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A safety and pharmacokinetic dosing study of glucagon-like peptide 2 in infants with intestinal failure☆

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ABSTRACT

Background & Aims: Glucagon-like peptide 2 (GLP-2) analogues are approved for adults with intestinal failure (IF), but no studies have included infants. This study examined the pharmacokinetics (PK), safety, and nutritional effects of GLP-2 in infants with IF.
Methods: With parental consent (Health Canada Protocol:150,979), parenteral nutrition (PN)-dependent infants were treated with 5–20-μg/kg/day GLP-2 for 3 days (phase 1), and if tolerated continued for 42 days (phase 2). Nutritional therapy was by primary caregivers, and follow-up was to one year.
Results: Six patients were enrolled, age 5.4 ± 3.2 months, bowel length: 27 ± 12% of predicted, PN dependent (67 ± 18% of calories). GLP-2 did not affect vital signs, nor were there significant adverse events during the trial. Dosing 5 μg/kg/day gave GLP-2 levels of 52–57 pmol/L, with no change in half-life or endogenous GLP-2 levels. Enteral feeds, weight, Z scores, stooling frequency, and citrulline levels improved numerically. The trial was discontinued early because of a drop in potency.
Conclusions: GLP-2 was well tolerated in infants, and pK was similar to children with no changes in endogenous GLP-2 release. The findings suggest that GLP-2 ligands may be safely used in infants and may have beneficial effects on nutritional status. Further study is required.
Level of evidence: 2b Prospective Interventional Study.

Intestinal failure (IF) because of nutrient malabsorption following intestinal resection and the subsequent requirement for prolonged parenteral nutrition (PN) is a common problem in neonates [1]. Worldwide, there is an increasing incidence of such babies, because of the increase in survival of premature infants, and the associated increased incidence of necrotizing enterocolitis (NEC); NEC is the most common cause of IF [1]. The provision of nutritional support (PN) and allowing time for the remaining intestine to heal and adapt are the fundamental therapies for this patient group [2,3]. The process of intestinal adaptation is a fundamental up-regulation of the nutrient absorptive capacity of the remnant intestine; this process occurs naturally only in response to enteral nutrient stimulation. However, in sick neonates, feeding is often delayed because of many factors. There are no standard pharmacologic therapies to improve the function of the residual intestine or hasten the course of healing or adaptation. The ability to pharmacologically stimulate intestinal adaptation would be a major advance in therapy for this patient population.

Glucagon-like peptide-2 (GLP-2 1–33) is an enteroendocrine hormone which is a key regulator of growth and function in the intestinal mucosa [4,5]. It is synthesized by the L-cells of the small intestine, which are most numerous in the terminal ileum. L-cells are situated on the basal aspect of the intestinal mucosa but project to the lumen to 'taste' the intestinal content [6]. They release GLP-2 (and GLP-1) in response to sensing undigested nutrients, especially free long chain fatty acids, in the intestine. In turn, GLP-2 activates a specific receptor, which is exclusively expressed on enteroendocrine cells, the enteric neuronal system and myofibroblasts of the intestine, but not on the epithelium directly [7]. Acutely GLP-2 slows motility, increases mesenteric blood flow and reduces enteric secretions; chronically it is trophic for the small intestinal mucosa [8–10]. In animals following intestinal resection, GLP-2 levels are strongly correlated with the amount of partially digested nutrients in the intestinal lumen; chronic elevations in GLP-2 levels stimulate an increase in intestinal surface area. This then increase absorption, and decreases the amount of residual nutrients in the lumen at the terminal ileal L-cell sensor location, and GLP-2 levels fall [9–12]. Thus GLP-2 can be thought of as a regulator in a feedback

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system or 'axis' controlling intestinal nutrient absorption, similar to the insulin–glucose axis.

In infants, this GLP-2 'axis' of nutrient-stimulated GLP-2 production in response to feeds is highly active. In fed premature infants, postprandial levels of GLP-2 are very high (up to 450 pM/L), but are low in fasted infants [13]. In infants with IF, serum GLP-2 concentrations correlate with residual small intestinal length, intestinal absorption and ultimately survival [13,14]. Thus, we hypothesized that exogenous GLP-2 therapy would improve intestinal function in this population. However, there are no studies examining the safety, metabolism, or physiologic effects of GLP-2 in infants.

