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Review

On the horizon: trophic peptide growth factors as therapy for neonatal short bowel syndrome.

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Abstract

Introduction: Short bowel syndrome (SBS) occurs more commonly in human neonates than in adults. There are currently no approved therapeutic agents aimed directly at stimulating intestinal adaptation in this population.

Areas Covered: A brief review of SBS and intestinal adaptation is first presented. We then present candidate peptide growth factors that are suggested to augment intestinal adaptation in SBS, with a particular focus on glucagon-like peptide-2, as well as insulin-like growth factor-1 and epidermal growth factor. The normal physiology of these peptides and our understanding of their roles in intestinal adaptation are discussed. We further consider the roles of these peptides in the ontogeny of the gastrointestinal tract and we present the limited preclinical data on the effects of administering these peptides in neonatal SBS.

Expert Opinion: The clinical translation of trophic peptide therapies in neonatal SBS will require several challenges to be overcome. The optimal dose, timing and route of administration for the likely peptide, or combination of peptides, to be administered will be paramount. Despite their cost to patient care, trophic peptides have shown promise in preclinical models of neonatal SBS and may be especially beneficial for neonates that lack remnant ileum and suffer from irreversible intestinal failure.
Key Words: neonatal, short bowel syndrome, intestinal failure, glucagon-like peptide-2, teduglutide, insulin-like growth factor, epidermal growth factor, growth factors

1. Introduction

The intestine is vital for absorption of fluid and nutrients in the neonate, allowing for normal health and development. When the neonatal intestine becomes compromised, either quantitatively or functionally, adequate absorption of nutrients may become jeopardized, resulting in varying degrees of intestinal insufficiency [1,2]. Intestinal failure refers to a compromise in the small intestine’s normal absorptive capacity, such that intravenous fluid or nutrient support is required [3]. This may result from anatomical (e.g. short bowel syndrome; SBS), mucosal (e.g. mucosal enteropathy) or neuromuscular (e.g. dysmotility) intestinal diseases [3]. In human neonates, SBS is the most common cause of intestinal failure and is, therefore, the focus of the present review. All neonates with intestinal failure require total or supplemental nutrition support via parenteral nutrition (PN) to maintain the child’s growth and development whilst permitting structural and functional adaptation of the remnant intestine.

SBS occurs following the massive resection of intestine, which, in neonates, is often consequent to necrotizing enterocolitis (NEC; Table 1). SBS is a heterogeneous condition, marked by variation in etiology, age of occurrence, and the length, anatomy and motor function of the remnant intestine [1,2]. The remnant intestinal length and anatomy in particular directly impact disease severity and prognosis [2,4]. Although three subtypes have been described based on anatomical differences in the region of
resection (Table 2; Figure 1), the pathologies that lead to intestinal resection and subsequent SBS in neonates most commonly involve the ileum (e.g. NEC), thus resulting most frequently in Type 2 and 3 SBS [3]. Both Type 2 and 3 SBS result in severe diarrhea, fluid and electrolyte imbalance, and greater malabsorption than in Type 1 SBS due to complete loss of the ileum (Table 2).

Adaptation of the remnant intestine in SBS is a process whereby structural and functional changes occur, enhancing nutrient absorptive capacity [5]. In animal models, structural adaptation results in increased surface absorptive area, secondary to increases in intestinal villus height and crypt depth as a result of crypt cell proliferation. Functional adaptive changes include the up-regulation of digestive enzymes and nutrient transporters and reductions in intestinal permeability [5]. In humans, the observed mechanisms of adaptation include intestinal dilatation, mucosal hyperplasia, and remnant mucosal hyperfunction. Expansion of intestinal stem cells following intestinal resection has been suggested as the initiating event in the adaptive process [6]. In contrast, suppression of enterocyte apoptosis does not appear to be a mechanism underlying such intestinal adaptation [5]. Indeed, following resection, rates of apoptosis have been reported to increase [7]. Furthermore, when this increase in apoptosis is abolished, as observed in p38-intestinal knockout mice, greater increases in cell proliferation, and crypt and villus lengths are observed after a 50% Type 1 resection [8]. Finally, intestinal adaptation following massive intestinal resection is variable and can take months to years. As intestinal adaptation occurs and intestinal nutrient absorption improves, infants with SBS can gradually wean off PN as they achieve enteral autonomy.
As intestinal adaptation is a slow-occurring process, many infants with SBS require PN support for extended periods of time. This increases the risk for developing the two major co-morbidities associated with long-term PN therapy: intestinal failure-associated liver disease and central venous catheter-related thrombosis, infection and sepsis, both of which are associated with high mortality (Table 1) [4,9]. Furthermore, a significant limitation in the management of infants with SBS is the lack of treatment of options. Currently, PN is utilized as the mainstay of therapy to support growth and development and replenish fluid and electrolyte losses [1,9]. At this time, there are no therapeutic agents that augment intestinal adaptation in neonates with SBS.

Recent attention has focused on the development of peptide factors with intestinotrophic actions as possible therapy for adults with SBS. Although several growth factors are believed to stimulate and augment intestinal adaptation, interest in the intestinal hormone, glucagon-like peptide-2 (GLP-2), has come into focus by approval of a synthetic analogue (teduglutide) for adult SBS. Yet, despite the fact that SBS is far more prevalent in the neonatal population, with higher rates of comorbidities and more often leading to consideration of intestinal transplantation, trophic peptides remain inadequately studied and none are approved for this vulnerable population [10].

2. Trophic peptides

The list of peptides that exert growth and/or adaptive effects on the gut continues to grow. The purpose of this review is to examine those factors that have garnered the most
attention and research, and particularly those with greatest potential for translation to use in pediatric patients with SBS. We begin by exploring the normal physiology of key intestinotrophic factors, including GLP-2, insulin-like growth factor (IGF) and epidermal growth factor (EGF), followed by a discussion of their known benefits in animal models of SBS and the available data from preclinical models of neonatal SBS, including the limited clinical trials involving children.

2.1 Glucagon-like peptide-2

2.1.1 GLP-2 physiology

GLP-2 is a peptide hormone released by enteroendocrine L cells in response to oral nutrient ingestion [11]. L cells are found along the length of the gastrointestinal tract, but predominate in the ileum and proximal colon (Figure 1). Circulating GLP-2 is inactivated by dipeptidylpeptidase IV (DPP-IV)-mediated removal of the first two amino acids [12]. Active GLP-2 (1-33) has a half-life of 7 minutes in humans, whereas that of GLP-2 (3-33) is 27 minutes. GLP-2 is also cleared by glomerular filtration [12].

