University of Alberta

Role of Glucagon-like Peptide-2 and Elemental Formula in Short Bowel Syndrome – Using Neonatal Piglets as an Animal Model

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science

in

Medical Sciences - Paediatrics

Spring 2012
Edmonton, Alberta

ABSTRACT

Recovery from short bowel syndrome (SBS) requires intestinal adaptation, dependent on enteral nutrition (EN) and peptides, like glucagon-like peptide-2 (GLP-2). The purpose of this thesis was to investigate endogenous GLP-2 production and compare elemental versus polymeric formula in a neonatal animal model of SBS. Piglets were assigned mid-intestinal resection (JI); distal-intestinal resection (JC); or to a sham group. Postoperatively piglet's commenced parenteral nutrition (PN), tapering as EN (elemental or polymeric) was increased. JI piglets had shorter PN duration ($p<0.01$), longer villi ($p<0.01$), deeper crypts ($p<0.01$) and higher plasma GLP-2 ($p<0.001$). Adaptation did not occur in JC piglets, while polymeric formula increased their duration of PN support ($p<0.05$) and plasma GLP-2 ($p<0.05$). This thesis shows that endogenous GLP-2 production is increased in association with adaptation in SBS piglets with ileum (JI). Polymeric formula increased endogenous GLP-2 in piglets without ileum, but did not benefit adaptation in neonatal SBS.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor, Dr. Justine M Turner, a very nice physician and mentor. She gave me the chance to study in the University of Alberta, so I had the opportunity to come to Canada, viewing this beautiful country. She introduced me to this pretty interesting research area: neonatal short bowel syndrome. During the past two and half years, she taught and guided me in how to do research, helped me to improve my writing and presentation skills, let me attend national and international conference, and inspired me to overcome difficulties and gain confidence. Without her help and supervision, it would have been impossible for me to finish my thesis on time with good quality.

I want to thank Dr. Paul W Wales in the University of Toronto, a very nice surgeon and co-supervisor. He taught me the surgical technique during the surgery, which I was much enjoyed. He also taught the technique in data analysis and software use, making me can understand my results more preciously.

Thank you to Dr. Diana R Mager, a registered dietitian. She is very kindly and introduced me to Dr. Justine M Turner, so I got the opportunity to meet this lovely team and work with them. She also kindly offered me the help with the nutritional aspect in my thesis. I would like to thank Dr. Ron Ball. He helped me with course selection, and data analysis. He also gave me helpful advice in result interpretation and result presentation.

Thank you to my colleagues in the lab, Pamela Wizzard, Charlane Gorsak, Rhian Ormond, and Christine. Pamela Wizzard taught me a lot about research technique and made me feel comfortable doing research with piglets and working in the lab. Rhian Ormond and Christine helped me with daily care during the weekend, so made me not in a muddle when Pam was not in the lab. Dr. Patrick Nation in our team did all the histological analysis for us and I am very

appreciated his help. He watched thousands of slides and it was very time consuming.

Moreover, I want to thank Dr. David Sigalet in the University of Calgary, and his technician Laurie Wallace in the Dr. Sigalet's lab. They help to have a better understanding about GLP-2 and the technique in ELISA, RT-PCR and slide preparation.

I want to thank my parents especially. They were very supportive when I decided to study aboard after I got my undergraduate degree. During the time in Canada, they always encouraged me when I had problem and without their care and support, I was not able to focus on my study and research.

Also, I thank my boyfriend, who read as a master's student in parallel. He encouraged me to go to Canada to receive a high-quality education. Otherwise, this memorable journey would have been impossible. He helped me a lot when I was very busy with courses studying and lab work. I am sure I would not be able to finish lab work with good results in courses without his support. Moreover, he made my life in Edmonton enjoyable and colorful, instead of boring and lonely!

I extend special thanks to all my friends in the department, in Edmonton, and in China. They helped me a lot when I confronted technical and personal difficulties. All of them helped and encouraged me to keep learning and to live happily!

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LIST OF ABBREVIATION

SBS - Short bowel syndrome

CAPS - Canadian Association of Pediatric Surgeons

TPN - Total parenteral nutrition

NEC - necrotizing enterocolitis

PN - Parenteral nutrition

PNAC - Parenteral nutrition associated cholestasis

ICV - Ileocecal valve

EN - Enteral nutrition

SCFAs - Short-chain fatty acids

LCFAs - Long-chain fatty acids

CO_2 - Carbon dioxide

EGF - Epidermal growth factor

IGF - Insulin-like growth factor

KGF - Keratinocyte growth factor

GH - Growth hormone

TGF-a - Transforming growth factor-a

NT - Neurotensin

PYY - Peptide tyrosine tyrosine

GRP - Gastrin-releasing peptide

CCK - Cholecystokinin

GLP - Glucagon-like peptide

CCPR - Crypt cell proliferation rate

7-TM - 7 transmembrane

PC4/TIS7 - Pheochromocytoma cell-4/TPA Induced Sequence 7

DPP-IV - Dipeptidyl peptidase IV

JI - Jejunoileal anastomosis

JC - Jejunocolic anastomosis

DAB - Diaminobenzidine

RT-PCR - Reverse transcription polymerase chain reaction

Ct - Cycle threshold

ANOVA - Analysis of variance

SD - Standard deviation

GGT - Galactosylhydroxylysyl glucosyltransferase

ALP - alkaline phosphatase

ALT - Alanine aminotransferase

E - Elemental formula

P - Polymeric formula

SBL - Small bowel length

SBDW - Small bowel dry weigh

pM - pmol/L

GLP-2R - GLP-2 receptor

eNOS - Endothelial nitric oxide synthase

1 LITERATURE REVIEW

1.1 Short Bowel Syndrome

1.1.1 Introduction

Amongst the clinical problem managed by pediatric specialists, short bowel syndrome (SBS) is a serious one that consumes time and resources (1). The definition of SBS by the Canadian Association of Pediatric Surgeons (CAPS) is the need for total parenteral nutrition (TPN) longer than 42 days, or a remaining small intestinal length less than 25% of expected for gestational age after intestinal resection (2). One cause for pediatric SBS is congenital anomalies, such as gastroschisis and atresias. Another course is acquired inflammatory disorders, like necrotizing enterocolitis (NEC) and Crohn's disease, for which infants or children need to undergo bowel resection (1). Amongst these causes, NEC is the most common one for neonatal SBS, particularly for preterm infants, accounting for at least 30% of reported cases (3-6).

Short bowel syndrome along with other intestinal disease, like severe extensive motility disorders and congenital diseases of enterocyte development, can causes intestinal failure (7). Intestinal failure is a condition of severe intestinal malabsorption with parenteral nutrition (PN) required for survival, growth and development, as a result of reduced functional gut mass below the minimal amount necessary for digestion and absorption to maintain these functions (8). In pediatrics, SBS is the most common cause of intestinal failure (6).

1.1.2 Incidence of Pediatric Short Bowel Syndrome

Estimating the incidence of SBS accurately is not easy because the definition of SBS differs from study to study, comprehensive follow-up is often lacking and the study population heterogeneous and not easy to clearly define (6). According to a population based (Toronto, Canada) estimate of the incidence of SBS, the overall incidence is 24.5/100,000 live births, with a higher incidence of 353.7/100,000 live births in preterm

infants (<37-week gestation) and lower incidence, of 3.5/100,000 live birth, in term infants (2). Based on the same study, SBS patients were more premature than the patients without SBS (30.7 vs. 35.9 weeks) (2). Gestational age was used to stratify surgical NICU admissions and premature newborns had a higher incidence of SBS (43.6/1,000 vs. 3.1/1,000 admissions) (2). Moreover, extremely low birth weight (401 to 1,000 g) was related to an increased risk of surgical SBS of approximately twice that of infants with birth weight between 1,001 and 1,500 g (1.1% vs. 0.4%) (9). Besides, SBS associated complications are also higher in preterm infants. For example, liver disease or parenteral nutrition associated cholestasis (PNAC) is more common amongst premature infants than older infants (10). The reason for this difference likely relates to the physiological immaturity of the liver in preterm infants (11). Finally, the overall incidence of SBS is higher in infants with NEC (8%), than infants without NEC (9). For extremely low birth weight infants, the incidence of NEC is 10%, and the related mortality as high as 30% (12, 13). In addition, since neonatal intensive care medicine has been improving recently, the neonatal mortality rate in preterm infants due to cardiac and respiratory diseases may actually decrease, while the proportionate mortality due to SBS will increase (14, 15).

1.1.3 Nutrients Digestion and Absorption

The small intestine, the main organ for nutrients digestion and absorption, has three major components - duodenum, jejunum, and ileum. Most macronutrients, including most protein, carbohydrates and some fat, are absorbed in the jejunum, which has longer villi, a larger absorptive surface area, higher permeability, higher enzyme concentration and more transporters, than the distal small intestine (16, 17). On the other hand, the ileum in the very distal small intestine, has shorter villi, less ability for nutrient absorption, except for specific absorption of vitamin B_{12} and bile salts (16). The ileum and colon are main sites for fluid and electrolyte absorption due to the tight junctions of the epithelium (16).

1.1.3.1 Carbohydrate

Carbohydrates are an essential energy source for maintenance and growth in human, and they include starch, cellulose, glycogen and free disaccharides. These carbohydrates are hydrolyzed into monosaccharides, glucose, galactose and fructose, by carbohydrases in the brush border of the enterocyte, especially in the proximal small intestine (18). After digestion, these monosaccharides can across the brush border membrane and basolateral membrane by either active transport, facilitated diffusion or passive diffusion. The active transporter sodium glucose cotransporter (SGLT1) is a sodium-coupled glucose-galactose cotransporter, which can transport D-glucose, glucoside, and D-galactose (19). Glucose transporter GLUT5 is a low affinity brush border membrane transporter responsible for specific facilitative fructose transport, again with its highest concentration being in the jejunum, (18). In the basolateral membrane, GLUT2, is a sodium-independent transporter for glucose, galactose and fructose (20).

Apart from the absorption in the upper small intestine, carbohydrates can also be taken up and used by the colon. Dietary fiber and unabsorbed carbohydrates reaching the colon can be fermented by resident bacteria to short chain fatty acids (SCFAs) and reabsorbed by colonocytes, accounting for potentially up to 5-10% of total dietary energy in adult humans (20).

1.1.3.2 Fat

Fat is another important dietary energy source, representing 30-40% of the daily calorie intake in an adult (21). The proximal jejunum is the main site for fat absorption, but the ileum can also absorb fat well if the dietary amount ingested is increased (22, 23). Compared with carbohydrate, the digestion and absorption of lipids is very complex. Lipid in the intestinal lumen must undergo many steps prior to absorption, including emulsification, hydrolysis of fatty acid ester bonds by esterases and aqueous dispersion of the lipid products to form micelles with bile acids (20). The pancreatic enzyme lipase plays an important role in hydrolysis as it splits each triglyceride into two molecules of

fatty acid and one of 2-monoglyceride (21). The formation of micelles, which needs the bile acids, is a critical step that makes fatty acids and monoglycerides able to move across the aqueous layer adjacent to the microvilli of the enterocytes, a process called aqueous dispersion. Fatty acids and monoglycerides can then simply diffuse into the enterocytes, through the lipid based microvillus brush border membrane, after dissociation from the micelles. The absorbed fatty acids and monoglycerides in the enterocyte must then be re-synthesized into lipid and bind to apolipoproteins and other molecules, followed by the delivery into chylomicrons, for eventual lymphatic transport (21).

Bile acids are hence, a very important component in fat absorption and digestion. They are secreted from the liver into the duodenum (24). After dissociated with fatty acids and monoglycerides, bile acids in the intestinal lumen can be reabsorbed, principally in the ileum utilizing a specific receptor, and finally be transported back to the liver. This process is called enterohepatic circulation and it is a critical process in bile acid homeostasis, as the amount of reuptake determines the total bile acid pool size, the activity of the bile acid synthesizing enzymes and the rate of bile flow from the liver (25).