The present study was planned as a phase I–II trial with the primary aim of examining the safety and pharmacokinetics of native glucagon-like peptide-2 (GLP-2) (1–33) in infants with anatomic short bowel syndrome or intestinal failure. Because this is the first study in human infants, we began with a low dose of 5 μg/kg, and planned to use higher doses once the preliminary safety study was completed. The dosing was based on a safety study using 40 μg/kg/day in neonatal piglets, supported from day 2 of life through to weaning and on to day 44, which showed that PK and metabolism in these neonatal animals was similar to that seen in adult humans [15]. Additional support for the safety in pediatric patients was provided by our recent positive experience using a dose of GLP-2 at 20 μg/kg/day in children with IF [16]. The secondary aim of the study was to obtain preliminary data to determine the nutritional effects of GLP-2 therapy, to guide future investigations in infants.

1. Methods

With institutional ethic board approval (Clin trials # NCT01573286, Health Canada Reference GLP-2-01 150,979, University of Calgary Conjoint Health Ethics Board #21691), families of infants who, on the basis of residual intestinal length had anatomic short bowel syndrome (SBS) or had demonstrated IF on the basis of not weaning from PN, were approached to participate in the study. Patients were less than one year corrected age at the start of therapy, with SBS/IF post-surgery (resection or repair of gastroschisis). Short bowel syndrome was defined as a total remaining small intestine less than 40% of predicted for gestational age, based on the expected length as published by Strujis et al. [17]. Intestinal failure was defined as a requirement for >50% of calories by PN more than 45 days from the last intestinal surgery [1,3]. The exclusion criteria were related to co-morbidities (either systemic diseases or intestinal mucosal) which would interfere with the potential for the recovery of normal intestinal function (Table 1).

1.1. Subjects

Subjects were primarily inpatient post-surgical patients who had been transitioned from the NICU to the pediatric ward for care following a resection. During the trial they were treated following the standard nutritional care protocol of the institution's intestinal rehabilitation team. Patients were enrolled at 2 sites, following a common treatment protocol. After the study, patients were cared for by the IF care team, with clinic visits for study specific review at 1, 6 and 12 months post therapy. At enrollment, demographics and anatomic data were extracted from the chart, including operative measurements of bowel length, and nutritional parameters of weight, weight gain, tolerance of enteral formula and stool output. Bowel length was expressed as both the absolute length as measured in the operating room, and as a percentage of the expected small intestinal length, based on the weight of the child [17]. Lab parameters followed were routine PN blood work (complete blood counts, electrolytes, creatinine, urea, AST, ALT, bilirubin, gamma GT, protein and albumin levels). Twice weekly, nutritional parameters and an overview of the general clinical status of the infant was obtained. Clinical status evaluation was primarily for episodes of sepsis, any unusual swelling or edema, or other potential adverse events.

During the study there were no study mandated changes in nutritional support of the patients. Advancement of feeds and tapering of PN were done by the primary care team, independent of the study. Similarly, the use of other medications, including the use of antibiotics and motility enhancing agents was at the discretion of the primary care team.

1.2. Study design

A quasi-experimental interrupted time series design with patients from a convenience sample was used. The therapeutic agent, native human glucagon-like peptide 2 (1–33), was produced as a lyophilized powder by solid state synthesis (CS Bio, Menlo Park CA) (>98% purity) and prepared as a sterile solution in alkalinized saline (0.9% NaCl alkalinized to pH 8.5–9.5 by addition of 0.05 M NaOH). Powdered GLP-2 was dissolved in individual vials (1.5 mg in 1.5 ml) following good manufacturing practices (GMP) by the experimental therapeutics program of the British Columbia Cancer Agency, Vancouver BC (Lot IC115, May 2009). The peptide was stored as a frozen solution at −20 °C until used. Stability was demonstrated by repeated testing using an identical mixing and freezing sequence, using mass spectroscopy at 6 month intervals following manufacture, with minimal loss of potency (>90% of nominal peptide concentration) until July 2014. (Testing done by Maxam Corporation, Burnaby BC, following GMP standards.)