The intestinotrophic effects of an unidentified glucagon-related peptide were first described in rare individuals who developed intestinal mucosal hyperplasia secondary to proglucagon-expressing tumors. However, it was not until 1996 that GLP-2 was identified as the proglucagon-derived peptide capable of exerting intestinotrophic effects in mice [11]. Subsequent studies in healthy rodents demonstrated that administration of either native GLP-2 or a DPP-IV resistant GLP-2 analog (i.e. human Gly²-GLP-2, used synonymously with native GLP-2 in this discussion; teduglutide refers to recombinant
human Gly²-GLP-2) increases small and large intestinal weight, mRNA and protein, induces villus hypertrophy and crypt expansion, and increases epithelial cell proliferation as well as decreasing apoptosis [11,13,14]. Of key importance to the potential clinical use of GLP-2 therapy, the intestinotrophic actions of GLP-2 are lost in mice after discontinuation of treatment [14]. Finally, while little is known about the role of endogenous GLP-2, several studies have indicated that it mediates the intestinal adaptive growth response to oral refeeding after a prolonged fast [15,16].

In addition to its trophic effects, acute treatment with GLP-2 increases glucose transport via activation of SGLT-1 and GLUT-2 transporters [17]. Chronic administration of GLP-2 (for 10-14 days) further increases carbohydrate, protein and lipid absorption through enhanced expression of epithelial nutrient transporters and increased activity of brush-border digestive enzymes [13,17]. GLP-2 also increases plasma triglyceride and apoB48 lipoprotein levels in rodents and humans given an oral fat load.

The intestinotrophic effects of GLP-2 have also been studied in animal models of total PN (TPN) feeding. In piglets, GLP-2 administration reverses TPN-associated mucosal hypoplasia and reduction in intestinal function. Administration of human GLP-2 increases intestinal weight, lengthens the villi, deepens the crypts and decreases apoptosis [18]. GLP-2 treatment also enhances intestinal function in these animal models, via increased expression of epithelial nutrient transporters and digestive enzymes [13,17,19].
In addition to direct effects to promote intestinal function, GLP-2 administration to animals stimulates mesenteric blood flow. Interestingly, this effect appears to be site-specific, with significant increases in blood supply to the duodenum and jejunum and to the intestinal serosa (versus mucosa), but no effect in the ileum or colon [20]. Increased mesenteric blood flow has also been observed in human patients with SBS with GLP-2 therapy [21]. Furthermore, in mice, pigs on PN, and humans, GLP-2 administration decreases gastric emptying and proximal intestinal motility [22]. Collectively, these effects are thought to further enhance the actions of GLP-2 to increase nutrient absorption.

Finally, additional beneficial actions of GLP-2 administration include increases in intestinal barrier function and decreases in intestinal inflammation. In both healthy mice and animal models of impaired barrier function, GLP-2 decreases intestinal permeability in association with enhanced tight junctional protein expression [23]. In addition, GLP-2 exerts local intestinal anti-inflammatory effects that are mediated by vasoactive intestinal polypeptide (VIP), as will be subsequently discussed.

2.1.2 GLP-2 signaling and secondary messengers

GLP-2 receptor (GLP-2R) expression is mainly localized to the gut, with only limited expression in the lung and hypothalamus, thereby conferring high specificity of its actions to the intestinal tract. Importantly, although receptor desensitization following ligand activation has been demonstrated \textit{in vitro}, the GLP-2R does not appear to exhibit tachyphylaxis \textit{in vivo} [14,24]. The greatest abundance of GLP-2R expression occurs in
the jejunum relative to other portions of the gastrointestinal tract, with its trophic actions limited to the mucosa (Figure 1). However, the mechanism of action of GLP-2 is not fully understood, due to expression of the GLP-2R in multiple gut cell types that do not include the crypt or villus epithelium [25-17]. Numerous studies in rodents, pigs and humans have now localized the GLP-2R to intestinal epithelial enteroendocrine cells, enteric neurons and subepithelial myofibroblasts (SEMFs), as well as vagal afferents and colonic submucosal glia, suggesting the involvement of indirect mediators in the trophic effects of GLP-2. These second messengers are believed to be released by the cell types expressing the GLP-2R and include IGF-1 and -2 (SEMFs), fibroblast growth factors (SEMFs) [28], VIP (enteric neurons) [29], and ErbB ligands (SEMFs) [27], as discussed in the following section. Studies in PN-fed neonatal piglets also suggest that endothelial nitric oxide synthase (eNOS) may mediate the GLP-2-associated intestinotrophic response to enteral nutrition (EN), including changes in portal blood flow [27,30].

Finally, numerous studies have shown that GLP-2R activation leads to cyclic AMP generation and protein kinase A signaling [24,25]. However, GLP-2 has also been reported to activate Erk1/2 in several cell models [26]. Furthermore, the effects of GLP-2 on both SEMFs and enteric neurons appear to be mediated by the Akt pathway [29,31,32]. In addition, GLP-2 administration has been reported to increase mucous cell number as well as VIP expression in enteric neurons via a mechanism requiring phosphatidylinositol-3 kinase-gamma [29]. Our limited understanding of the various intracellular signaling pathways that lie downstream of GLP-2R activation has been recently reviewed [33].
2.1.3 GLP-2 in adult models of SBS

In animal models of Type 1 SBS (Table 2), proximal intestinal resection itself augments intestinal adaptation in the remnant intestine, characterized by increases in villus height, crypt depth, enterocyte proliferation, nutrient transporters and digestive enzymes [34]. Furthermore, elevated plasma GLP-2 levels are associated with this adaptation, suggesting a role for endogenous GLP-2 in this response. Consistent with this hypothesis, structural adaptation is diminished upon GLP-2 immunoneutralization in rats with a 75% proximal intestinal resection [35]. In contrast, the literature on GLP-2 levels in Type 2 SBS is conflicting. In rodents, there was no difference in plasma GLP-2 following a 60% distal intestinal resection with jejunocolic anastomosis; however, there was also no adaptation in the remnant jejunum [36]. In contrast, higher GLP-2 levels, with morphologic but not functional adaptation, have been reported in rodents after a distal intestinal resection leaving 10-20 cm of jejunum [37]. The adaptive potential following a distal intestinal resection may therefore be decreased in Type 2 SBS compared to a proximal intestinal resection due to the absence of ileum and removal of a significant proportion of the intestinal L cell mass, although up-regulation of colonic GLP-2 release in this setting cannot be discounted (Figure 1). Finally, the potential benefits of exogenous GLP-2 therapy in SBS have also been studied in rodent models. Hence, PN-fed rats with 70-90% proximal intestinal resection demonstrate increases in the growth, and digestive, absorptive and barrier functions of the remnant intestine after chronic treatment with GLP-2 [38].
2.1.4 GLP-2 in adult humans with SBS