1.1.3.3 Protein

Protein is necessary in the diet, to maintain positive nitrogen balance and provide essential amino acids. In the human body, protein is used for cellular structure, formation of antibodies and formation of protein mediators or enzymes, hence the control of cellular metabolism (26). In the stomach zymogen precursors are antocatalytically activated into gastric proteases and begin the digestion of protein (27). This is known as the gastric phase of digestion, leading to a mixture of polypeptides as well as small amount of oligopeptides and amino acids entering the intestine (27). This phase is not as important in protein digestion as the subsequent digestion in the intestine, the intraluminal phase (27). In the duodenum, with its neutral or slightly alkaline environment, gastric pepsins are inactivated and the pancreatic inactive zymogens are activated by a brush border

enteropeptidase (28). In the intestinal lumen, the activated pancreatic proteases, including trypsin, chymotrypsin, elastase, and carboxypeptidase A and B, further digest protein to free amino acids or oligopeptides (2-6 amino acids) (29). After this process, these free amino acids and oligopeptides can reach the small intestinal mucosa and are finally digested to amino acids, dipeptides, and tripeptides by brush border peptidases (28). Carrier-mediated peptide transporters then absorb the hydrolyzed amino acids, dipeptides, and tripeptides. In humans, although the absorption rates of amino acid in the proximal jejunum is higher than in the ileum, the absorption of peptides appears to be efficient in both the proximal and distal small intestine (27, 30).

1.1.3.4 Minerals and vitamins

Similar to the macronutrients, the minerals calcium, magnesium and iron are maximally absorbed in the proximal small intestine (31). Calcium is a essential nutrient for bone mineralization and its absorption involves an active transcellular transport process, as well as a passive paracellular process (32). Compared with the active process, which mainly occurs in the duodenum, the passive process occurs throughout the small intestine, although overall the major site for calcium absorption is the upper small intestine (33). In the small intestine, iron can only be absorbed in the form of Fe^{2+}, reduced from Fe^{3+}, by a brush border membrane protein (34). Zinc can also be absorbed by the iron transporter, but with lower affinity (20). Copper, playing a role in enzyme catalyzing reactions, is absorbed similar to calcium by two processes, the active process competing with zinc (32, 35). The absorption of phosphates is carried out by the sodium-phosphates cotransporter (20). In mineral absorption competitive interactions exists, for example, copper absorption can be impaired by high levels of zinc; zinc absorption can be reduced if the intake of iron is 2 times more than zinc (36).

Most water-soluble vitamins, except vitamin B12, are also absorbed in the upper small intestine. In the intestinal lumen, some vitamins are absorbed in an active manner,

such as biotin and nicotinic acid; some vitamins are absorbed as a facilitated way, such as riboflavin (37). Vitamin B_{12} is a necessary nutrient for DNA synthesis, red blood cell production and nervous system cellular maintenance, and unlike the other water soluble vitamins it is mainly absorbed in the ileum (38). The fat-soluble vitamins, A, D, E, and K, not surprisingly, have a similar manner of absorption as dietary lipid in the intestine.

1.1.3.5 Water and electrolytes

Under normal conditions, most electrolytes are absorbed in the small intestine, but the capacity to absorb sodium is limited (39). The colon, on the other hand, has a greater ability to absorb water and sodium, and is the main site for water and electrolyte absorption. More specifically, the absorption of water, sodium, and chlorine is higher in the right than left colon (40). Sodium, is an essential nutrient for blood volume and pressure, osmotic equilibrium and pH regulation and growth in children. It is absorbed by enterocytes, co-absorbed with glucose or amino acids, or as an exchanger in the brush border membrane (32). The relative locations for digestion and absorption of nutrients in the healthy intact gastrointestinal tract were shown in **Figure 1.1.3-1**.

1.1.4 Impact of Congenital Loss or Intestinal Resection

1.1.4.1 Nutrition deficiency

After extensive surgical resection, loss of absorptive surface area will decreased microvillus enzymes and transporters, thus maldigestion and malabsorption occur and lead to malnutrition and chronic dehydration, in SBS. Children, especially those with extensive small intestinal resection, are particularly vulnerable to malabsorption, malnutrition and growth failure (41). Usually the degree of malabsorption is determined by the amount and site of resection, as well as the presence of the ileocecal valve (ICV) and colon (42). Studies in rodents show fat and energy malabsorption happens with 90% proximal small intestinal resection and protein malabsorption with a 50% resection (43). More than a 75% proximal intestinal resection increased water content in the stool, and

thus caused diarrhea (43). For adult humans who are maintained only on oral intake with less than 100cm of remaining small intestine, they can absorb 40-50% of enteral energy, resulting in the need for almost twice the amount of enteral energy intake (44).

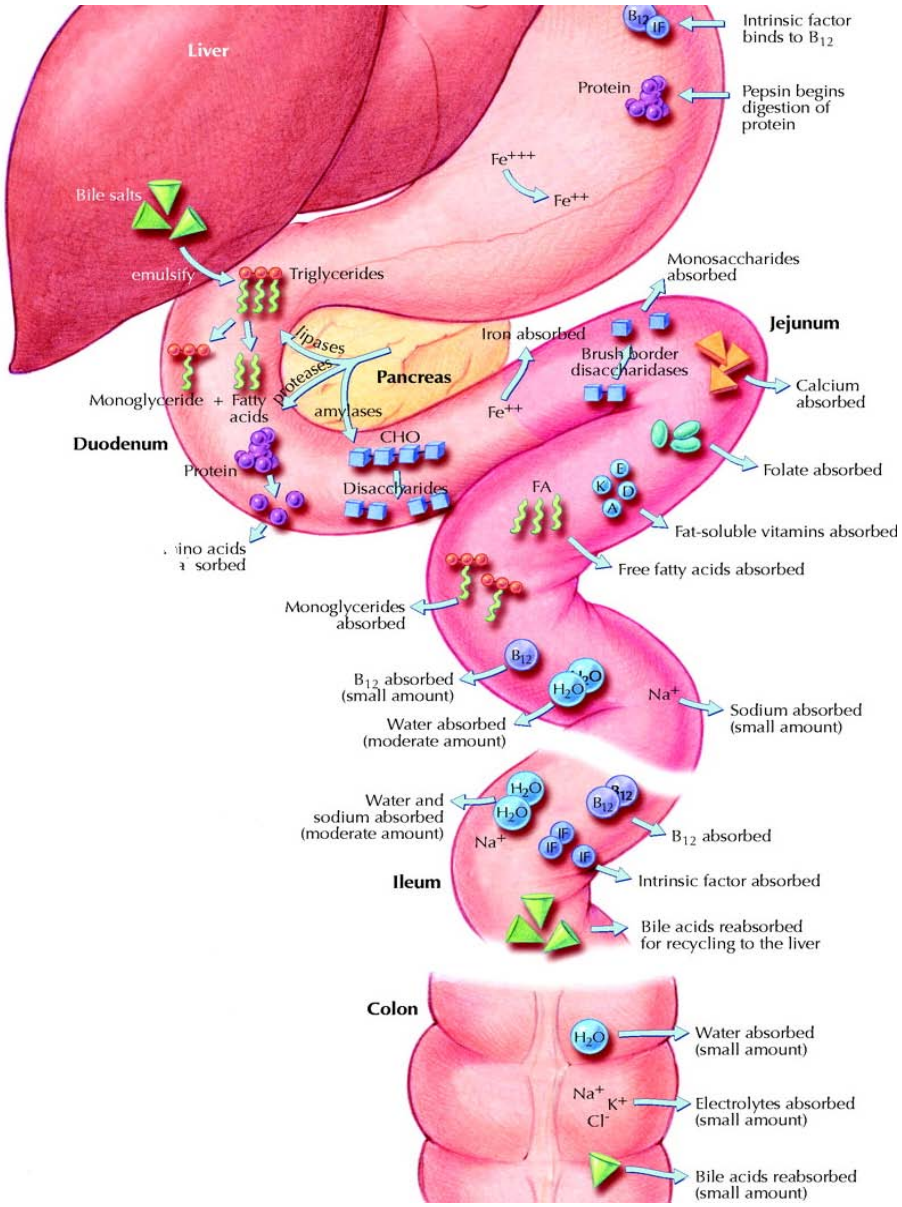

Figure 1.1.3-1 Relative locations of digestion and absorption of nutrients in the healthy gastrointestinal tract http://ecmaj.ca/content/166/10/1297/F1.large.jpg

Considering the resection site, as aforementioned, the absorptive capacity for most nutrients is highest in the jejunum and less in the ileum (45). However, while absorption

from the jejunum can be compensated by terminal ileum after a resection of the jejunum, the function of the terminal ileum cannot be replaced by the jejunum (46).

1.1.4.1.1 Effect of proximal small intestine loss

The proximal small intestine is the main site for protein absorption. Therefore resection of the proximal intestine will lead to diminished protein content in cells and organs, thus impairing cellular function, so called protein energy malnutrition, that leads to increased morbidity and eventually to death (26). Apart from protein, the duodenum and proximal jejunum are also important for magnesium and calcium absorption, so for patients who lose these parts they will have hypomagnesemia and hypocalcemia. This leads to muscle weakness, osteoporosis and depression (44, 47). The proximal jejunum is also the main site for water-soluble vitamin and iron absorption, but deficiencies of these nutrients are not common unless there is very extensive resection, as absorption occurs very proximally. Zinc deficiency may cause poor growth and impair wound healing and the process of intestinal adaptation, that compensates for the reduced absorptive surface area by proliferation of the remaining mucosa (48, 49).

1.1.4.1.2 Effect of distal small intestine loss

After terminal ileum resection, which is particularly common in neonatal SBS, the absorption of vitamin B_{12} and bile salts will be impaired. Although the synthesis of bile salts can increase after intestinal resection, the increased hepatic synthesis will not keep up with large losses. Neonates and infants are particularly vulnerable as compared with adults, their bile salt pool size is very small and the bile acid concentration in the duodenal juice also very low (50). To participate in fat absorption, a minimum concentration of bile acid is required (51). Therefore, fat malabsorption will occur, the result of impaired fat emulsification and steatorrhea will eventually developed (24). This condition is common in newborn infants, especially premature infants, even without intestinal resection (24). So as fat is an important energy source for growth, worsening

steatorrhea will markedly decrease energy uptake in young SBS patients and cause fat-soluble vitamin (A, D and E) deficiency (52). The loss of ileum is also related to an increased risk of biliary sludge and gallstones, given the role of ileum in bile salt reabsorption and hence bile flow rates (53). Further still in the colon unabsorbed long-chain fatty acids (LCFAs) can bind magnesium and calcium and aggravate potential hypomagnesaemia and hypocalcaemia.

For SBS patients, the presence of the ICV has traditionally been considered important for patients' nutritional status because of its role in inhibiting gastric emptying, so called ileal break. Loss of the ICV will therefore decrease intestinal transit time, impair the mix of chyme and enzymes, and thus further impair digestion (54, 55).

1.1.4.1.3 Effect of colon Loss

The colon is the main site for water and electrolytes absorption and colonic resection, as a result of resection or discontinuity (as with a diverting stoma), will lead to large fluid and electrolyte losses or even large volume diarrhea with hypotension and renal failure (44). These losses often leads to complications like dehydration, sodium imbalance or metabolic acidosis (1, 44, 56-58). During the early phase of intestinal failure post resection, fluid and electrolyte balance is a major problem, as it may continue to be through the entire period, prior to adaptation (1). In patients who have extensive intestinal resection, particularly with colon loss or discontinuity, excessive fluid secretion may be life threatening (59).