Table 1
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Patients less than 1 year of age (corrected gestational age) AND	Significant extra-intestinal disease (e.g. grade IV intraventricular hemorrhage, severe hypoxic encephalopathy)
Anatomic SBS, with less than 40% of expected bowel length (for gestational age) and a requirement for >50% of calories by PN, more than 45 days from last intestinal surgery OR	Significant cardiovascular, hemodynamic or respiratory instability as noted by 1) requirement for dopamine >4 mcg/kg/min, 2) high frequency ventilatory support, 3)extracorporeal membrane oxygenation
Intestinal Failure with a requirement for >50% of calories by PN, more than 45 days from an intestinal resection, independent of the length of remnant small intestine OR	
Gastroschisis with a requirement for >50% of calories by PN, and more than 45 days from last abdominal/intestinal surgery	Hepatic disease defined as direct bilirubin >100 μmol/L (5.2 mg/dL)
	Renal disease defined as BUN >80 or creatinine >90 μmol/L (1.5 mg/dL)
	Inborn errors of metabolism necessitating protein restriction or other special diet
	Ongoing sepsis syndrome, as noted by refractory hypotension, thrombocytopenia, acidosis, and/or bacteremia
	Primary motility defect such as intestinal pseudo-obstruction
	Absorptive defects (such as microvillus inclusion disease)
	Coagulopathy which precludes the use of subcutaneous injections
	Allergy to GLP-2 or any of the constituents of the GLP-2 IC-115 preparation

1.3. Phase I

The first 3 days of GLP-2 administration examined the effects on vital signs, general well-being, and the pharmacokinetics of GLP-2. In the week prior to the first dose, plasma citrulline and endogenous GLP-2 levels were measured (fasting, and post-prandial if the child was feeding). The study drug was prepared by the research pharmacy of the University of Calgary, with dosing (5 to 20 μg/kg/day in divided doses) for the week based on the measured body weight and adding one half of the body weight gained over the previous week. A single child was randomized to a dose of 20 μg/kg/day prior to a change in the study design. The appropriate dose was prepared in 1-ml syringes, and then refrozen. Individual doses were thawed within 1 h of use; all injections were given using a 0.22-μm filter (Millipore, Etobicoke, Ontario, Canada) and a 23 gauge needle (Becton Dickinson, Mississauga, Ontario, Canada) using an s.c. Insuflon catheter (Unomedical, Lejre DK). Injection site preparation was done following a standard protocol for administration of s.c. medications; sites were rotated at least weekly. In order to compensate for the dead space of the filter and the needle, each injection of GLP-2 was followed by a flush of 0.6 ml of drug diluent, also prepared by the experimental pharmacy. Patients were injected twice daily at approximately 0800 and 1700, typically before meals. During the initial 3 days of the treatment, vital signs were assessed before, and at 15, 30, 60, 120 and 180 min following the injection. On a daily basis, the child's temperature, appetite, nutritional indices inclusive of general well-being, caloric intake, eating, stooling patterns, complaints of nausea and inspection of the injection site were monitored daily. Nutritional parameters monitored included weight, Z-scores, tolerance of enteral nutrition, requirement for PN, and stool output. Prior to starting the study, biochemical parameters that included electrolytes, creatinine, and liver function studies were done, following the protocol of the PN monitoring in the 7 days prior to starting the study. A PK study was done on day 3, modified for use in children to minimize the volume of blood required. Samples were taken prior to GLP-2 administration and at 60, 90 and 180 min post injection. Blood was drawn using a micro collection technique (0.5 ml/sample) and immediately aliquotted into tubes containing diprotin-A (0.1 mM; Sigma–Aldrich Inc., St. Louis, MO) and aprotinin (500 KU/mL blood; Bayer Inc., Toronto, ON) and kept on ice until processed. Samples were centrifuged at 2500 g for 10 min at 4 °C and the plasma stored at −80 °C until analysis. Plasma GLP-2 concentrations were measured using a GLP-2 (1–33) specific radioimmunoassay with an intraassay coefficient of variation (CV) of 5% as previously described [18].

1.4. Phase 2

After the first 3 days of therapy, the infants were reviewed, and if no adverse events had occurred, they were moved into phase two. GLP-2 therapy continued for a further 39 days. Vital signs and monitoring of systemic endpoints (appetite, fluid balance, general well-being, and stooling patterns) and nutritional endpoints were recorded by the study team. Patients were monitored for any adverse events including edema, pain or nausea following injections. Biochemical parameters were extracted from the chart. Any potential adverse events were reviewed by a Data Safety Monitoring Board (DSMB), which examined the potential causality of GLP-2 therapy to the adverse event. After each review, the ongoing participation of the patient in the study was at the discretion of the DSMB. Plasma citrulline levels, and the production of endogenous GLP-2 both fasting, and post-prandially, with a repeat pK post-injection of exogenous GLP-2 were repeated in the final week.