Although parameters of growth are more difficult to study in humans, administration of teduglutide for 21 days increased wet weight absorption, and decreased fecal wet weight and energy excretion [39]. These positive findings led to full randomized, placebo-controlled trials, in which teduglutide treatment for 24 weeks was found to reduce PN volume by >20%, as well as to increase small bowel villus height and surface area [40,41]. Progressive decreases in PN volume were also found in a 28-week double-blind extension study. Furthermore, chronic teduglutide treatment increased citrulline levels, a marker of intestinal growth [40,41]. Whether these changes are associated with alterations in epithelial cell apoptosis is not known. Nonetheless, exogenous treatment with a long-acting GLP-2 analog has been found to improved measures of intestinal growth and function in adult patients with SBS.

2.2. Insulin-like growth factor family

The IGF family encompasses three structurally similar ligands (IGF-I, IGF-II and insulin) and two high-affinity cell surface receptors: the IGF-I receptor (R) and the IGF-IIR, the latter of which is largely a clearance receptor for IGF-II [42]. The IGFs are widely-expressed growth factors with important functions in tissue, organ and whole body growth and differentiation, from development to adulthood. IGF-I and IGF-II are synthesized and secreted by many cells in the body, including the gastrointestinal tract. In vivo, the majority of circulating IGF-I is bound to IGF-binding proteins (IGFBPs) that modulate the activity of IGF-I. In humans, six IGFBPs both protect the IGFs from degradation and regulate interactions between the IGFs and their receptors.
2.2.1 IGF-I

Circulating IGF-I is predominantly synthesized in the liver, where its secretion is regulated by growth hormone (GH), insulin and protein and caloric intake [42]. Studies in liver-specific IGF-I null mice have suggested that hepatic IGF-I exerts many of the endocrine functions related to GH-mediated somatic growth, as well as negatively feeding-back on GH synthesis in the pituitary. IGF-I binds with highest affinity to IGF-IR, which signals through activation of the Akt and Erk1/2 signaling pathways [31,42,43].

Whole-body overexpression of IGF-I or exogenous administration of either IGF-I or LR3IGF-I, an IGF-I analog with decreased affinity for IGFBPs, induces significant intestinal lengthening, and increases intestinal wet weight, villus height, crypt depth and crypt cell proliferation in the jejunum, but does not affect disaccharidase activities [44,45]. However, IGF-I is also locally produced in the intestine by SEMFs, consistent with expression of the IGF-IR on the basolateral membrane of the enterocyte [42,46,47]. IGF-I also promotes intestinal fibrogenesis by increasing collagen synthesis and secretion from these cells [42]. Locally-expressed IGF-I is thus believed to act in an autocrine and/or paracrine manner to regulate intestinal mucosal growth responses via the intestinal epithelial IGF-IR. Furthermore, IGF-I is also produced by intestinal myofibroblast cells, with both over- and under-expression studies indicating a role to stimulate proliferation and reduce apoptosis of the submucosa and muscularis propria [45,47]. Thus, unlike the
intestinal mucosa-specific actions of exogenously administered GLP-2, the growth effects of IGF-1 are considered to be pleiotrophic.

Within the intestine, IGF-I has been found to exert many similar biological effects to GLP-2. Thus, in PN-dependent rats, IGF-I administration reverses structural intestinal hypoplasia, normalizes ion transport and permeability, and increases jejunal glucose absorption [48]. IGF-I may also play a role in the adaptive response to re-feeding, as mucosal growth in re-fed rats is associated with increased IGF-I mRNA expression [49]. Finally, in neonatal pigs, oral administration of IGF-I increases small intestinal weight, DNA and protein content and villus height although, unlike GLP-2, IGF-I levels do not increase following intestinal resection in this model [50].

Similar to GLP-2, IGF-I administration also augments the adaptive response following intestinal resection. After a 70-80% Type 1 resection in rats, treatment with either native or long-acting IGF-I stimulates intestinal crypt cell proliferation in the remnant intestine, and increases digestive enzyme activities in the ileum [51]. Similarly, intravenous administration of IGF-I to rats with a 60% Type 2 resection promotes small intestinal growth, while IGF-I given orally increases colonic adaptation [52]. However, unlike the mucosal-specific actions of GLP-2, mice that over-express IGF-I in the myofibroblasts demonstrate a 50% increase in intestinal length after resection, suggesting that IGF-I expression in the muscularis plays a role in intestinal lengthening post-resection [47]. The findings of a concomitant decrease in longitudinal muscle thickness, with unchanged

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cellularity, suggests that longitudinal stretching of individual smooth muscle cells may be a mechanism underlying the intestinal lengthening [47].

Finally, both IGF-I and long-acting IGF-I increase circulating IGFBP-1-5 levels after 70-80% Type 1 intestinal resection in rats, although IGFBP4 levels are not increased in rats with a Type 2 resection [51,52]. Conversely, decreased IGFBP-3 mRNA expression has been suggested as an adaptive mechanism for increasing post-resection local IGF-I bioavailability for intestinal adaptation this model [42]. These discrepancies may relate to the autocrine/paracrine effects of IGF-I within the intestine as compared to the hormonal effects exerted by circulating IGF-I.

Given the similar biological effects of GLP-2 and IGF-I in the intestine, and the differential expression of GLP-2R in SEMF cells as compared to the IGF-IR in the intestinal epithelial cells (IECs), IGF-I has been proposed to serve as a downstream mediator of the intestinotrophic actions of GLP-2. Hence, the intestinal growth-promoting effects of GLP-2 are reduced in IGF-I knockout mice, while the growth and barrier effects of GLP-2 are impaired in IEC-IGF-IR null animals [43,53,54]. GLP-2 also increases the expression and secretion of intestinal IGF-I from SEMFs through a phosphatidylinositol 3 kinase (PI3K)/Akt-dependent pathway [27,28,32,43]. Finally, IGF-I mediates the effects of GLP-2 on crypt cell beta-catenin nuclear translocation and activation of Akt, both of which play key roles in crypt cell proliferation [27,31]. Collectively, these findings are consistent with a paracrine role for IGF-I as a mediator of the intestinal growth and functional effects of GLP-2.
2.2.2 IGF-II

In contrast to the role of IGF-I in crypt cell proliferation, endogenous IGF-II appears to regulate crypt fission, thereby increasing overall crypt number. IGF-II also exhibits parental imprinting and, when maternal IGF-II silencing is lost, there is an increase in IGF-II that is associated with tumor development including colorectal cancer [55]. However, the intestinal adaptive effects of GLP-2 are only partially abrogated by loss of IGF-II, and it has been surmised that loss of IGF-II reduces the total number of crypts that can respond to other growth factors such as GLP-2 or IGF-I [27,43]. Furthermore, while IGF-II administration to normal mice does not affect small bowel length or weight, villus height or crypt depth, colon weight and crypt depth are actually decreased [56]. The potential clinical utility of IGF-II for the treatment of either adult or neonatal SBS therefore remains uncertain.