Considering all these nutritional complications together, SBS infants, especially the extremely low birth weight or premature infants, who are also most likely to have distal small intestine loss from NEC, will experience growth failure compared to their peers without SBS (9).

1.1.4.2 Disturbed motility and bacterial overgrowth

In neonatal SBS, disturbed motility of the remaining intestine and secondary bacterial overgrowth are major problems. Bacterial overgrowth is almost always present when motility is slowed, the bowel is dilated, the propulsion of chyme in the gut is delayed or the ICV is absent (1). Usually, prevention of excess bacterial proliferation in the normal gut requires the combination of normal peristalsis, gastric acid, production mucosal barrier and immune function and the ICV (1). The ICV acts as a natural barrier to stop bacteria in the colon migrating up into the small intestine. Patients without the ICV will have bacterial overgrowth in their small bowel, these bacteria will compete for nutrients in the gut and hence this problem will worsen malnutrition (60).

Disturbed motility of the remaining intestine and bacterial overgrowth can also lead to feeding intolerance, hence frequent episodes of fasting, and hence increase the risk of biliary sludge, gallstones and PNAC. Bacterial overgrowth may be a major factor in the production of colitis or ileitis in SBS with resulting inflammation similar to seen in Crohn's disease. This bacterial induced mucosal inflammation will also exacerbate malabsorption in SBS (1). Furthermore, bacterial overgrowth may lead to bacterial translocation and increase the risk of sepsis and its complications, with both bacteria and bacterial derived toxins gaining access into the portal and systemic circulation (1).

In addition, bacterial overgrowth will result in the deconjugation of bile salts and thus deplete bile salt pool, impair micellar solubilization and result in steatorrhea and malabsorption of fat-soluble vitamins (vitamins A, D, E and K) (1). Bacteria may also compete with the host for some nutrients, especially vitamin $B_{12,}$ and therefore absorption of this important nutrient may be compromised, even when ileum is present (1). Other complications of bacterial overgrowth include D-lactic acidosis and colitis or ileitis (1, 61). D-Lactate, which is only produced by bacteria, can accumulate in the bloodstream, resulting in neurological symptoms such as disorientation and frank coma (1). All these

factors combine together with sepsis, liver cholestasis and liver failure the most severe consequences of SBS, related to bacterial overgrowth (62).

1.1.5 Short Bowel Syndrome in the Preterm Infants

For SBS patients, different patient ages and varying developmental maturation of the intestine, relates to different degrees of severity and risk of complications. Preterm infants with low birth weight, especially extremely low birth weight, are at the most risk due to gut immaturity and existing nutritional deficits at birth (63). The reason for this immaturity is missing of the important intrauterine developmental phases in gut maturity, which takes place principally during late gestation. Therefore, preterm infants who are born prior to completion of this developmental phase have impaired intestinal maturity. Moreover, while absorption and digestion, have already developed well by twenty-eight weeks of human gestation, motor function develops many weeks later, so preterm babies have an increased risk of intestinal stasis and bacterial overgrowth (64, 65). Furthermore, compared with term infants, premature infants are more likely to have NEC, a common causes of neonatal SBS likely to lead to extensive resection of ileum and colon (2, 66). Therefore these premature babies are more sensitive to loss of intestinal length, microbial colonization, metabolic and endocrine changes, dysmotility of the gastrointestinal tract and malnutrition (65, 67).

1.1.6 Clinical Management

Since the major problems for SBS patients are impaired enteral nutrient usage, the goal of treatment is to improve absorption, optimize nutritional status and support normal growth and development; as well as control related complications and comorbidities. Amongst all these treatments, inducing adaptation of the remaining intestine is perhaps the most important goal as it leads to recovery of the intestine both structurally and functionally, and hence enteral autonomy (68).

1.1.6.1 Nutritional approaches

The nutritional management of SBS is a multistage process, beginning with TPN (1). Providing adequate calories and amino acids for growth, PN has enabled SBS patients to survive. However, PN support should be limited, due to its specific complications Ideally it should be replaced by enteral nutrition (EN) as soon as possible. Specific nutritional therapies may be required, such as for patients who have lost ileum, when long-term exogenous vitamin B_{12} supplementation will be necessary. However, intestinal failure management relies principally on adjusting caloric intake to maintain normal weight gain, the EN being increased as the PN is decreased (1). This process takes place over a period of weeks to years, often starting with continuous feeds and then weaning to bolus feeding and introduction of solids (1). The process that allows autonomy from PN is of course intestinal adaptation.

Luminal nutrition is essential for intestinal adaptation but in addition, will maintain saturation of transport carrier proteins in the small intestine and thus can promote better absorption independent of adaptation (1). We consider EN as an essential stimulant of adaptation in SBS as it can induce adaptation and will prevent atrophy of the remaining intestine. For SBS patients, providing EN also seems to reduce the complications of PN. Luminal administration of a significant percentage of total calories, usually between 20 and 30% of the total daily requirements, can reduce the risk of PNAC (1). Even lower amounts reduce the risk of sepsis. Therefore, early introduction of EN and ensuring patients are able to progress towards meeting all nutritional requirements without PN, are crucial objectives of SBS clinical management.

Methods to control high output, as a result of increased fluid and electrolyte losses, especially after colon resection, include restriction of hypertonic fluid intakes, which promotes stool osmotic fluid and electrolyte losses, particularly when containing simple sugars. To maintain hydration at least it appears advantageous to provide enteral glucose-saline solutions with a sodium concentration of at least 90 mmol/L throughout

the day (69). EN also plays an important role in the regulation of secretory function of the small intestine as intestinal hypersecretion can be caused by starvation or no EN in experimental animals (59, 70, 71). Therefore, after resection early introduction of luminal nutrition, even non-nutritive feeds (that is not providing amounts that support nutrition), is beneficial to reduce high output, as long as they are not hyperosmolar.

1.1.6.2 Pharmacological therapies

Giving drugs to reduce intestinal motility or secretions can also be used to reduce high output (44, 72). Medical strategies to control diarrhea include loperamide, a medication to slow rapid transit and cholestyramine to deal with bile salts malabsorption induced diarrhea, by binding malabsorbed bile salts (44). A variety of oral antibiotics have been used to treat bacterial overgrowth, commonly, metronidazole is used for anaerobic overgrowth or gentamicin for gram-negative organisms (68). In order to normalize the flora and limit bacterial overgrowth, probiotics, including lactobacilli and saccharomyces, have been used, although efficacy is unclear (73).

There are additional medical therapies that remain unproven to date, like recombinant growth hormone to stimulate adaptation and ursodeoxycholic acid to reduce PNAC (74). Systematic review showed human growth hormone can benefit adult SBS patients for weight gain and absorption, but the effect does not last and patients return to their baseline shortly after the therapy is stopped (75). Unfortunately, the ideal duration and safety of this hormone is also unclear. Data in the infant population is currently not available (75). Even less commonly used therapies include pancreatic enzymes to compensate for the inadequate mixing caused by rapid transit and ursodeoxycholic acid to prevent gallbladder stasis and PNAC, despite negative clinical trials (42, 76).

1.1.6.3 Surgical therapies

Surgical therapies can augment the above medical therapies. In the past, several surgical procedures have been attempted to improve nutrient absorption. These include

reverse segment procedure, which reversed segment of small bowel, and colonic interposition, all designed to slow transit (42, 68). However, these are rarely used today.

Since, with adaptation after resection, the remaining intestinal caliber can increase, it may result in ineffective peristalsis and cause intestinal stasis, malabsorption, bacterial overgrowth and sepsis (77). Therefore, surgical procedures that can reduce the luminal diameter, especially with preservation of all of the mucosa, are actually of greater relevance. Bianchi described a procedure that can increase small intestinal length and reduce intestinal caliber by tapering bowel without mucosal loss (78). In this procedure, the dilated intestine was split longitudinally with the blood supply preserved on each side, and two hemi-loops, new intestinal tubes, created by auto-suture stapling along the longitudinal axis. Finally, an end-to-end anastomosis of the two new intestinal tubes was created, in an isoperistaltic arrangement (78). For this procedure, a remaining small intestine $\geq$ 40 centimeters, with dilated segment $\geq$ 20 centimeters and diameter $\geq$ 3 centimeters is required (79).

Recently, a novel intestinal lengthening procedure, named the serial transverse enteroplasty, has been reported (80). This procedure can significantly increase intestinal length with varying tapering determined by the surgeon. In this procedure, dilated bowel is converted to a zig zag-like channel of about 2-2.5 centimeters in diameter, by stapling in a sequential alternating in opposite direction, transverse and partially overlapping fashion (80). Compared with the previous procedure, this one does not require minimum length of intestine. Instead of merely doubling the dilated length, the extent of increase in intestinal lengthen depends on the amount of dilation and diameter of the new intestinal tube decided by the surgeon (81). Clinical application of this procedure in SBS children shows promising clinical and biochemical outcomes, but long-term follow-up is not yet available, so further studies are warranted (80).

Finally, when all these conservative therapies fail, patients with impending or overt liver failure and/or irreversible intestinal failure without the possibility of adaptation and recurrent complications, like line sepsis, recurrent dehydration, loss vascular access or a gastrointestinal tract that cannot be reconstructed, will need liver and/or small bowel transplantation, in order to survive (42). These patients may need isolated intestine, combined liver-intestine, isolated liver or multivisceral transplants (42). Generally, SBS children who need isolated intestinal transplantation have somewhat better prognosis than those needing combined liver and intestinal transplantation (82, 83). Similarly, compared with an isolated intestinal transplant, a combined liver and intestine transplant is often related to a longer time of PN, more related morbidity and a more prolonged recovery time from the operation (84). Although recent reports do suggest outcomes of intestinal transplantation have improved, there are still many problems, especially for young children; not the least of which is a long time waiting time for an appropriate small-sized donors to be found. It is estimated that half of all children listed for an intestinal transplant will die on the waitlist, especially from complications of liver failure for patients with PNAC awaiting a combined liver-intestinal transplant (84). Meanwhile, postoperative management of these patients, including immune suppression, remains very challenging, with more complications and reduced long term survival, compared with other organ transplantation (84). The survival rate one year following intestinal transplantation is around 93%, while long-term survival rates is only 50-60% (68, 84). Given the relatively high morbidity and mortality of intestinal transplantation, SBS infants will not be considered for this unless as a last resort (84).

1.1.7 Parenteral Nutrition and Its Complications

After its was first introduction in the late 1960s, PN was recognized as life saving therapy for SBS infants (85, 86). PN can promote the same energy balance, net utilization of substrates for oxidative metabolism and nitrogen balance as EN (87). Moreover, PN

calories and composition can be changed according to developmental requirements during growth. Previous studies have already shown the ability of TPN to support optimum growth in infants during their first two years of life (88). Given the intravenous route for this nutrition, patients who cannot receive enough nutrition from bowel absorption can expect normal nutritional status. The length of duration of PN therapy is determined mostly by the length and site of intestinal resection. In most infants with SBS, PN is absolutely necessary at some time for survival, but for those with very short intestinal length, particularly without colon in continuity, PN dependency is for extended periods of time (42). As intestinal adaptation occurs, patients can gradually increase EN and decrease PN. Eventually, if they can rely on EN to grow normally, the PN can stop. For neonatal SBS, small bowel length less than 40 centimeters is related to long duration of PN support (89).

While the long-term survival rate of SBS has improved, the life-threatening problems for infants and children are mostly related to complications of the PN (62). The major complications are: PNAC, which may present steatosis, cholestasis or even cirrhosis; and catheter-related problems, particularly sepsis, often exacerbated by bacterial overgrowth; and metabolic derangements (1, 90, 91).