1.5. Post study monitoring

Patients remained as inpatients for 2 to 4 months following the study completion, and so data collection was done from the inpatient chart. Following discharge, they were typically seen at monthly intervals by their primary care team. Study-specific repeat assessments of physiological and nutritional parameters, biochemical indices, citrulline levels, and post-prandial GLP-2 levels were repeated at 1, 6 and 12 months.

2. Statistics and data analysis

Results were collected during the patient visit and recorded in a secure database. Descriptive statistics using mean ± standard deviation were calculated to provide information about the primary endpoints of the pharmacokinetic and safety results related to the therapy. Pre- and post-GLP-2 therapy values were compared using the Wilcoxan signed-rank test, with a p value of 0.05 set as significant.

3. Results

A total of 6 subjects were enrolled over a nineteen-month period; the patient population was one of short bowel syndrome (SBS) resulting in severe intestinal failure from birth (Table 2). All patients had severe SBS, with remnant small intestinal length averaging less than 40 cm. All received the majority of calories by PN (Table 2). The requirement for PN had been stable for 2 weeks in all patients prior to enrollment. The average chronological age at starting the GLP-2 therapy was 5.4 months. The patients tolerated the GLP-2 therapy without any effects on routine vitals. There were no changes in physiologic parameters, including heart rate, blood pressure, or temperature with injection in any patient throughout the study (Table 3).

The Insuflon injection system worked well; there were no problems with the injections or the injection site. No child developed sensitivity to the GLP-2 preparation, or evidence of an allergic reaction. There were no consistent changes in mood or appetite with the injections.

Baseline fasting GLP-2 levels were within normal limits, but endogenous post-prandial levels were low at the beginning of the study (31 ± 23 pM) neither fasting nor post-prandial levels changed from day 0 to week 6 (Table 4). Five infants were allocated to treatment with 5 μg/kg/day, or 2.5 μg/kg/dose of GLP-2; this significantly increased GLP-2 levels ($p < 0.001$); the peak levels at day 3 and day 42 were similar (52 ± 40 and 57 ± 40 pmol/L). The number of observations was insufficient to calculate a meaningful half-life. (Fig. 1). One patient was assigned to be treated with 20 μg/kg/day; after treatment with 10 μg/kg/dose; levels were 250 pM/L at 42 days; a lab error occurred on the day 3 study preventing accurate measurement of the levels. (See Table 5.)

Table 2
Demographics.

Patient ID	1	2	3	4	5	6	Mean ± STD
Birthweight (kg)	2.009	2.745	1.170	1.300	1.240	0.850	1.55 ± 0.70
Gestational age(weeks)	34	35.5	35	29.1	29	25.5	31.3 ± 4.0
Etiology of IF	Jejunal atresia	Midgut volvulus	Meconium ileus	Gastroschisis, atresia and NEC	NEC	NEC	
Remnant bowel length (cm)	21	65	45	25	38.5	31.5	37.7 ± 16
Remnant bowel length (%) [17]	12	40	41	18	35	20	27 ± 12
Age starting GLP-2 (months)	5.5	1.5	6	5	11	4	5.4 ± 3.2

Table 3
Vital signs post-injection.

VITALS	Time post injection	Day 1	Day 2	Day 3
Temperature (C)	0	36.8 ± 0.3	37.0 ± 0.2	36.7 ± 0.5
	15 min	36.9 ± 0.2	36.8 ± 0.1	36.8 ± 0.2
	60 min	37.0 ± 0.3	37.1 ± 0.2	36.7 ± 0.1
Heart rate (BPM)	0	127 ± 31	122 ± 27	132 ± 26
	15 min	131 ± 19	117 ± 31	140 ± 23
	60 min	126 ± 12	127 ± 37	123 ± 25
Respiratory rate (breaths/M)	0	41 ± 9	42 ± 11	39 ± 13
	15 min	43 ± 9	39 ± 5	42 ± 14
	60 min	41 ± 7	42 ± 6	35 ± 8
Blood pressure (MAP, mmHg)*	0	65 ± 12	61 ± 6	67 ± 22
	15 min	68 ± 14	65 ± 8	58 ± 6
	60 min	67 ± 6	64 ± 11	67 ± 16

n = 6 for each determination, Data: Mean ± StDev.
(Values shown 0–60 min, for days 0–3 of the study, no further changes were seen in the values out to 180 min after injection, and in the repeated assessments to the one year follow-up).
* Mean arterial pressure (MAP) = [(2X diastolic) + systolic]/3.