2.3 Epidermal growth factor family

The EGF family of peptides, also known as the ErbB ligands, includes a number of intestinotrophic peptides: EGF, transforming growth factor-alpha (TGF-α), amphiregulin, heparin-binding EGF (HB-EGF), epiregulin, betacellulin, neuregulin and neuregulin-2. All of these growth factors have been shown to stimulate crypt cell proliferation and inhibit apoptosis [57]. The major sites of EGF synthesis are the salivary glands and kidney, with immunoreactive EGF detectable in most biological fluids, including plasma. EGF is not synthesized by the normal small intestinal epithelium (except in duodenal Brunner’s glands and, possibly, in response to injury), whereas most gastrointestinal cells
synthesize and secrete TGF-α [58]. Amphiregulin is also detectable in differentiated, surface colonocytes. The main EGF receptor (EGFR; ErbB1) is present on the majority of epithelial and stromal cells, as well as some smooth muscle and glial cells, and is critical for the development and physiology of the gastrointestinal tract [59]. Within the gut, EGFR is expressed on the basolateral surface of enterocytes, where it plays a role in intestinal repair and homeostasis. Activation of the EGFR stimulates its intrinsic tyrosine kinase activity, leading to activation of multiple downstream cellular substrates involved in proliferation, apoptosis and gene expression including, most notably, the Akt and Erk pathways [60].

Increased salivary EGF production, decreased urinary EGF excretion and increased ileal EGFR activation have been observed following a 50% Type 1 proximal resection in mice, suggesting increased intestinal utilization of EGF and a possible role for endogenous salivary EGF in intestinal adaptation [60]. Consistent with this notion, removal of the submandibular glands significantly reduces circulating EGF levels and attenuates the intestinal adaptive response to massive intestinal resection, and this is rescued equally by oral and systemic exogenous administration of EGF [58]. EGF therapy has also been shown to promote both structural and functional adaptation in rodent models of SBS. In a rat Type 1 resection model, enteral EGF administration increases villus height and crypt depth and attenuates enterocyte apoptosis, with greatest effects when given early after resection [61]. In a rat Type 2 resection model, enteral EGF administration prevents weight loss and improves both carbohydrate absorption and intestinal barrier function [62]. The adaptive effects of EGF are also dependent on a
functional EGFR, as oral administration of a selective EGFR inhibitor (ZD1839) abrogates the adaptive response after massive resection [63].

Similar to IGF-I, the ErbB signaling pathway has been suggested to be a downstream mediator of GLP-2 effects in the intestine. In mice, treatment with either GLP-2 or EGF up-regulates expression of amphiregulin, epiregulin, and HB-EGF, as well as of their downstream immediate-early gene transcriptional targets, c-fos, egf-1 and Phlda-1, in jejunum and colon. These effects are reduced in Waved-2 mice that express a mutated EGFR, and are absent in GLP-2R knockout mice. Nevertheless, Waved-2 mice still exhibit a trophic intestinal response to GLP-2 [64]. However, treatment with a pan-ErbB receptor tyrosine kinase inhibitor, CI-1033, abrogates the intestinal response to both exogenous GLP-2 and EGF administration. Subsequent studies using GLP-2R knockout mice revealed that EGF and not IGF-I administration rescues adaptive jejunal mucosal regrowth during re-feeding after a 24-hour fasting period. Furthermore, CI-1033 administration inhibits the adaptive crypt cell proliferation and increase in ErbB ligand gene expression following re-feeding, further supporting a role for the ErbB pathways in mediating the adaptive effects of endogenous GLP-2 [65]. Collectively, these findings indicate that signaling by the EGF family of ligands and receptors is required for the trophic effects of GLP-2, although the exact contribution of each family member remains to be fully elucidated, as does the exact relationship between the EGF and IGF families of trophic peptides in this pathway. In contrast, Hare et al. suggested that the intestinotrophic effects of GLP-2 occur independently of ErbB signaling because GLP-2
administration reverses the atrophy observed in the proximal intestine with administration of gefitinib, an EGFR tyrosine kinase inhibitor [59].

2.4 Other Peptides
Vasoactive intestinal peptide (VIP), a 28-amino acid peptide widely expressed in the enteric nervous system and that regulates gut motility, has been suggested as a downstream mediator of the anti-inflammatory actions of GLP-2. Thus, GLP-2 treatment increases the number of VIP-expressing neurons and administration of a VIP antagonist abrogates the anti-inflammatory effects of GLP-2 [29]. Yusta et al. has shown that VIP knockout mice exhibit abnormal villus morphology with increased crypt cell proliferation and enhanced expression of both IGF-I and keratinocyte growth factor (KGF). However, the growth-promoting actions of exogenous GLP-2 administration are not affected in VIP knockout mice, suggesting that VIP is not required for these effects of GLP-2 [66].

Several members of the fibroblast growth factor (FGF) family have also been implicated in the remnant intestinal adaptive response after resection. Administration of FGF-7 (also known as KGF) to rats after 55-85% Type 1 resection augments both structural (morphology and histology) and functional (basic ion transport and glucose and alanine absorption) indices of adaptation [67]. FGF-10 expression is also increased in the base of the crypts after a 50% Type 1 resection, and rat IECs treated with recombinant FGF-10 in vitro demonstrate increased proliferation and phosphorylation of Raf and Akt [68]. Finally, KGF has been implicated as a potential mediator of some of the trophic actions of GLP-2, as administration of a KGF antibody abolishes the growth effects of GLP-2 in
the colon [28]. Notwithstanding, there is only limited evidence suggesting a potential clinical role for any of the FGF family members in the treatment of SBS.