PNAC is especially common in children receiving long-term PN, and the incidence also increases in younger children and particularly preterm infants (5). PNAC occurs in 40% to 60% of infants receiving prolonged PN. In one study children with SBS were shown to depend on PN for a longer period (an average of 16.5 weeks) with an incidence of PNAC of 67%; compared to children without SBS shorter PN duration and rates of PNAC of only 30% (92). Similarly, this problem is more severe given SBS. Another study showed SBS patients have 5-fold increased risk of developing liver failure in PNAC than other abdominal surgery patients without SBS (93). Infants with PNAC can have early onset histologically observed liver damage that include cholestasis, fibrosis

and eventually cirrhosis (91). A rise in conjugated bilirubin, the first sign of cholestatic liver disease, can be seen as early as after only two weeks of TPN in infants (94). Infants with SBS on PN commonly develop biliary sludge, 20% develop cholelithiasis, while steatosis is more common in adults (92, 95). The cause of cholelithiasis is multifactorial, related to malabsorption of bile acids, altered bilirubin metabolism and gallbladder stasis (1). Biliary disease in this group of patients is frequently complicated and associated with increased morbidity (96). Biliary sludging is progressive with a incidence of 6% at 3 weeks and 100% by 6-13 weeks after starting PN (97).

A variety of causes for PNAC with cholestasis and progressive liver fibrosis have been postulated. These include direct toxicity of PN amino acids or other components such as lipids and mineral contaminants; competition of amino acids and bile acids for transport across the canalicular membrane; production of toxins in the unused bowel, particularly secondary to bacterial overgrowth and bile acid decongugation; toxic substances that contaminate the PN solutions, such as phytosterols; and lack of EN with lack of stimulation from gastrointestinal hormones that would normally increase biliary flow (1). Recently, the use of novel omega-3 lipids has been proposed as treatment for PNAC, with the suggestion that it is the high omega-6 content of current parenteral lipid emulsions that is related to the liver disease (98).

Catheter-related complications are also a significant problem in children requiring prolonged PN, occurring at a rate around 67.8% for SBS infants and 23.7% for infants without SBS (1, 93). These complications include catheter breakage, central venous thrombosis and catheter-related bacterial or fungal sepsis (91). These complications are again highest in infants under a year old (99). Compared with neonatal patients with abdominal pathology, but no SBS, neonates with SBS had increased risk of gram-positive, gram-negative and fungal sepsis, with an average of 0.5 septic events per month (93). Not surprisingly, in young children with SBS, the mortality rate from liver failure, sepsis, and

multiple organ dysfunction is as high as 37.5% while the mortality rate in patients without SBS is 13.3% (93). Liver failure accounts for 60.0% of the observed mortality in SBS (93).

1.1.8 Prognosis and Outcome

The prognosis and outcome of neonatal SBS is determined by several factors, like the remaining small intestinal length, the presence of the ICV and colon, PNAC, sepsis, central venous catheter related complications, underlying diseases and availability of multidisciplinary intestinal rehabilitation. Pediatric patients with less than 12 cm of small intestine are hardly able to wean off PN (100-102). In another study, presence of colon in continuity is associated with shorter duration of PN support (53). Similarly, in another pediatric SBS study, the presence of the ICV and the remnant small bowel length were both related to duration of PN support (41). However, in contrast another study using multivariate models of predictive factors for intestinal adaptation in a large neonatal cohort did not find the presence of colon to be predictive (103). One reason for these disparate findings could be the that the absence of the ICV, or disruption of continuity, is actually more a marker for resection of ileum, with reduced potential for adaptation (6).

A requirement for longer periods of PN support, especially in infants requiring higher amounts of lipid for growth, places children with SBS at higher risk of developing PN associated complications and hence of poor outcome (104). Actually, excluding death shortly after surgery as a result of complications, increased mortality rate found at around 8-12 months post surgery as the result of sepsis and PNAC (6). Once again, in young children with SBS, the mortality rate from liver failure, sepsis, and multiple organ dysfunction is much higher than the mortality rate in patients without SBS, and liver failure accounts for 60.0% of deaths (93).

1.1.8.1 Mortality rate

Similar to incidence, mortality rates are difficult to estimate, because follow up of all participants is necessary to avoid observation bias with an adequate duration for the for the event to occur (6). In SBS mortality rates are reported to be quite different between studies, probably in part due to patient selection that often excludes early deaths, and hence underestimate the total burden of mortality (6). The mortality rate of surgical SBS infants compared to non-SBS surgical infants is 5 times greater (2). The mortality rate during the initial or first hospitalization was 25% in extremely low birth weight infants (9). More infants with NEC (20%) are reported to die during initial hospitalization than those infants without NEC or with SBS (12%) (9).

There are temporal trends in mortality that may also relate to trends in treatment over time, particularly multidisciplinary care teams. In 1955, the mortality rate was as high as 50% given intestinal length was less than 40 cm, especially without ICV (105). In 1991, according to Goulet et al, the overall mortality rate in neonatal SBS was 20%, and for those with remnant intestine longer than 40 cm it was only 8%, compared to 34% for those with remnant intestine less than 40 cm (106). In 1999, anther study showed the mortality rate was zero when children had a remnant intestine longer than 13 cm or had ICV remained (107). In that study four out of five with less than 13 cm of remaining intestine and four of nine without an ICV died. The overall mortality rate was 23.5% (107). Collecting data from 1986 to 2002 Schalamon et al showed the overall mortality rates of SBS in pediatric samples range from 15% to 25% (62). A more recent cohort study observing neonates with SBS between January 1977 and July 2011 showed a mortality of 37.5% (93).

However, intestinal length is not the only factor to consider, in fact considering absolute remaining intestinal length independent of developmental potential, especially for preterm infants, is rather nonsensical (6). A neonatal SBS study that applied a percentage of the expected length at each gestational age, rather than an absolute length,

showed that a small bowel length less than 10% of expected for gestational age is the main predictor of death (41). In addition it must be considered: what region was resected, what is the functional capacity of the remnant intestine, what is the gestational age of the patient, hence their growth potential, and what is the primary diagnosis (6).

1.1.8.2 Quality of life and cost

The treatment for SBS and the prognosis of these infants has improved because of the development of specialized multidisciplinary care teams (108). Data shows that the overall mortality rate has improved significantly during recent decades (62, 89). Even for children with extensive intestinal resection long-term survival is not impossible. However, pediatric SBS remains one of the most lethal conditions in early childhood. The coordinated implementation of successful surgical, nutritional and pharmacological treatments must be important factors, beyond the patients specific factors mentioned above (109). However, there is no doubt that long term dependence on EN and PN, often continuously delivered for infants, with frequent hospital and team visits is a burden for SBS patients and their families (3). Such therapy is also very expensive, the cost of care of SBS patients ranges from $100,000 to $150,000 per year per patient (62).

1.2 Intestinal Adaptation

Small bowel resection induced adaption is a complex process dependent upon the extent and site of resection, the presence of luminal nutrients, the release of enterotrophic peptide factors, pancreaticobiliary secretions, cytokine and other tissue derived cell signaling molecules, as well as neuroendocrine signals that include both sympathetic and parasympathetic pathways (110-112). Although intestinal adaptation will happen after any intestinal resection, and there is no minimal length required to promote this process, the intensity of the adaptation process is correlated to the extent of resection (41). Importantly, the potential for mucosal adaptation is less in the jejunum than in the ileum, so jejunum resection will have better overall outcome than terminal ileum resection

(113-116). This discussion will focus on nutrition and specific enterotrophic factors in promoting adaptation.

1.2.1 Adaptation Process

1.2.1.1 Structural adaptation

Adaptation is a resection-induced compensatory response for the reduced absorptive surface area and this process includes both structural and functional responses. The structural adaptation is best characterized by epithelial hyperplasia, with longer villi and deeper crypts (1, 117-121). Adaptation is a crucial process following resection and it can be an important indication of survival potential and prognosis for the SBS patient (122). Although histological features of adaptation are rarely studied in humans, due to the need for tissue collection, they are well described in animal models, including some dynamic morphological parameters, such as crypt cell proliferation rate (CCPR) and the enterocyte migration rates (123). Intestinal adaptation is dependent on many factors, including patient age and intestinal development, the site and length of the remnant intestine, the patients nutritional status, underlying diseases, and the presence of hormones and growth factors involved in intestinal adaptation (112). Intestinal adaptation is the most important factor that determines if patients with SBS and intestinal failure can to get enough nutrients enterally for growth and keeping survival without PN (91, 100).

Structural adaptation is a necessary to compensate for the loss of absorptive mucosal mass following intestinal resection. The typical changes of structural adaptation include increased villus height, crypt depth and intestinal cellularity, with increased CCPR, and thus increased intestinal mass and absorptive surface area. **Figure 1.2.1-1** shows the normal small intestine and adaptive small intestine with increased caliber. **Figure 1.2.1-2** shows three different histological sections that show normal and adapting villi and crypts. In animals we observe specific alterations in intestinal cell turnover, with cell hyperplasia at the base of the crypts, cellular migration up the crypts, and elongation of villi, as well

as suppression of epithelial apoptosis (49, 112). While evidence of structural adaptation in human are scarce, it has been shown that dilatation and lengthening of the residual small intestine occurs (112, 124-127). Unlike rodent which undergo hypertrophy during adaptation, the process in humans is hyperplasia without hypertrophy (128). The total number of cells increases with proportional increase in mucosal DNA and protein, so the number of epithelial cells increases per unit length of crypt/villus (129).

Adaptation begin with stem cells, localized at the bottom of crypts (130). Normal maintenance of mucosal health also occurs from stem cells, a process of both cell differentiation and migration. It takes about seven days for all types of differentiated cells to move in a continuous wave up the crypt to villus tip, where eventually they are shed (131-133). These normal proliferation and differentiation events are balanced by apoptosis of senescent cells (134). The balance favors proliferation in adaptation.

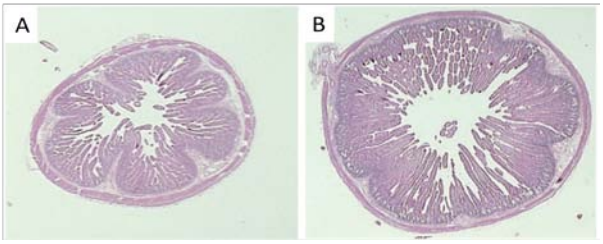

Figure 1.2.1-1 Morphological comparison of intestinal adaptation. Hematoxylin- and eosin-stained A: TPN fed piglet; B: TPN fed piglets with human GLP-2 had higher villi (135).

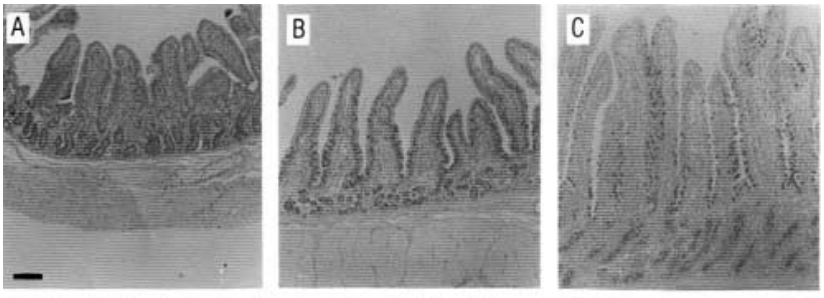

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Figure 1.2.1-2 Histological comparison of intestinal adaptation

A: normal villi; B: villi after intestinal transection; C: villi after intestinal resection, significant higher than normal villi and villi after intestinal transection (136)

1.2.1.2 Functional adaptation

Functional adaptation, represents the improvement in absorption function and an improvement of that function per unit mass. During this process, activities of intestinal enzymes, brush border membrane fluidity and transporters are altered (49). During the first three months following resection a decreased stool volume over time will occur, the result of the improved ability to absorb fluid and electrolytes, the earliest functional adaptation responses seen post resection (112).