Nutritionally, there was appropriate weight gain in all subjects, with a statistically non-significant ($p = 0.44$) increase in the Z score for weight and a similarly non-significant decrease in the number of stools per day ($p = 0.63$), which was maintained once the therapy was discontinued (Table 3). There were also an increase in the citrulline levels with treatment which was maintained post therapy (7.8 ± 1.7 to 10.5 ± 3.4 μmol/L, $p = 0.55$). There was an increase in the proportion of calories taken enterally (Table 3) ($p = 0.40$) which declined in the month after GLP-2 was discontinued. There were no changes in electrolytes (data not shown), creatinine, liver function tests, serum protein, or albumin levels (Table 4).

During the study phase there were no adverse events attributed to the GLP-2 therapy. There were no episodes of sepsis during the study course, but two patients developed sepsis in the month following GLP-2 discontinuation. Patient 5 developed severe sepsis related to the central line, and died from sepsis 3 months after the trial was completed. Patient 3 had ongoing severe nutritional difficulties and ultimately underwent intestinal transplant. These events were reviewed by the Data Safety monitoring board, and judged not to be related to the trial. During the trial, the formulation was tested every 6 months for stability, and dosing was adjusted accordingly. At month 24 testing showed a significant decrease in potency of the peptide (<75% of original peptide content) and so the trial was suspended.

4. Discussion

This study shows for the first time in infants that treatment with glucagon-like peptide 2 over a 6 week period is safe, with a pharmacokinetic profile similar to that seen in adults. While the trial was not designed or powered to show changes in nutritional status, there were encouraging suggestions that GLP-2 may have a clinical benefit, as demonstrated by increased tolerance of enteral feedings, improved Z scores, a reduction in stooling frequency and a possible trophic effect on the gastrointestinal mucosa as evidenced by the increase in citrulline levels. These will require an adequately powered trial to evaluate for clinical relevance.

There were no measurable changes in vital signs at any point throughout the trial, demonstrating that the therapy did not have cardiovascular or central nervous system effects in these infants, which are the youngest subjects to have been treated with GLP-2. Additionally, there were no adverse events attributed to the GLP-2 therapy. Specifically, there were no episodes of pain or unexplained distress following the GLP-2 injections, which has been reported in adults [19,20]. These episodes have been attributed to pain from changes in bowel size and motility from the actions of GLP-2, in the setting of multiple abdominal adhesions and scarring post-operation. Chronic changes may be because of an actual increase in mucosal mass [20]. No complaints of cramping or vomiting were noted in any patient. Further, as seen in the pediatric patients treated with GLP-2, there was no evidence of fluid retention, edema, or peripheral swelling in these patients. In the present cohort, renal function, as reflected by creatinine levels was normal, and did not change with GLP-2 therapy. This is in contrast to findings seen in adult SBS patients, who often are volume depleted, which is improved with GLP-2 therapy, but there is an incidence of associated peripheral edema [15]. There were no changes in any of the hematologic or liver function values.

The pharmacokinetic data are novel; this is the first trial using GLP-2 or a similar hormone metabolized by dipeptidyl peptidase IV (DPP-IV), in human infants. The result suggests that the distribution and metabolism of GLP-2 in infants is similar to the findings in children and adults [15,18,21]. While it is difficult to compare peak levels directly the present results suggest that they may be lower in infants. The average peak level in adults was approximately 1400 pM, after an s.c. injection of 8 μg/kg of GLP-2: in children the peak levels were 600 pM following an injection of 10 μg/kg [16,21]. In the current study peak levels were in the order of 150 pM after receiving 2.5 μg/kg. Further, the apparent half-life after injection in the adult studies was ~120 min, while in the current study, the apparent half-life was ~45 min. It is extremely difficult to do pK studies in this age group, because of the restricted sampling that is possible in these small patients. Within these limitations, the results suggest a greater volume of distribution, and a more rapid clearance of GLP-2 delivered by s.c. injection in infants, compared with pediatric patients. These differences may be because of age-related variations of the volumes within specific tissue compartments, the activity of DPP-IV, subcutaneous blood flow, or the difference in the formulation and the carrier solvent for the GLP-2 used in these studies. It is unlikely that the differences were because of the assay; the methodology used was consistent in all of these studies.