Finally, hepatocyte growth factor (HGF) and its receptor, c-Met, are expressed in the small intestine, and HGF been shown to be trophic for the small intestinal epithelium. More importantly, HGF administration to rats following an 80% Type 1 resection augments both structural and functional measures of adaptation [69]. Vascular endothelial growth factor (VEGF) has also been suggested as a downstream mediator of GLP-2, and delivery of VEGF to rats following an 80% Type 1 resection increases villus height and sucrase activity in the remnant intestine [70]. Finally, given its trophic effect on the intestinal mucosa, oral insulin was found to augment indices of structural adaptation in rodent models of Type 1 SBS [71].

3. Role of Trophic Peptides in Gastrointestinal Development

Within the context of SBS in neonates, it is critical to consider the role that trophic factor peptides play in gut development and neonatal intestinal physiology. Although our understanding of these complex processes remains limited, many of the candidate trophic peptides being studied for a role in intestinal adaptation are either present in breast milk or have known roles in gut development or neonatal gut physiology. Neonates, with their innate intestinal growth potential, may thus have an advantage in recovering from SBS in comparison with adults. A better understanding of the role of trophic peptides in gut development and neonatal intestinal physiology, including GLP-2, IGF-I and EGF, may aid in the application of these growth factors to neonatal SBS, as well as serve as a
foundation to assess the roles of other candidate peptides that harbor intestinotrophic potential.

Enteroglucagon, an L cell proglucagon-derived peptide and, thus, a surrogate marker for GLP-2, is found in low concentrations in the human fetus at 8-11 weeks of gestation, but circulating concentrations are two-fold greater than those in the maternal blood by 19-21 weeks of gestation [72]. Coincidently, the earliest time at which premature human fetuses are able to support themselves by oral feeding is 25 weeks of gestation, suggesting that enteroglucagon may play a role in human intestinal development.

Although L cells appear late during development in rodent models, in the last third of gestation, the functionality of these cells is demonstrated by secretion of GLP-2 in vitro. Furthermore, as in humans, neonatal rats exhibit high circulating levels of GLP-2, which then fall over several months to adult levels [73]. GLP-2R mRNA is also highly expressed in the fetal and neonatal rat intestine, declining to adult levels by 21 days of life. However, more importantly, administration of GLP-2 to neonatal rats increases intestinal weight and both small bowel and colon length. Altogether, these studies reveal that the developing and newborn rat intestine express a functional GLP-2-GLP-2R axis. However, the biological role of GLP-2 in fetal gastrointestinal development remains to be determined, as mice lacking either GLP-2 or its receptor demonstrate normal macroscopic and histologic intestinal development [16].
The neonatal piglet has served as an important translational model of the human infant gastrointestinal system due to its greater similarities in anatomy, ontogeny and physiology, as compared to rodents [74]. Notably, term neonatal piglets secrete GLP-2 in response to EN, and both circulating GLP-2 concentrations and intestinal mucosal growth correlate with the percentage of EN intake [30]. In TPN-dependent premature piglets, intravenous GLP-2 increases intestinal growth by suppressing proteolysis and apoptosis rather than by stimulating protein synthesis and cellular proliferation, particularly in the jejunum [18]. However, although the GLP-2R is expressed in fetal and neonatal pig intestine, there is no detectable circulating GLP-2 until the last 2 weeks of gestation. Furthermore, administration of GLP-2 to fetuses or premature piglets does not induce any trophic effects in the mucosa, suggesting that the preterm GLP-2R is either not functionally coupled to pathways involved in epithelial proliferation or is under tonic inhibition. These findings suggest that GLP-2 does not play a pivotal role in piglet intestinal development [19]. However, GLP-2 administration increases mucosal mass, villus height, digestive enzyme expression and Akt signaling in neonatal piglets, indicating maturation or activation of the GLP-2R signaling pathway at parturition.

The relevance of the findings in the piglet model, as compared to the human neonate, remains undetermined. However, early studies revealed that, as in these animal models, the human intestinal L cell is stimulated by first-feeding in human neonates. Hence, 5 ml/kg of breast milk given to term infants increases circulating levels of enteroglucagon. In contrast, these changes are not observed when 2.5 mL/kg of breast milk is given to preterm infants [75]. Conversely, significant increases in plasma enteroglucagon were
observed in both orally fed term and preterm infants but not in preterm infants on TPN. Whether the human GLP-2R is expressed and functionally coupled to trophic signaling pathways at this time remains to be established. However, in human infants (mostly premature) undergoing intestinal resection, there is a strong correlation between GLP-2 levels and residual small bowel length. When a meal is given, circulating GLP-2 levels also correlate with tolerance to EN and nutrient absorption, although not with indicators of intestinal permeability. GLP-2 levels also correlate with likelihood for infants to achieve TPN independence, with a postprandial GLP-2 level of 15 pmol/L being discriminatory. Collectively, these findings suggest that the human GLP-2 – GLP-2R axis is functional in the human neonate [10].

A role for IGF-I in intestinal development is suggested by the expression of IGF-I and, to a greater extent, IGF-II in the human fetal (16-20 weeks gestation) intestine [76]. Similarly, both TGF-α and EGF mRNA are detectable in the human fetal intestine at 15-20 weeks of gestation. The presence of all of these growth factors in milk also suggests a role in the postnatal development of the neonate and the neonatal gastrointestinal tract [77].

4. Trophic peptide therapy in preclinical models of neonatal SBS

The neonatal piglet and the human infant share similar features of the intestinal adaptive process following surgical resection, although this occurs much faster in piglets (weeks versus months to years). This is in contrast to adult rodents, wherein the adaptive process occurs within days following even a 90% proximal intestinal or 60% distal intestinal
Furthermore, rats adapt via increased mucosal surface area with decreased brush border enzyme activity, whereas humans and piglets adapt via villus hypertrophy leading to increased nutrient uptake. These findings further suggest that preclinical studies utilizing piglets provide an important translational model for the potential use of trophic peptide therapy in neonatal humans with SBS. Similar to the three subtypes of SBS encountered in humans (Table 2), piglets with Type 2 and 3 anatomies are difficult to maintain due significant fluid and electrolyte losses, malabsorption and reduced intestinal adaptation. However, as in humans, piglets with Type 1 SBS anatomy are easier to maintain due to a highly pro-adaptive remnant intestine, which may be secondary to the presence of the retained ileum and L-cell mass producing endogenous GLP-2 (Figure 1). Importantly, in a juvenile (4-week old) piglet model with Type 1 SBS anatomy, GLP-2 therapy increases the number of proliferating cells in the remnant ileal epithelium. However, GLP-2 treatment was associated with several negative outcomes in this model, including decreases in weight, serum albumin levels and ileal digestive enzyme expression, as well as ileal villus atrophy. One of the caveats of this study was that weaned juvenile pigs were used, which precludes translation to human neonates with SBS, and subsequent studies have therefore utilized piglets at an earlier age, either neonatal (2-5 days old) or preterm (92% of gestation) to better approximate the age group in humans most commonly affected by SBS.