Functional adaptation can be viewed in two separate categories, non-specific and specific (137, 138). Non-specific adaptation increases the uptake of all nutrients simply by the process of structural change, that increases the intestinal mucosal mass and/or absorptive area. Specific adaptation is a process that change the uptake of particular nutrients by regulating the specific nutrient transporters (137-139). For example, th sodium-dependent glucose transporter in the brush border membrane increases (140). In some studies, the specific activity of enzymes are actually observed to decrease with mucosal hyperplasia (129, 141, 142). One reason could be that the hyperplasia after resection leads to a increased production of functionally immature enterocytes.

1.2.2 Role of Temporal Regulation in Intestinal Adaptation

Adaptation commences immediately after resection, functional adaptation starts within 24h and within 24-48h, enterocytes begin to increase replication in crypts (143). Within 2 weeks in experimental animals, mucosal mass can be seen to dramatically increase, brush border membrane surface area as well as absorptive area increase by day 10 after resection, and the small intestine can have a fourfold increase in mucosal surface area by two weeks (1, 144).

Although intestinal structural adaptation is presumed to happen simultaneously with functional adaptation, it does not always appear to be the case (117, 145). Actually, after resection, it seems that there are separate signals for both acute and chronic adaptation, as

well as for structural and functional adaptation (146). In addition, different from the structural response, which appears to be maximal within weeks, functional adaptation can continue for months to years before full adaptation is achieved (112). In children it can take about 1 to 4 years to reach the maximal adaptation (112).

1.2.3 Role of Nutritional Regulation in Intestinal Adaptation

As a general principal, the most important stimulus to adaptation is the presence of food in the intestinal lumen, with an additional effect of endogenous secretions circulating hormones and gut-derived peptide factors (147). The presence of luminal nutrients can greatly stimulate the process of adaptation. Actually, EN is absolutely necessary for maintaining mucosal mass and promoting mucosal recovery in both acute and chronic enteropathies (110, 115, 147-149). Although EN might not be an indispensable factor in adaptation, adaptation can be greater with stimulation from EN than without EN (147). The converse is true and EN deprivation will impair intestinal adaptation (150, 151). In normal circumstances, mucosal hyperplasia will not happen without enteral nutrition, even some degree of mucosal atrophy may happen if no nutrients are provided enterally (1, 148). This mucosal atrophy can happen very rapidly, with reduction in CCPR within hours of food withdrawal (152-155). In human studies both starvation and TPN cause a fall in brush border enzyme activity (156, 157).

Three major mechanisms are involved in the EN stimulated intestinal adaptation: firstly, direct stimulation of hyperplasia through contact of the epithelial cells with intraluminal nutrients; secondly, stimulation of release of upper gastrointestinal secretions, that are trophic to the small intestine; and thirdly, stimulation of the secretion of trophic gastrointestinal hormones, principally from the distal small intestine and colon (115, 158-162). The stimulation of EN as a result of direct contact with luminal nutrients may even not require active absorption or local mucosal metabolism of the substrate (158). Therefore, the difference in ability to adapt seen between jejunum and terminal

ileum may also be related to the presence of nutrition in the gut. After the jejunum is resected the ileum is exposed to much more chyme than normally, but after the ileum is resected, the luminal chyme in the jejunum won't change much, or exposure may actually decrease, given rapid intestinal transit (163).

The content of the EN, including carbohydrates, protein and fat, can also influence the process of intestinal adaptation because they can regulate their corresponding transporters in the intestinal tract (164).

1.2.3.1 Role of enteral protein in intestinal adaptation

Protein has a role in the intestinal morphology and amino acid transport activity (165, 166). One study using rats showed that in the jejunum, increased amino acid uptake, especially non-essential amino acid uptake, occurs with a higher protein diet (167). In addition, high concentrations of polyamines, a compound with two or more primary amino groups, including spermine, spermidine and putrescine, are found in rapidly proliferating tissue such as the small bowel epithelium and stimulate mucosal hyperplasia (168-170).

Many contradictory studies exist regarding the different effects of polymeric (intact protein) and elemental (amino acids) formula on small intestinal growth and adaptation (171). In the clinical management of intestinal failure elemental formula is often recommended (16, 82). The most obvious reason for this is that less process of digestion is required of elemental formula before absorption. In infant rats hydrolyzed casein appeared to be as trophic as whole protein in stimulating intestinal adaptation (171). On the other hand in more mature rats compared with an amino acid diet, protein diet was associated with less digestibility and a longer duration of diarrhea (17 vs. 12 days), hence greater muscle protein loss (172). Although this difference did disappear after a period of time (172). Such intolerance might be related to increased intestinal transit as a result of

SBS with less time for digestion of protein, gastric hypersecretion impairing protein digestion or the increased osmotic load (173). Given better tolerance, amino acid based formula may help SBS children wean from PN (173). However, studies using piglets and rats suggest that post resection polymeric formula is superior to elemental formula in promoting morphological and functional adaptation of the remaining intestinal (174-176). In fact, intact protein in the gastrointestinal tract may protect endogenous or exogenous growth factors by blocking the active sites of pancreatic enzymes that usually inactivate these protein growth factors during fasting, and thus helps maintain their function (177). Nevertheless, there is also a short term study in children with SBS children that showed difference between intact protein and amino acids in intestinal permeability, weight gain, energy and nitrogen balance (178). Therefore, the benefits of either elemental or polymeric formula need further exploration.

1.2.3.2 Role of enteral carbohydrate in intestinal adaptation

Carbohydrate also plays an important role in intestinal adaptation. Adult SBS patients, with a preserved colon, can use unabsorbed carbohydrate to salvage energy from SCFAs by bacterial fermentation (179-181). In dogs a diet enriched with fermentable fiber increased glucose uptake and expression of GLUT-2 transporter (182). Furthermore, directly providing SCFAs to rats can increase glucose uptake and GLUT-2 expression after bowel resection (183). Amongst the SCFAs, butyrate is believed to have more potential ability to increase GLUT-2 mRNA than acetate or propionate (184). In addition, SCFAs have a high caloric content, can be quickly absorbed, stimulating water and electrolyte absorption and can be readily metabolized by the intestinal epithelium (185).

In adult SBS patient ingesting 50g carbohydrate, up to 20% of ingested carbohydrate is estimated to pass into the colon; almost 48% of this unabsorbed carbohydrate will be fermented in the colon (181, 186). More importantly, there appears to be no upper limit for carbohydrate absorption for adult SBS patients (180). As a result, fecal excretion of

carbohydrate will not increase as carbohydrate intake increase, making high carbohydrate diets reduced fecal energy loss compared to high fat diets (180). However, controversy about the benefits of high carbohydrate diets exists, particularly for infants. Patients with a high carbohydrate diet have increased flatulence, abdominal pain and potential increased D-lactic acid (187). Besides, osmotic diarrhea due to the carbohydrate load can decrease the uptake of bile salts, fat and fat-soluble vitamins (188). The benefits of high carbohydrate diets are not proven for infants, rather an adverse effect may exist, due to the risk of NEC related to increased carbohydrate malabsorption and fermentation, especially in preterm infants (189). Compared with term babies and adults, preterm infants have already limited ability to digest lactose, because they lack the late gestation phase of lactase development (190).

1.2.3.3 Role of enteral lipid in intestinal adaptation

Lipid is the most important macronutrient for dietary energy and appears to play a pivotal role in intestinal adaptation (191). One characteristic of lipid is its involvement in the alteration of the fatty acid composition of membrane phospholipids, which can further influence the activity of membrane transporters (192, 193). The fluidity of the brush-border membrane can be influenced by the ratio of unsaturated to saturated fatty acids, as well as the cholesterol and ganglioside / glycosphingolipid content in the EN (194). Changed fluidity may influence the permeability of the membrane and the expression of binding sites for proteins. As a result, the uptake of luminal nutrition will change according to the composition of dietary fatty acids. For example, compared with saturated fatty acids, polyunsaturated fatty acids reduce glucose and galactose uptake (195). Moreover, dietary lipid can alter metabolism, growth, cell differentiation and hence intestinal adaptation by regulating gene expression (196).

Considerable evidence suggests that among all the lipids, long-chain triglycerides are the most trophic (191). However, unlike carbohydrate, which can be used well by the

distal intestine, LCFAs cannot be completely used by the remaining intestine after resection. This limitation may be even worse in infants, especially preterm infants, with lower luminal concentrations of pancreatic lipase and bile salts (197). Alternately, medium-chain fatty triglycerides, despite lack of evidence of benifit for adaptation, have been suggested as a good dietary fat source for SBS patient (191). This because they are more readily absorbed (directly into the portal blood) and hence are potentially better tolerated.

According to the argument of benefit and adverse effects of each dietary component, choosing the appropriate diet to optimize the outcome in SBS is complex and requires individualization, considering age or developmental stage, underlying disease, extent and site of resection.

1.2.4 Role of Humoral Mediators Regulation in Intestinal Adaptation

Many humoral mediators and exocrine factors, such as enteroglucagon, gastrin, epidermal growth factor (EGF), insulin-like growth factor (IGF) and so on, have been shown to play important roles in small intestinal adaptation (1, 198-202). Pancreatic and biliary secretions entering the distal bowel in higher concentrations also appear to stimulate villus hyperplasia, especially in response to stimulation of release by EN (1). This review shall focus on identified gut derived humoral mediators and trophic factors.

1.2.5 Role of Gut Hormones or Peptide Factors Regulation in Intestinal Adaptation

Numerous gut hormones and peptide factors have been shown to have a potential role in intestinal adaptation, including IGF-I, EGF, keratinocyte growth factor (KGF), growth hormone (GH), transforming growth factor-a (TGF-a), neurotensin (NT), peptide YY (PYY), gastrin-releasing peptide (GRP), cholecystokinin (CCK), vasoactive intestinal peptide (VIP) and gastrin (203). IGF-I, which is synthesized from the gut, can enhance mucosal growth, increase small bowel length, weight, and CCPR (204, 205).

Peptides of the EGF family are mainly secreted from the submandibular gland, duodenal Brunner's glands, epithelial and Paneth cells and can induce intestine growth and functional development by stimulating crypt cell proliferation as well as suppressing apoptosis (203, 206, 207). The EGF family of peptides also plays a role increasing nutrient absorption and reducing intestinal permeability and weight loss (208-210). In particular it is believed that EGF, which is present intrauterine in amniotic fluid, plays a role in regulation of transport function before and after birth, by inducing mucosal growth and specific transport processes, as well as suppressing apoptosis (206, 211). PYY, which is secreted from endocrine L-cells of the ileal, colonic, and rectal mucosa, can slow gastric emptying and intestinal transit, and CCK has been shown to stimulate the release of PYY (203, 212). Similarly secreted from the L-cells of the ileum and colon, glucagon-like peptide-2 (GLP-2) is an important trophic peptide that can induce intestinal regulation by regulating cell proliferation, apoptosis, nutrient absorption, motility, as well as epithelial and intestinal permeability (43, 213, 214).

1.3 Glucagon-Like Peptide-2 (GLP-2)

1.3.1 Introduction

Amongst all the endogenous factors that are involved in stimulating intestinal adaptation after resection, GLP-2 seems to be a very important and also importantly to be intestine specific. GLP-2 is a 33-amino acid member of the pituitary adenylate cyclase-activating peptide glucagon superfamily (215). It is a post-translationally spliced from the proglucagon gene (216, 217). Compared with other peptides, GLP-2 is a potential therapy for intestinal disease as it is a enteroendocrine peptide uniquely trophic for the intestine (218). The post-translational processing of proglucagon as well as the GLP-2 peptide sequence of human and domestic animals are shown in **Figure 1.3.1-1** and **Figure 1.3.1-2** respectively. GLP-2 is secreted from proglucagon-immunopositive enteroendocrine L cells in epithelial layer of the distal ileum and proximal colon (219).