Results related to the pharmacokinetics of GLP-2 suggest a difference in the volume of distribution and metabolism of the GLP-2 between adults and infants; however this will require further direct study. These findings also highlight the limited understanding of the activity of the DPP-IV system in the developing human [21]. As drugs and hormones are developed which are metabolized via this pathway, there will be a need to examine it in greater detail in pediatric patients.

Table 4
Nutritional and growth indices.

	Day 0	Day 42	1 m post	6 m post	12 m post
Number of patients	6	6	6	5	4
Weight (kg)	4.7 ± 1.6	5.3 ± 1.2	5.8 ± 1.4	8.2 ± 1.5	9.9 ± 0.9
Z score (weight)	−2.34 ± 2.0	−2.0 ± 1.9	−2.0 ± 2.4	−0.6 ± 0.5	−0.7 ± 0.4
% calories by PN	66 ± 16	54 ± 17	59 ± 22	46 ± 28	25 ± 18
% calories by EN	34 ± 16	46 ± 22	41 ± 40	54 ± 32	75 ± 46
Stools/Day	5 ± 0.7	4.3 ± 1.0	4.5 ± 1.7	4.2 ± 1.5	3.8 ± 2.2

Data: mean ± StD.

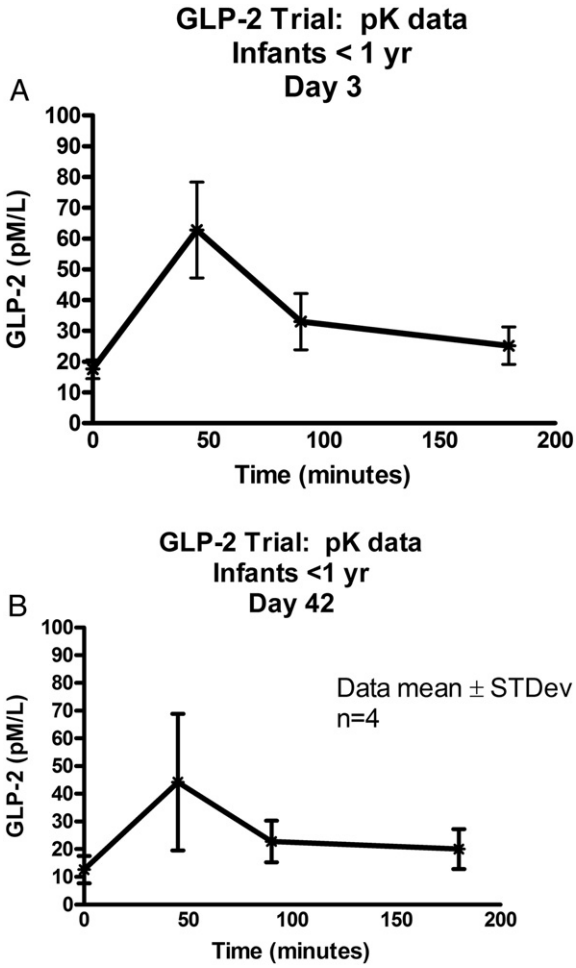

Fig. 1. Pharmacokinetic studies. PK studies done at a) 3 days of therapy b) 42 days of therapy. Data: mean of plasma concentrations of intact GLP-2 (1–33) after injection of GLP-2: 2.5 μg/kg given subcutaneously, measured at times: 0 (pre-injection), 60, 90 and 120 min after injection. Plasma GLP-2 levels in pmol/L as determined by radioimmunoassay [18]. Values: mean ± STD, n = 4 at each time point.

The secondary endpoints of this study related to the effects of GLP-2 on nutrition; while limited by the small sample size and short treatment period, the results are encouraging. All of the patients showed an improvement in their tolerance of enteral nutrition, all gained weight at an appropriate rate for their age, and 5 of the 6 had an improvement in Z scores for weight. Stooling frequency or the volume of stoma output also decreased. A trophic effect on the intestinal mucosa is suggested by the trend of an increase in citrulline levels over the study; interestingly this did not reverse when therapy was stopped, and indeed continued to improve over the 1-year follow-up of the study [22]. This may indicate that the structural effects of GLP-2 are more long lasting in this age group, when compared to older children and adults. Alternatively, a long-lasting increase in intestinal mucosal mass may also have been stimulated by the trophic effects of the nutrients themselves. Regardless of the mechanisms, the observed improvements in tolerance of enteral nutrition, and increase in mucosal mass, are precisely the hallmarks of intestinal adaptation that are sought after as therapeutic endpoints in the care of patients with IF.