The effects of intravenous human GLP-2 therapy have recently been examined in neonatal piglets (2-5 days old) with Type 1 and Type 2 SBS anatomies consequent to 75% intestinal resection. Following GLP-2 therapy for two weeks, Type 2 SBS
piglets exhibit fewer days on PN, more days on EN alone with more EN overall, and fewer days of diarrhea in comparison to saline-treated surgical controls. These improvements in clinical outcomes were not observed in Type 1 SBS piglets following the same GLP-2 therapy regimen, although GLP-2 therapy increased intestinal length, jejunal villus height, crypt depth and markers of cell survival in both models. The effect of exogenous GLP-2 has also been studied in a piglet model of Type 3 SBS, an anatomy that reflects the clinical scenario commonly seen in newly-resected infants with SBS. In this setting, preterm (92% gestation) piglets on TPN do not display remnant intestinal adaptation and there is an absence of compensatory endogenous GLP-2 production in response to both resection and enteral stimulation. However, continuous infusion of GLP-2 for 5 days increases the relative absorption of fluid, energy and macronutrients, commensurate with increases in intestinal mass, villus height and crypt depth (but not proliferation) and digestive enzyme activity [82]. These results are in a contrast to a similar study using neonatal piglets in which subcutaneous teduglutide increased bowel weight per length, and protein synthesis, but decreased DNA concentration and had no effect on digestive enzyme activity, intestinal permeability or nutrient absorption [83]. Finally, whether the adaptive effects of teduglutide are complemented or enhanced by concomitant administration of EN was examined in 2-day old piglets with a Type 1 anatomy and on either PN alone or 80% PN with 20% EN. Interestingly, the adaptive effects of teduglutide and EN were complementary with regards to increased glutamine transport, while synergistic effects on intestinal and colonic structural growth responses were observed. Through principal component analysis, crypt depth emerged as a highly predictive outcome of neonatal intestinal adaptation. However, teduglutide alone had a
positive effect only on ileal mass and cell differentiation and the only long-term functional benefit was an increase in ileal peptide transport at 7 days [84]. Collectively, these are the first studies to show clinically-relevant benefits of GLP-2 therapy in preclinical models of SBS that mimic human neonatal SBS. These studies further suggest that continuous infusion of GLP-2 may provide significant benefits as compared to intermittent administration. The potential benefits of other growth factor therapies such as EGF and IGF-I, either alone or in combination with GLP-2, using these relevant preclinical models remains to be undertaken.

5. Trophic peptides in human infants with SBS

Clinical trials and human studies investigating growth factor therapies in infants and children with SBS remain extremely limited. GH administration, in combination with glutamine, was the initial therapy studied in children with SBS. A prospective study in children determined the intestinal adaptive effect of GH therapy to be transient, and a subsequent prospective randomized open-label multicenter study found that 4 months of GH therapy had no effect on the weaning of PN in children with SBS [85,86]. The potential utility of other intestinotrophic growth factors also remains largely unexplored in children with SBS. One small study in children found that oral EGF improves carbohydrate absorption and EN tolerance [87]. Conversely, an open-label observational study did not observe a consistent decrease in PN requirement following oral insulin therapy in children with SBS [88]. Recent focus has thus been on the potential utility of GLP-2 therapy in pediatric clinical trials, given its recent approval for adults SBS. Sigalet et al. reported a Phase I-II safety study in children, revealing that subcutaneous
GLP-2 treatment of children with SBS, at a single dose, appeared to be well tolerated with minimal side effects and a pharmacokinetic profile similar to adults with SBS [89].

6. Conclusion

Short bowel syndrome and consequent intestinal failure remains a common condition seen in human neonates. Children who do not regain intestinal autonomy require long-term PN therapy but many succumb to complications. There are currently no approved therapies aimed at augmenting adaptation of the remnant intestine. Growth factors, such as GLP-2, IGF-I and EGF, are trophic to the intestinal mucosa and stimulate increases in functional capacity. The exogenous administration of such peptides has shown promise in inducing both structural and functional adaptation in rodent models of SBS. The neonatal piglet serves as a translational animal model for the human neonate and the limited studies of GLP-2 administration in piglet SBS models suggest that GLP-2 therapy may be beneficial to neonates with SBS. The study of trophic peptide therapies in preclinical models of SBS and human SBS trials is summarized in Table 3.

7. Expert Opinion

A number of different growth factors have been investigated with respect to their potential for the treatment of adult SBS, although there remains only limited data regarding the utility of such factors in neonates or children with SBS. The majority of preclinical models studied also have limited relevance to the neonate. Nevertheless, GLP-2 therapy has shown promise in translational piglet models of neonatal SBS. Notably, piglets with Types 2 and 3 SBS respond to GLP-2.
There are limitations to trophic peptide therapies to consider. The high cost of treatment may restrict the use of growth factor peptides to a last-resort therapy, when children are long-term PN-dependent and show no signs of continuing adaptation (most seen with Types 2 and 3 SBS). Furthermore, the potential requirement for life-long treatment if PN weaning is not completely successful will also be a consideration. Given the mitogenic properties of growth factors, a significant concern must also be the potential for dysplasia. Preclinical studies suggest that, while GLP-2 does not induce carcinogenesis, it accelerates the growth of preexisting tumours [90]. However, new dysplastic lesions were not detected in 77 adults with SBS receiving teduglutide for 6 months and only a single hyperplastic polyp was found in a 7-month extension study with 52 patients [91]. Considering other peptides, both the EGF/EGFR and IGF/IGF-IR axes have been implicated in the development and biology of a variety of cancers. The evidence therefore underlines the importance of carefully selecting candidate patients for growth factor therapy, potentially excluding those with pre-existing malignancy and suggesting a requirement for frequent, pre-emptive screening.

Several aspects of treatment that are unique to the pediatric SBS population will need to be considered as GLP-2 therapy is advanced from current approval in adult SBS to pediatric trials. The timing of GLP-2 administration relative to the onset of SBS is one factor to consider. The landmark randomized clinical trials in adult patients with SBS selected patients that were several years post-major intestinal resection who were considered to be stable in terms of PN requirements, the so-called chronic phase of
intestinal adaptation [40,41]. However, the preclinical studies suggest that exogenous administration of either GLP-2 or EGF is most effective when given immediately after intestinal resection, to augment the acute phase of intestinal adaptation [6,61]. Early administration of therapy may also have more potent adaptive effects in neonates and children than in adults, given the intrinsic developmental potential of the neonatal and pediatric intestine for growth.