The release of this peptide can be induced by both proximal enteric neuronal signaling and direct stimulation from nutrients in distal bowel, especially LCFAs (220-223). The release of GLP-2 is shown in **Figure 1.3.1-3**.

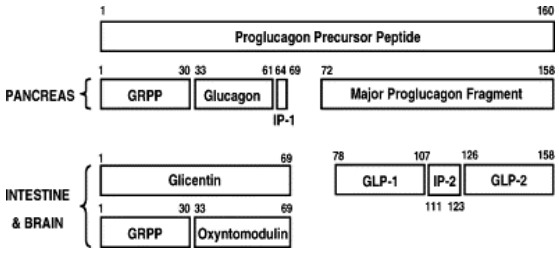

Figure 1.3.1-1 The posttranslational processing of proglucagon (213)

Human	HA DGS FSDEM	NTILD NLAAR DFINW LIQTK ITD
Rat (97%)	HA DGS FSDEM	NTILD NLATR DFINW LIQTK ITD
Guinea pig (97%)	HA DGS FSDEM	NTILD NLATR DFINW LIQTK ITD
Mouse (94%)	HA DGS FSDEM	STILD NLATR DFINW LIQTK ITD
Pig (88%)	HA DGS FSDEM	NTVLD NLATR DFINW LLHTK ITD
Cow (88%)	HA DGS FSDEM	NTVLD SLATR DFINW LLQTK ITD
Dog (88%)	HA DGS FSDEM	NTVLD TLATR DFINW LLQTK ITD
Chicken (58%)	HA DGS FTSDI	NKILD DMAAK EFLKW LINTK VTQ

Figure 1.3.1-2 GLP-2 peptide sequence of human and domestic animals

Line box encompasses N-terminal sequence with greatest homology among species. Underlined residues are different from human sequence (213).

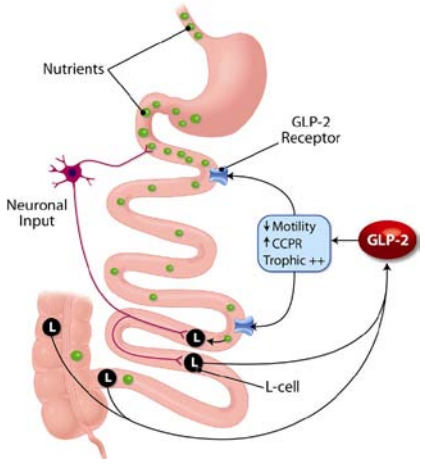

Figure 1.3.1-3 Release and basic function of GLP-2

Glucagon-like peptide-2 release by enteral nutrients and signaling effects (224).

1.3.2 The Function of GLP-2

GLP-2 was first bought to attention because the increased enteroglucagon levels in patients with glucagonomas could be related to intestinal mucosal hyperplasia (199). Further reports of enteroglucagon related mucosal growth of the small intestine followed (225). GLP-2 is considered to be the only peptide that significantly increases mucosal thickness throughout the small intestine, with the most obvious changes in the proximal jejunum and distal ileum (223, 226). Besides, GLP-2 has a function in physiological regulation of intestinal growth, gastric and small intestinal motility, permeability and nutrient absorption (218, 227). GLP-2 can also increase the activity of specific transport proteins, thus increase glucose uptake, and increase intestinal blood flow (228, 229). Nutrient absorption is increased also by the increased height and density of microvilli due to GLP-2 induced adaptation (230). Many animal studies have found both endogenous and exogenous GLP-2 play a role in intestinal adaption after resection (111, 214, 226, 231-233). In adult SBS patients, after the use of a GLP-2 analogue, the absolute and percentage intestinal absorption, as net stool weight, as well as the percentage of absorption of energy and nitrogen, were increased (234). Therefore, GLP-2 can increase both intestinal absorptive surface area and absorptive capacity of the residual intestine, and as GLP-2 levels are related to the degree of intestinal adaptation, a rise in plasma GLP-2 can be an indicator for the early phase of the adaptive response (216). Besides, data from both human and pigs showed that GLP-2 can increase digestion and absorption of nutrients by increasing mesenteric blood flow (229, 235, 236).

In addition, the function of GLP-2 is not limited to structural and functional adaptation. GLP-2 administration can decrease intestinal permeability and may therefore prevent bacterial translocation and thus reduce the enteric-induced infections in SBS patients (237, 238). Moreover, GLP-2 has been found to cause a reduction in paracellular transport of ions and small molecules and inhibition the uptake of macromolecules (239).

These functions may be helpful in reducing diarrhea, bacterial translocation and its potential complications.

1.3.3 The Mechanisms of GLP-2 Related Adaptation

The reason why GLP-2 has these functions is because it can increase protein and DNA content and the CCPR, leading to increased villus height, crypt depth, mucosal mass per centimeter and small intestinal length and weight (43, 217, 219, 226, 240, 241). In parenterally fed immature pigs, exogenous GLP-2 decreased proteolysis and apoptosis, thus promoting intestinal growth, whereas in enterally fed piglets, GLP-2 administration promoted intestinal protein synthesis and cell proliferation, as well as decreased apoptosis (214). In studies using a neonatal pig model, there was no increase in CCPR with low and intermittent dosing of GLP-2, but there was an apparent increase in CCPR with a higher intermittent dose or continuous infusion (214, 242). Considering the relationship between the GLP-2 and CCPR, it is reasonable to consider GLP-2 as an important factor in the initiation of intestinal adaptation and CCPR as a fundamental mechanism that leads to the nonspecific intestinal adaptation and thus increased nutrient availability (110, 115).

Apart from the effect in increasing CCPR, GLP-2 may also have differential effects on apoptosis (214, 233, 243, 244). Studies suggest that continual GLP-2 stimulation caused an increase in crypt compartment apoptotic rates in the jejunum, which could be considered crypt cell “selection” for enterocyte absorptive function cell lines (110, 115, 132, 245). Production of all intestinal cell lines is increased by the activation of the crypts, which means during the initial maturation phase, the crypts select for apoptosis of nonabsorptive phenotypes (e.g., enteroendocrine cell and mucus producing cells) and this in turn alters the transport capability of the villus (218, 245, 246).

At the molecular level, GLP-2 achieves its function by binding to a 7 transmembrane (7-TM) G protein-coupled receptor which is comprised of 550 amino acids (247).

Although the downstream mechanisms of G-coupled protein, particularly for altering CCPR are poorly understood, some data exists suggesting its activation inhibits cell apoptosis, through a cAMP-dependent pathway (244).

The exact pathways of GLP-2 induced adaptation are unclear. Some studies suggest that GLP-2 may act on intestinal epithelial cells directly and others suggest it acts via intermediate enteroendocrine cell activation (248-251). Since the expression of GLP-2 receptor (GLP-2R) is not found in crypt cells or enterocytes, the function of GLP-2 must be mediated indirectly by paracrine and/or neural pathways (252). GLP-2R expression is found in both enteric neurons and enteroendocrine cells in humans (236). Some studies suggest that pheochromocytoma cell-4/TPA Induced Sequence 7 (PC4/TIS7), a member of fibroblast genes, is critical for intracellular signaling during intestinal adaption (253, 254). It is shown that both the cell division cessation and cytodifferentiation onset need the PC4/TIS7 pathyway (255-257). After small intestinal resection, the expression of PC4/TIS7 is significantly increased in remaining adaptive intestine (254). It is possible that this is a common downstream molecular mechanism, as many intestinal growth factors may share this pathway (253). In vitro, compared with other growth factors, GLP-2 shows the most potential ability to induce PC4/TIS7 expression (253).

The function of GLP-2 on small intestinal growth is related to other growth factors, in particular: IGF-1, IGF-2, the IGF-1 receptor, EGF and other ErbB ligands (258). In studies using knockout models, the trophic effects of GLP-2 on the intestinal mucosa are dependent on local effect of IGF-1, which is believed to be released from myofibroblast close to the crypt cells in the distal intestine (205, 259). Similar to the function of GLP-2, some studies show EGF-induced increases in brush border surface area, brush border SGLT1 content and glucose maximal uptake, sudgesting they overlap with GLP-2 (213, 230). The effect of GLP-2 as an anti-inflammatory and to increase blood flow are

suggested to depend on VIP and nitric oxide, respectively (258). **Figure 1.3.3-1** shows the localization of GLP-2R and action of GLP-2 in the intestinal adaptation.

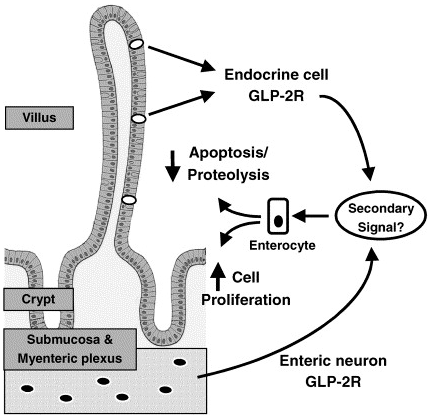

Figure 1.3.3-1 GLP-2R Localization and action of GLP-2 in the intestinal adaptation (213)

1.3.4 The Characteristics of GLP-2 Related Adaptation

Although the function of GLP-2 in adaptation appears impressive, there are still many questions to answer before final usage of it as a treatment, particularly for children. One of the unsolved questions is the timing of administration after resection to maximize adaptation (223). Comparing later post-resection administration (the second week post resection), with early post-resection administration (the first week after resection), early GLP-2 effects on intestinal growth were maximal, increased intestinal length and the expression of the GLP-2R (223). Most importantly, these effects persist after withdrawal of GLP-2 administration (223). However, CCPR changes were similar between both early and late treatments and crypt depth and villus height were significantly increased in the late treatment group; although these increases could not compete with the early treatment, considering the total small intestinal surface increased by gut growth (223).

Age or developmental stage is another important factor to be considered when talking about the effects of GLP-2 administration. During late gestation in the fetal piglet,

plasma GLP-2 levels gradually increase, with a peak level achieved at about two days after birth (260). In addition, the effect of exogenous GLP-2 on intestinal adaptation is also highly age specific, with the most pronounced effect during the first few weeks after birth; while pharmacological levels of GLP-2 have no trophic effect on intestine in fetal or weanling pigs (261). Exogenous GLP-2 has no effect on fetal mucosal growth, intrauterine, and limited effect on brush border enzyme activities (262). Comparison of the PN fed preterm and term piglets show that exogenous GLP-2 in the preterm piglet has less advantage for intestinal nutrient uptake, especially the carrier-mediated uptake of glucose (263). Therefore, it is reasonable to suggest that the role of exogenous GLP-2 in intestinal adaptation may be restricted to the postnatal period. This indicates the importance of time-dependent GLP-2 treatment in SBS infants.

In addition, findings suggest region-specific effects of GLP-2 exist. In the jejunum, GLP-2 can increase nutrient absorptive capacity by increasing mucosal surface area, rather than increasing the density of transporters per unit surface area (226, 228, 264). However, in the ileum, GLP-2 may be able to increase both surface area and SGLT-1 transporter expression (264). Besides, exogenous GLP-2 will increase apoptosis in the jejunum but not in the ileum (264). Therefore, the relationship between adaptation of different segments of the intestine and GLP-2, as well as the mechanisms by which GLP-2 impacts intestinal adaptation all need further investigation.

1.3.5 GLP-2 Receptor (GLP-2R)

GLP-2R, the binding protein for GLP-2, may contribute to the differential function of GLP-2. The distribution of GLP-2R appears region specific, since it was recently shown that GLP-2R were found throughout the small and large intestine in human, mice, and rats, with the greatest expression in the proximal small intestine (265).

