be accomplished by comprehensive assessment of feeding practices as well as relevant infant and maternal factors. Furthermore, a novel risk scoring tool will be easy to use since similarly designed tools have been successful in predicting and reducing the incidence of falls in the elderly[15] and pressure ulcers in adults.[16]

Following pilot testing, the tool was demonstrated to be simple to use and valued by nurses. Its accuracy, consistency, inter-rater reliability, and predictive validity were in acceptable ranges. Clinical testing revealed clear modifications that could be made to the tool to improve its clinical utility, which were implemented during optimization of the tool.

Though the tool could likely be improved through implementation at additional institutions and subsequent further refinement, in its current form, the tool is instrumental in raising awareness of the factors that contribute to feeding intolerance and NEC, helping nurses to put disparate clinical signs into context, and communicating risk of these conditions to providers. Though some factors of feeding intolerance and NEC development are unmodifiable (gestational age at birth, for example), nursing focus should be on those that can be modified through alteration of clinical practice. Of particular importance as indicated by the tool, are ensuring that all infants receive a complete course of antenatal glucocorticoid therapy, and preventing sepsis and hypoxia.

Even as research in this area advances, nurses will remain critical first-line defenders in detection of feeding intolerance and NEC and important advocates for their patients. Providing nurses with an objective means with which to communicate risk of feeding intolerance and NEC

should improve the likelihood that their observations will be taken into consideration by NICU providers.

FUTURE DIRECTIONS

The tool could be further improved by following up on interest already expressed by other institutions to strengthen the validity of the tool to ensure that it's broadly applicable to infants on a larger scale. Specifically, implementing the tool at additional institutions would also allow for exploration of the possibility of including an institutional risk factor on the tool,[17,18] which may account for multiple risk factors, such as the use of standardized feeding protocols[19-25] and prioritization of human milk feeding,[26] in a single variable, thus simplifying scoring.

The tool could also be used to justify research regarding investigation and application of new NEC treatments, including the use of teduglutide, according to NEC risk categorization. Additionally, the tool could be used to validate potential biomarkers of NEC such as urinary creatinine and fatty acid binding protein,[27-29] or fecal calprotectin[30] and volatile organic compounds.[31,32] Such biomarkers have been previously proposed to be predictive of NEC onset or severity, but being able to correlate biomarker levels with infant NEC risk and subsequent outcome could further justify the use of such markers. Additional emerging techniques and biomarkers such as the use of proteomics[33] may also serve as means to assess infant feeding intolerance and NEC risk. Though not all of these biomarkers could be evaluated by the bedside nurse, combining them with the tool may maximize clinical NEC predictive capabilities. The future directions outlined here will be important in ensuring a broadly applicable risk scoring tool that will maximally improve both short- and long-term preterm infant outcomes for minimal time and financial cost.

CONCLUSION

This work established a novel tool to assess preterm infant feeding intolerance and NEC risk though evaluation of relevant infant and maternal factors. Though it will continue to be refined, it also lays the groundwork for identification of infants who may benefit from prophylactic therapies in order to promote gastrointestinal maturation in an effort to prevent feeding intolerance. In addition, this work also establishes teduglutide, already approved for use in PN-dependent adults, as a potential means by which to promote enteral autonomy in the pediatric population, specifically through promotion of primarily structural, but to a lesser degree functional, measures of intestinal adaptation. The data presented here furthers knowledge in the area of preterm infant feeding intolerance and identifies future directions for this line of research.

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