The route and duration of administration will also be important factors to consider. Preclinical studies have shown that continuous GLP-2 exposure is required for the maintenance of intestinal adaptation in rodents, as cessation of GLP-2 therapy leads to reversal of the adaptive effects [14,92]. Similarly, the intermittent subcutaneous teduglutide administration employed in the trial with adult SBS patients reduced PN volume dependence and enhanced structural adaptation but, 4 weeks after drug discontinuation, there was a need to increase PN volume [39,41,91]. Furthermore, preclinical studies using piglets have demonstrated that continuous intravenous GLP-2 therapy stimulates both structural and functional adaptation, whereas intermittent subcutaneous teduglutide administration stimulates structural adaptation only [81-83].

In contrast to teduglutide, which has only been administered parenterally, both intravenous and oral administration of EGF to rats augment intestinal adaptation, consistent with the basolateral localization of the EGFR on the enterocyte and the detection of EGFR activity on the apical surface [61]. In further support of these findings, oral administration of EGF improved tolerance of enteral feeds and
carbohydrate absorption in 5 children with SBS [87]. Despite its candidate role as a secondary mediator of GLP-2 actions, EGF is the least studied trophic peptide.

Given the widespread effects of IGF-I in the body, oral administration of this growth factor may be preferred in order to restrict its mitogenic effects to the intestine. In addition, a recent study investigating a role for oral insulin therapy in intestinal adaptation demonstrated extensive digestion and proteolysis, which will be a concern for any potential oral growth factor treatment unless efforts are made to protect the peptide [88]. Nevertheless, preclinical studies utilizing oral IGF-I administration have demonstrated a concomitant rise in plasma IGF-I suggesting that the oral route may be feasible [52]. For drugs such as teduglutide, which are eliminated by glomerular filtration, careful determination of the appropriate dose in infants who are at varying stages of renal maturation is also pertinent.

The notion of administering several growth factors in combination remains a further consideration of potential trophic peptide therapies. The rationale behind combination therapy is that growth factors may act synergistically or the actions of individual peptides may complement one another. Thus, in mice, long-acting GLP-2 in combination with either GH or IGF-I augments intestinal growth as compared to GLP-2 alone [56]. EGF and IGF-I administered in combination has also been shown to have a synergistic effect [93]. Finally, due to the variant pathophysiology, growth factor therapies have differential effects depending on the SBS type in piglet models. This leads to the
possibility that growth factors given in combination may have a synergistic effect, depending on SBS type, although this remains unexplored.

In summary, the successful translation of peptide growth factor therapies for neonatal SBS will require a number of challenges to be overcome. Therapeutic considerations for the neonate, especially timing, target anatomy and indication, cannot be based on data from mature adult patients. Determination of the growth factor or combination of growth factors, optimal dose, route and timing, will most likely be dependent on developmental stage. Better understanding of neonatal gut development is therefore paramount. In reality, these beneficial therapeutic opportunities will have to be carefully balanced against the high costs for patient care and the potential for the development of dysplasia in the setting of potential life-long treatment with these orphan intestinotrophic agents. In this regard, trophic peptide therapies may become an important option in the armamentarium for infants with Types 2 and 3 SBS, the types most commonly encountered in human neonates and most at risk for developing irreversible intestinal failure.

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interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

**Article Highlights Box**

- Short bowel syndrome (SBS) and consequent intestinal failure occurs more frequently in neonates and children, and diseases leading to SBS in neonates often require removal of ileum.
- Unlike adults, there are currently no approved therapies aimed at stimulating intestinal adaptation in children with short bowel syndrome.
- Intestinotrophic peptides, such as glucagon-like peptide-2, insulin-growth factor-1 and epidermal growth factor have shown therapeutic benefit in preclinical models of adult short bowel syndrome.
- The limited preclinical studies investigating the potential efficacy of trophic peptide therapies in neonatal short bowel syndrome show promise with glucagon-like peptide-2 therapy, notably in short bowel syndrome without remnant ileum.
- The clinical translation of trophic peptide therapies targeted for neonates and children with short bowel syndrome will require the determination of the ideal growth factor or combination of growth factors, and of the optimal dose, route and timing of administration.
- Trophic peptides may become an important option for neonates with short bowel syndrome that lack remnant ileum and are most at risk for irreversible intestinal failure.
Reference

Reference annotations

* Of interest

** Of considerable interest


* Neonatal short bowel syndrome is a disease with high morbidity and mortality. The management of these patients is complex and requires a multidisciplinary approach. Recent advances in medical and surgical treatment options have improved outcomes. This review highlights salient points in the management of this challenging patient population.


* This article reviews the normal physiology of the intestine and how pathophysiology in SBS occurs when distinct regions of the gastrointestinal tract are resected.

* This review provides a concise summary of the epidemiology of SBS (incidence, ethology) and factors that are associated with better outcomes. The study also explores current limitations of published SBS data.


* This review effectively summarizes the functional definition of “intestinal failure”, as well as provides a comprehensive overview and organization of the causes of intestinal failure in children, of which SBS is the most common.


** This is a concise review of intestinal adaptation and the changes that occur at the structural and functional level. The time course of adaptation, hormonal control of adaptation and measures of intestinal adaptation are also discussed.


** This is the first report identifying glucagon-like peptide-2 as the proglucagon-derived peptide responsible for intestinal growth and proliferation.


* This is an important article outlining the localization of the glucagon-like peptide-2 receptor in humans. GLP-2R localization was limited to enteroendocrine cells of the gastrointestinal tract.


** This important recent review on glucagon-like peptide-2 by Drucker is a comprehensive and extensive review of GLP-2 biology and physiology, in regards to both its intestinal and extra-intestinal effects.


* This is an important study in rats showing that GLP-2 immunoneutralization abrogates the intestinal adaptive response following major resection.


**This comprehensive review summarizes the physiological and pathophysiological roles of IGF-I, IGF-II, and their receptors, IGF-IR and IGF-IIR, in the intestine.**


**This key paper demonstrates a role for IGF-I as a downstream mediator of the intestinotrophic effects of GLP-2.**


** This is one of the first preclinical studies to study the effect of combining trophic peptides (GLP-2, IGF-I, IGF-II, EGF and GH) on intestinal structure in mice.

* This review provides a thorough introduction to the ErbB family of ligands, with a focus on EGF, TGFα, HB-EGF, and amphiregulin, and their roles in the gastrointestinal tract.