Considering the potential effect of different age or developmental stage in GLP-2R expression, a study using fetal and neonatal piglets showed that GLP-2R is expressed in both the fetal and neonatal gastrointestinal tract (266). Similar results have been found in rats, with GLP-2R mRNA transcripts reach peak levels during the fetal and neonatal period, decreasing to normal levels, observed in mature rats, at about 3 weeks after birth (267). However, though GLP-2R mRNA is expressed in the fetus, evidence to support the presence of biologically active GLP-2 in the fetal circulation does not exist (266). Furthermore, despite the presence of GLP-2R in the fetus, exogenous GLP-2 treatment intrauterine shows no affect on mucosal growth. Perhaps there is limited function of GLP-2R to couple with secondary pathways (266). These GLP-2R changes may explain the different effects of GLP-2 observed during different stages of growth in pigs. Importantly resection induced GLP-2R expression changes also exist. Using rats Munroe et al, showed there is a significant up-regulation in the expression of GLP-2R mRNA day 3, maximal by day 10 post resection, with the highest density measured in the jejunum (247).

1.3.6 Regulation of GLP-2

1.3.6.1 Role of intestinal resection

Apart from stimulation from proximal enteric neuronal signaling and direct nutrients in distal bowel, resection itself also affects the amount of endogenous GLP-2 production. Research shows that plasma GLP-2 levels were higher in resected animals than transected animals fed the same diet, with adaptation taking place on both sides of the resected small bowel (82, 114, 227). Moreover, the site of small bowel resection can influence the endogenous plasma GLP-2 response. This is not surprising as the synthesis and secretion of GLP-2 occurs mainly in the L-cells of ileum and colon (217, 268). Hence, animals undergoing proximal small bowel resection with ileum and colon intact show a greater increase in plasma GLP-2 levels than those that undergo a distal small bowel resection

(227). It has been demonstrated that as long as ileum can be kept after resection, exogenous GLP-2 alone will stimulate adaptation, even without enteral feeds (264). Conversely, after patients undergo distal ileal resection production of GLP-2 is reduced, even given EN (268).

1.3.6.2 Role of anatomical factors

It is known that GLP-2 is secreted from distal ileum and colon predominantly, and the ileum has a greater capacity to adapt than the jejunum. However, concerning GLP-2 production from the ileum relative to the colon, there is controversy. In the adult human, the colon has been shown to play an important role in GLP-2 release after resection (268, 269). On the other hand, in infants with SBS, with or without colon there is no evident effect on GLP-2 release (231). This difference could be attributed to the different aged populations with different maturity of the colon.

Another interesting finding is regarding the remaining small intestine surface area and GLP-2 production. Since we believe GLP-2 is produced by the remaining small intestine, more remnant small intestine length should produce more GLP-2, but this is in fact not clear. One study using rats showed an inverse correlation between postprandial GLP-2 level and the calculated intestinal surface area (43).

1.3.6.3 Role of L-cells

Although there is an increase in endogenous GLP-2 after resection, believed to be from L cell in the distal intestine, the L-cell population of the remnant ileum does not appear to increase after resection or transposition, which is counter intuitive (270). One reason for this could be the remaining L cell population is more sensitive to proximal neuronal or increased humoral signaling factors after intestinal resection (270). However, this still warrants further clarification.

1.3.6.4 Role of neuronal signaling

The postprandial GLP-2 increase has two phases, with first phase happens 15 minutes after meal ingestion, the later phase around 60-120 minutes. Since ingested food needs time to go through the gut and reach the distal intestine, the later phase is believed to be related to the direct contact with luminal nutrients, the first to the neurohumoral stimulus (43). Both in humans and animals, the luminal chyme, which cannot all be absorbed in the upper intestine, can cause mechanical distension that induces the secretion of GLP-2 (220, 271, 272). When trying to find signaling for GLP-2 release, expression of *c-Fos*, the earliest response gene stimulated by a number of growth factors, peptides and the second messengers, was studied (245, 273). Using pre-treatment with tetrodotoxin, which abolished *c-Fos* expression in crypt cells, it has been shown that GLP-2 signaling in the intestine may depend on a transduction pathway requiring enteric neurons, such as parasympathetic neuronal regulation (245). Further study has shown that if the ileum could have direct contact with luminal nutrients post resection the vagal afferents become dispensable (163).

1.3.6.5 Role of nutrition

The presence of nutrients in the intestine seems to be a necessary factor in inducing GLP-2 release. In rodents the situation may be different as increased proglucagon mRNA expression is even found in the colon of the PN fed rats, but increased only in the colon (147). Nevertheless, luminal nutrients will elevate the release of GLP-2, both in the early post resection phase through neural regulation and the second phase by meal stimulation. Presence of EN in the gut can further augment the early increase in GLP-2, at 48 and 96 hours after resection, when oral feeding commences (274, 275). In enterally fed rats proglucagon mRNA increases about two times in the ileum one week after resection (274-276). Piglets given PN alone combined with GLP-2 infusion showed decreased proteolysis and apoptosis and thus intestinal growth, whereas in enterally fed piglets, GLP-2 infusion promoted intestinal protein synthesis, increased cell proliferation and

decreased apoptosis (214). Furthermore, compared with givng 40% of the total nutrient taken enterally, the circulating GLP-2 level was significantly reduced during TPN treatment (261).

It is believed that increased enteral nutrients that contact the distal gut can further stimulate the expression of proglucagon mRNA and the release of GLP-2 after resection (277). For patient with SBS, inadequate nutrient absorptive capacity cause the increased unabsorbed nutrients to reach the distal intestine and thus stimulate GLP-2 secretion by direct contact with L cells in ileum and colon (278). Interestingly, besides proximal small intestinal resection with functional ileum left, surgical transposition of ileum to the proximal intestine, can also stimulate GLP-2 release (227). In this case, the transposed ileum has more chance to have contact with luminal nutrients, just as the case when the proximal jejunum is resected. These findings further support the importance of nutrients in the intestine for SBS patients for adaptation, especially in the distal part of the intestine.

In addition, the amount of luminal nutrition can also influence GLP-2 release. In healthy adult humans, snacks less than 400 kcal could not induce GLP-2 release, while those of more than 400 kcal relate to an immediate response (279). It is possible that the release of the peptide requires a minimum caloric load from the diet (280). In the neonatal piglet there was also a strong correlation between the amount of enteral intake and circulating GLP-2 levels (261). Actually, it is not only the calories delivered but also the net absorption, that is important for GLP-2 release. In infants with SBS, there is a positive correlation between GLP-2 level and nutrient absorptive capacity (231). In contrast, in rodents an inverse correlation between postprandial GLP-2 level and fat or protein absorption is found (43, 281). Therefore, the relationship between the presence of nutrients in the distal small intestine and the absorptive capacity for the same nutrients and GLP-2 release also needs further investigation.

Furthermore, the exact dietary nutrient composition delivered to the gut and intestinal production of GLP-2 is related. Plasma GLP-2 levels are significantly increased in colostrum fed newborn and juvenile pigs, with or without intestinal resection, compared to all other diet groups, which included pig chow, non-polymeric infant formula and a commercial starter diet (216, 260). Also, some studies observed higher levels of circulating plasma GLP-2 levels and improved weight gain in animals receiving a diet with polymeric infant formula compared to elemental formula (216).

However, considering the individual component of diet in inducing GLP-2 production, controversial data exists. Studies in healthy human adults found the L cells were sensitive to either carbohydrate or fat in the diet, but not to protein (279). Similarly, another human study showed that carbohydrate and fat are more potent in stimulating GLP-2 release with glucose triggering almost double the response in total GLP-2 than triglycerides (282). Another member of the proglucagon super-family GLP-1 is concomitantly liberated with GLP-2 from the L cells and levels parallel GLP-2 production in the rat and pig (283-285). In healthy adult humans, plasma GLP-1 increases with an increased glucose uptake in a dose-dependent manner (280). However, for neonatal piglets, amino acids had less effect than carbohydrate, but greater effect than lipid in stimulating GLP-2 (286). Other studies also show amino acids can induce the production of glucagon-like peptide-1 (GLP-1), hence GLP-2 (287). One reason for the greater effect of carbohydrate in mature animals could be the presence of malabsorbed carbohydrate in the colon, fermented by anaerobic bacteria to SCFAs. It is know that SCFAs can act as a mediator of GLP-2 release from the L cells in the distal ileum and colon (288). Thus, increased GLP-2 could be the mechanism for the structural and functional intestinal adaptation noted after TPN plus SCFAs treatment in SBS adult rats, and when given by the parenteral route to SBS neonatal piglets (288, 289).

1.3.6.6 Role of other hormonal factors

PYY, a 36 amino acid gastrointestinal peptide which is also present in the L-cells of the mucosa of the ileum and colon, is considered to be co-localized with GLP-2 (290). Serum levels of both peptides increase within 15 minutes after meal stimulation (291-293). Prior studies have shown CCK induces the dose-dependent release of PYY, therefore it is also possible that CCK can induce the early phase release of GLP-2 (203).

1.3.7 GLP-2 Inactivation

Another important factor in regulating plasma GLP-2 level is clearance. The half-life of intact GLP-2^{1-33} is only 7 minutes and the half-life of cleaved GLP-2^{3-33} is 27 minutes in humans (294). In the rat, GLP-2^{1-33} level is regulated via both exopeptidase dipeptidyl peptidase IV (DPP-IV) and renal clearance (295). DPP IV, which is found in the intestine, especially ileum, and in the plasma, can cleave circulating GLP-2^{1-33} to the inactive form GLP-2^{3-33} (203, 279, 296). Active GLP-2^{1-33} has the amino acid alanine at position two and this makes it susceptible to degradation by the DPP-IV (297). Following inhibition of DPP IV, administration of exogenous GLP-2 initiates a significant increase in small bowel growth in rats (298). Similarly, knockout rodents deficient in DPP-IV have a higher serum GLP-2^{1-33} level, as well as increased small intestinal weight (297, 299). Thus, inactivation of GLP-2^{1-33} by DPP-IV is a limiting factor in regulating the function of GLP-2 in intestinal adaptation. Another interesting aspect of DPP-IV is the evidence it is influenced by intestinal resection. It is known that plasma GLP-2 levels increase after resection, obviously one reason could be increased GLP-2 production, however evidence exists there is also a role for reduction in DPP-IV level (300).

1.4 Summary and Knowledge Gaps

Overall, these findings show the potential to use GLP-2 or its analogues to increase intestinal mass and function following resection in SBS, particularly with loss or resection of ileum. Early treatment would appear to be beneficial, given the increased receptor expression and evidence for optimal growth of the residual intestine with early

treatment (223). This is probably most true for developing humans who have an innate growth potential. Following resection, early treatment with GLP-2 appears to augment mass, while continued treatment in combination with enteral nutrition as soon as possible, may up-regulate the transporter machinery for improved nutrient absorption.

SBS infants, especially preterm infants, are at particular risk of both the malabsorption syndrome and the life threatening complications of to SBS and long term PN. Since this condition requires expensive medical and surgical treatments, and carries the risk of death awaiting small bowel transplantation when such treatments fail, we are looking for solutions to enable these young patients to adapt and totally wean off PN. The process of adaptation is different from individual to individual. GLP-2 can play a pivotal role in intestinal adaptation and may have a potential role as a new treatment for these babies. However, at this time not enough is known about the relationship between endogenous GLP-2 production and the remnant intestinal anatomy after resection. Although GLP-2R expression is increased during intestinal development it is not known if the expression of the receptor will vary according to remnant bowel anatomy. This would impact the potential for GLP-2 to be a therapy for all infants with SBS. In considering the use of GLP-2 as a potential therapy it is important to determine if different diets will affect endogenous GLP-2 production, which has been inadequately studied, particularly in infants. Addressing these knowledge gaps is the purpose of this thesis and a neonatal piglet model is proposed as an appropriate animal model for reasons that will now be explained.