Another important secondary endpoint examined was the effect of exogenous GLP-2 administration on the production of endogenous GLP-2. Over the limited time course of the study, there were no effects on either fasting or post-prandial GLP-2 levels. Thus, exogenous GLP-2 does not appear to reduce the production of endogenous GLP-2, which may be important for long-term maintenance of intestinal autonomy. Further complicating the picture, after adaptation or up-regulation of nutrient absorption occurs, the levels of endogenous GLP-2 drop [9]. Other factors, such as an age-related change in endogenous GLP-2 production, or sensitivity may also be relevant. These factors should be included as potential confounding factors in planning studies future studies of the efficacy of GLP-2. A cross-over study design would seem most appropriate, to control for spontaneous adaptation that may occur during the study period. Additional considerations should include careful evaluations of nutritional requirements, absorption and growth over a longer time course, with protocol-driven reductions in PN if EN tolerance increases.

This study design was limited by the relatively short dosing period of 6 weeks, which was a regulatory limitation corresponding to the length of treatment in the supporting large animal dosing study [16]. Further, as the trial evolved, recruitment was slower than anticipated, and so the stability of the actual GLP-2 formulation became an issue. The decision to suspend the trial was made when the peptide content dropped below 75% of the original formulation. This was based both on the potential impact on efficacy (which could have been compensated for by increasing the dose), and also the difficulty in identifying the precise breakdown products which were accumulating.

In the neonatal and infant population, patients with apparently severe SBS often do much better than anticipated; conversely some patients who would seem to have adequate lengths of intestine may not show adaptation. With this study, we have established that GLP-2 is well tolerated in this age group; future studies should aim to establish the appropriate dosing. Ultimately, therapy to induce intestinal adaptation may broadly be aimed at two major therapeutic objectives: to allow adaptation and weaning from PN in those very severe cases of SBS who might be PN dependent for years, and to speed the adaptive process in less severe cases, to reduce the time of hospitalization and allow for more rapid resumption of a normal lifestyle for affected children, and their families.

Table 5
Liver and renal function.

	Normal Range	Day 0	Day 14	Day 42	1 m post	6 m post	12 m post
GLP-2 (fasting, endogenous) pmol/L	17 ± 5 *	19 ± 17	N/A	11 ± 7	N/A	N/A	N/A
GLP-2 (Post-prandial, endogenous) pmol/L	72 ± 8 *	31 ± 23	N/A	21 ± 7	37 ± 41	38 ± 29	39 ± 24
HGb (g/L)	106–225	103 ± 14	99 ± 19	107 ± 21	111 ± 22	109 ± 14	128 ± 16
WBC (10^9/L)	5.0–19.0	11.7 ± 3.3	11.4 ± 4.5	8.8 ± 2.7	14.7 ± 3.6	8.8 ± 4.9	7.9 ± 2.3
Platelets (10^9/L)	150–400	219 ± 61	217 ± 65	242 ± 51	227 ± 62	183 ± 83	202 ± 47
Creatinine (μmol/L)	20–60	17.2 ± 2.3	15.8 ± 2.5	16.0 ± 3.4	16.5 ± 3.5	16.0 ± 3.6	21 ± 4.0
AST (U/L)	10–65	179 ± 118	176 ± 121	128 ± 115	69 ± 39	116 ± 86	46 ± 10
ALT (U/L)	1–35	220 ± 166	210 ± 155	185 ± 204	71 ± 44	80 ± 47	41 ± 25
Bilirubin(total)	0–23	49 ± 27	43 ± 27	32 ± 29	14 ± 18	44 ± 18	3 ± 0.6
Gamma GT (U/L)	8–63	181 ± 58	157 ± 59	121 ± 82	80 ± 91	44 ± 38	22 ± 16
Total protein (g/L)	54–79	57 ± 7	58 ± 8	55 ± 4	56 ± 6	56 ± 7	60 ± 1
Albumin (g/L)	30–50	29.6 ± 2.2	29.4 ± 3.0	32.0 ± 2.1	30.0 ± 5.0	28.7 ± 12.1	33.5 ± 6.4
Citrulline μmol/L	6–35	7.8 ± 1.7	N/A	10.5 ± 3.4	10.8 ± 6.7	15.2 ± 9.9	18.0 ± 6.0

Data: Mean ± StDev.

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The authors declare no competing interests.

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