** This key paper implicates ErbB signaling as a downstream mediator of GLP-2 effects.


* This study provides insight into the how the developing rat intestine secretes and responds to GLP-2.

** This excellent review summarizes the various animal models (rats, mice, and piglets) used to study neonatal short bowel syndrome.


** This proof-of-concept paper illustrates the generation of the Type 1 and Type 2 SBS piglet models, and how they contrast with the disease spectrum seen in human neonates.


* This preclinical study using neonatal piglets shows that GLP-2 therapy improves clinically relevant outcomes and structural adaptation in a Type 2 SBS model.


* This preclinical study using preterm piglets demonstrates that continuous GLP-2 therapy stimulates measures of both structural and functional adaptation in a Type 3 SBS model.

* This preclinical study using neonatal piglets demonstrates that subcutaneous teduglutide treatment stimulates structural adaptation but not functional adaptation in a Type 3 SBS model.


Figure Legend.

Figure 1. Distribution of GLP-2-producing L cells and GLP-2R expression along the gastrointestinal tract, and their consequential removal in the varying types of short bowel syndrome. S: stomach; D: duodenum; J: jejunum; I: ileum; C: colon

Intestinal L cells, which produce GLP-2, are found along the entire gastrointestinal tract but the majority of the L cell mass resides in the distal ileum and proximal colon. In contrast, GLP-2 receptor (R) expression also occurs the entire gastrointestinal tract, with the greatest expression occurring the jejunum relative to other portions of the intestine. The three types of SBS have varying effects on the removal of L cell mass, which impacts pathophysiology. Type 1 SBS maintains ileum and retained L cells may secrete endogenous GLP-2 that promotes remnant intestinal adaptation. In contrast, Type 2 and Type 3 SBS involves removal of most or all ileum and the L cell mass, leading to a deficiency of endogenous GLP-2 and the lack of an endogenous adaptive response in the remnant intestine.
GLP-2 Receptor Expression

GLP-2-producing L-cell mass

Type 1 SBS

Type 2 SBS

Type 3 SBS
Table 1. Neonatal Short Bowel Syndrome: Key Facts

- **CAUSES:** congenital intestinal malformations (e.g. intestinal atresia), midgut volvulus or thrombosis, post-natal ischemia or necrosis (e.g. necrotizing enterocolitis, NEC)

- **PREVALENCE:** 24.5 per 100,000 live births (353 per 100,000 premature (<37 weeks gestation) births) [94]

- **MORTALITY:** 1.4% of all deaths in children less than 4 years of age [94]

- **CURRENT TREATMENT OPTIONS:** Mainly supportive.
  - Parenteral nutrition: to support growth and development, replenish lost fluids and electrolytes
  - Parenteral lipid minimization strategies
  - Use of fish oil rather than soy oil-based lipid emulsions
  - Introduce enteral nutrition as early as tolerated
  - Severe cases may require isolated bowel or combined liver/intestinal transplantation
Table 2. Neonatal SBS: subtypes, anatomy and clinical sequelae.\textsuperscript{1-4}

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Anatomy</th>
<th>Schematic</th>
<th>Clinical sequelae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>Removal of proximal or mid-small intestine (e.g. predominately jejunum and some ileum). Jejunoileo Anastomosis.</td>
<td><img src="image1" alt="Schematic" /></td>
<td>Self-limited diarrhea. Generally well-tolerated due to remnant ileal adaptation to enhance nutrient absorption.</td>
</tr>
<tr>
<td>Type 2</td>
<td>Removal of distal small intestine (predominantly ileum). Jejunocolic Anastomosis.</td>
<td><img src="image2" alt="Schematic" /></td>
<td>Vitamin B12 and bile salt malabsorption. Severe diarrhea, fluid and electrolyte disturbances associated with greater ileal loss.</td>
</tr>
<tr>
<td>Type 3</td>
<td>Removal of some jejunum, all ileum. Creation of a jejunostomy. Lack of colonic continuity.</td>
<td><img src="image3" alt="Schematic" /></td>
<td>Same as type 2. Significant output losses with higher-level jejunostomy.</td>
</tr>
</tbody>
</table>

Table 3. Summary of Available Studies on Exogenous Trophic Peptide Therapies in Animal SBS Models and Adult and Pediatric Human Studies.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Rodent SBS Models</th>
<th>Piglet SBS Models</th>
<th>Adult SBS Trials</th>
<th>Pediatric SBS Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-2</td>
<td>[34-38, 92]</td>
<td>[80-84]</td>
<td>[39-41, 91]</td>
<td>In progress</td>
</tr>
<tr>
<td>IGF-I</td>
<td>[51, 52]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td>[61, 62]</td>
<td></td>
<td></td>
<td>1 OL study [87]</td>
</tr>
<tr>
<td>VIP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGF-7/KGF</td>
<td>[67]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGF</td>
<td>[69]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>[70]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>[71]</td>
<td></td>
<td></td>
<td>1 OL study [88]</td>
</tr>
</tbody>
</table>

SBS: short bowel syndrome; GLP-2: glucagon-like peptide-2; IGF-I: insulin-like growth factor-1; EGF: epidermal growth factor; VIP: vasoactive intestinal polypeptide; FGF-7: fibroblast growth factor-7; KGF: keratinocyte growth factor; HGF: hepatocyte growth factor; VEGF: vascular endothelial growth factor; OL: open-label
Abbreviations List

Akt: protein kinase B
AMP: adenosine monophosphate
ApoB48: Apolipoprotein B48
DPP-IV: dipeptidylpeptidase IV
EGF: epidermal growth factor
EGFR/ErbB1: epidermal growth factor receptor
EN: enteral nutrition
eNOS: endothelial nitric oxide synthase
Erk1/2: extracellular signal-regulated kinase
FGF: fibroblast growth factor
GH: growth hormone
GLP-2: glucagon-like peptide-2
GLP-2R: glucagon-like peptide-2 receptor
GLUT-2: glucose transporter 2
HB-EGF: heparin-binding epidermal growth factor
IECs: intestinal epithelial cells
IGF-I: insulin-like growth factor I
IGF-IR: insulin-like growth factor I receptor
IGF-IIR: insulin-like growth factor II receptor
IGFBPs: insulin-like growth factor binding proteins
KGF: keratinocyte growth factor
NEC: necrotizing enterocolitis
PI3K: phosphatidylinositol 3-kinase
PN: parenteral nutrition
SBS: short bowel syndrome
SEMF: subepithelial myofibroblast
SGLT-1: sodium-glucose linked transporter 1
TGFα: transforming growth factor-alpha
TPN: total parenteral nutrition
VIP: vasoactive intestinal polypeptide