1.5 Piglets as a Model for Short Bowel Syndrome

Since ethical and practical limitations exist for the research possible in developing humans, an animal model would seem as a good tool to study SBS, where the outcome of importance requires invasive tissue collection. To assess intestinal adaptation after resection, many studies have used animal models (like rat, mouse, dog and swine) given

the ready availability of tissues for histology and molecular testing (216, 227). Among these the pig is the best non-primate model for humans, swine sharing similar body composition, gastrointestinal anatomy, physiology and metabolism (301-304). Interestingly, comparison between humans and pigs show the relative nutrient requirements of these two species are similar during infancy, growth, reproduction, and lactation (302). Specifically, both of the species show similar patterns of indispensable amino acid requirements and thus share similar amino acid metabolism (305). These findings are important for human nutrition research, especially for requirement studies at different developmental stages. The use of the piglet as an animal model for human infant and nutrition research in particular is thus well established (306-311).

Most importantly, in developing a pediatric SBS model, piglets share similar gastrointestinal development to the human baby (301). The newborn piglet shares similar body composition with the newborn human neonate during different developmental stages (302). The peak growth rate of the newborn piglet has not been reached at birth, so the neonatal piglet is less mature at term delivery than the human neonate (301, 305). The newborn piglet is in fact more similar to the premature human neonate with poor thermoregulatory mechanisms, high metabolic and growth rate, reduced glycogen storage and impaired immune function (312, 313). As premature babies account in large part for the burden of neonatal SBS it is useful to use newborn piglets to model the situation found in the premature rather than full-term baby (301).

After birth piglets have an excellent ability to grow. During the first 3-6 weeks post delivery piglets increase their weight 1000%, compared to the same time period for human neonates whose weight would increase only 50% (302). This means piglets are very sensitive to nutritional deficiency and this makes the newborn piglet a useful model to study human neonatal and nutritional disorders, over short research durations. In addition, since the intestine develops most rapidly during the neonatal period in the piglet,

it is also a good model to study intestinal disorders during development, such as neonatal SBS. In addition, piglets are large enough to ensure good outcomes with surgical interventions, easy to handle and adaptive to life in metabolic cages. Besides, during the first 48 hours of life the neonatal piglet can absorb immunoglobulins from maternal colostrum and emerge relatively immunocompetent by 3 days old, hence able to survive invasive procedures (314). Given these considerations the neonatal piglet would seem an excellent animal model for neonatal SBS. Furthermore, to be a realistic model for intestinal failure the piglet should be able to meet its nutrient requirements by the parenteral as well as the enteral route. Fortunately the neonatal piglet has been well established as a model for PN support, with nutrient requirements by this route well characterized. The piglet will have similar plasma amino acid concentrations and protein metabolism as human babies on TPN (305).

There are differences between the neonatal piglet and the human infant that might be of concern in developing an SBS model. The most notable difference is the higher metabolic rate, greater nutrient requirements and ability to develop and grow more rapidly than the human baby. However, this may in fact be an advantage as this will enable a short duration of experiments to reach relevant endpoints; in addition may be a factor in producing complications of PN therapy over a short period of time (315, 316).

Apart from neonatal piglet, the mature rodent rat is another commonly used model to study SBS. However, it is important to understand that the rodent model has little applicability to human infants. The neonatal rat is too immature at birth to tolerate surgical interventions, which are practically difficult regardless. Adult rats have significant physiological differences in gastrointestinal physiology, bile acid metabolism and nutrient usage, that all limit translational findings to humans (301, 317). Particularly given the previous discussion highlighting how the small intestine grows and adapts, how

GLP-2 is released and responses to GLP-2 administration, it should be noted that all are very different between infant and adult animals.

As early as the 1980s, the glucagon from pig intestinal tissue has been isolated and sequenced, which helped to further identify the amino acid sequence of GLP-2 in the intestine (285, 318). Importantly, similar to the human, swine have increased secretion of GLP-2 from L cells after similar nutrient, hormone and neural signaling. In swine, GLP-2 has similar functions as in humans, such as affecting digestive enzyme and nutrient transport activity, suppressing upper gastrointestinal motility and secretions, preventing mucosal villus atrophy and maintaining intestinal mass (214, 266, 319). Furthermore, the GLP-2R in both human and swine are strictly localized in the gastrointestinal tract and brain (251, 266). As a result, piglets can be considered an appropriate animal model for human neonatal SBS and the study of exogenous GLP-2 production in adaptation (320).

1.6 Summary

Neonatal SBS is one of the most serious diseases in children, especially in preterm infants. Adaptation following resection is important and determines the prognosis and outcome. Thus, studying factors involved in this process is valuable and can provide suggestions to improve clinical management and thus survival. At this time it is known that the ileum plays an important part in GLP-2 secretion and hence intestinal adaptation. SBS patient with some part of the ileum remaining appear to have better outcomes than patients without any ileum (113-116). Moreover GLP-2, derived from ileum, is a major factor mediating intestinal adaptation (234). SBS patients without ileum may have different levels of GLP-2 production compared to patients who have ileum and this may account for different potential for adaptation.

Therefore the goal of this thesis is to investigate whether different sites of small intestinal resection will lead to different plasma GLP-2 levels and thus different degrees

of intestinal adaptation. Our research group has already undertaken pilot work to develop viable neonatal piglet models of SBS: with 75% of mid-small intestinal resection (JI) or 75% of distal small intestinal resection (JC) (321). These models give us two comparative situations, one in which some part of the ileum remains (JI) and one with no ileum remaining (JC). Using these piglet models, we can see the impact of remnant intestinal anatomy on plasma GLP-2 levels and tissue GLP-2R expression.

Furthermore, the two resection models may have different capacity for nutrient digestion and absorption. As a result, they may have different diet tolerances and require different EN delivery during the same stages after surgery. However, the presence of EN in the lumen of the gastrointestinal tract is an important stimulus in GLP-2 release. Therefore it will be important to study different enteral diets with these models. Although specific dietary components have a different ability to induce endogenous GLP-2 secretion, research to date has been notably contradictory. In this research study elemental formula, which contains amino acids, glucose polymers and long chain triglycerides, will be compared to polymeric formula, based on commercial infant formula, containing intact protein, lactose, glucose polymers and long chain triglycerides. Amino acid solutions are easier to digest than intact protein, thus the neonatal animal may tolerate elemental formula better than polymeric formula. However, intact protein may stimulate more GLP-2 release and promote better adaptation, which would offset the prior advantage (177).

In summary, using neonatal SBS piglet models, this research will determine the levels of GLP-2 in each different anatomical short bowel group, as well as the effects of the two formula types on GLP-2 release and adaptation. Finally, this will provide evidence to better support the treatment of neonatal SBS in human babies.

2 RATIONALE AND OBJECTIVES

Given this background, in particular acknowledging the ileum as the principal site of endogenous GLP-2 release and recognizing the role of GLP-2 in intestinal adaptation, this thesis will characterize endogenous GLP-2 release in neonatal piglets with SBS, either leaving part of the ileum or completely removing the ileum. It will be determined how GLP-2 production under these two conditions is related to adaptation. Furthermore, given the pivotal effect of luminal nutrients from the diet for adaptation and GLP-2 secretion, the influence of different types of formula on GLP-2 release will be studied. Specifically elemental formula, which is amino acid based, will be compared to polymeric formula, with intact protein.

Specific hypotheses:

1. After intestinal resection, piglets with short bowel have increased plasma GLP-2 levels.
2. Short bowel piglets with ileum have the higher plasma GLP-2 levels compared to short bowel piglets without ileum.
3. Higher plasma GLP-2 level is related to increased small intestinal adaptation.
    a) Higher plasma GLP-2 is related to increased villus height and crypt depth in the jejunum and ileum of short bowel piglets with ileum.
    b) Higher plasma GLP-2 is related to increased villus height and crypt depth in the jejunum of short bowel piglets without ileum.
4. Compared with sham piglets, GLP-2 receptor expression will be increased in the remnant intestine in short bowel piglets, with an even higher level in the piglets without ileum.
5. In short bowel piglets polymeric formula will increase plasma GLP-2 level.

a) Polymeric formula leads to higher plasma GLP-2 levels independent of anatomical type.

b) Polymeric formula leads to increased small intestinal adaptation.

The overall objective of this thesis is to relate GLP-2 production in two anatomical sub-types of short bowel to the process of adaptation. Comparisons between three experimental groups: a normal sham control, short bowel piglets with ileum (JI) and short bowel piglets without ileum (JC) will address hypotheses 1-4.

Specific questions to be answered:

1. Is endogenous GLP-2 production different in surgical SBS when there is remnant ileum compared to when there is none?
2. Is the expression of the GLP-2 receptor different in surgical SBS when there is remnant ileum compared to when there is none?
3. Is the plasma level of GLP-2 production correlated to the amount of intestinal adaptation, such as mucosal hyperplasia?

By addressing these questions this research has important clinical relevance for designing treatment trials of GLP-2 or its analogues in human short bowel infants, with different sites of intestinal resection, as well as evaluating the prognosis of SBS infants.

A second objective is to determine if the type of formula feeding will be related to GLP-2 production (from the remnant ileum and/or the colon) and hence adaptation in the two anatomical sub-types of short bowel being studied. Comparisons within each experimental group of piglets by randomly assigning to two different types of formula will address hypothesis 6.

Specific questions to be answered:

1. Is endogenous GLP-2 production different in surgical SBS when given elemental compared to polymeric formula?

2. Is the expression of the GLP-2R different in surgical SBS when given elemental compared to polymeric formula?

3. Is the amount of intestinal adaptation, such as mucosal hyperplasia different in surgical SBS when given elemental compared to polymeric formula?

By addressing these questions this research has important clinical relevance for designing the appropriate diet for short bowel infants according to the intestinal resection.

3 MATERIALS AND METHODS

3.1 Animals and Surgery

All procedures were approved by the Faculty of Agricultural, Life and Environmental Sciences Animal Policy and Welfare Committee, University of Alberta, and were conducted in a bio-secure swine research facility, according to the guidelines of the Canadian Council of Animal Care. Male newborn Landrace-Large White cross piglets (4-6 days old) were obtained from the University of Alberta Swine Research and Technology Centre.

All piglets underwent general anesthesia, insertion of a jugular venous catheter, laparotomy for insertion of a gastrostomy feeding tube and intestinal measurement and resection, as has been previously described (321). After removal from the sow, piglets were immediately given an intramuscular injection of ampicillin, atropine and midazolam, masked and pre-loaded with oxygen. They were anaesthetized initially with halothane for intubation and maintained with isoflurane throughout the surgery. Intraoperatively piglets received an infusion of Ringers Lactate solution into the jugular vein catheter to maintain hydration and reduce operative stress. Trimethoprim/sulfadoxine, buprinex and ketamine were also given intraoperatively.

Piglets were randomly allocated into three groups: proximal intestinal resection, distal intestinal resection or sham group. A vascular catheter was implanted, following induction of anesthesia, into the left jugular vein for the purpose of PN infusion and blood sampling. Midline laparotomy was performed using a scalpel and then electrocautery. A gastrostomy tube was inserted into the stomach, secured with a purse string and then tunneled out the dorsal service to enable long term feeding.

Small intestinal length was measured in all of the piglets from the Ligament of Treitz to the ICV along the antimesenteric border, using a 3-0 silk suture. The proximal intestinal resection group had a 75% midintestinal resection with a jejunoileal anastomosis (JI), leaving the equal amount of jejunum and ileum (12.5%). The distal intestinal resection group had 75% of distal small bowel resected, including ileum, cecum and 5-cm of the spiral colon, with a jejunocolic anastomosis (JC). The sham group (Sham) is a control group without surgical resection but with central venous catheter insertion and laparotomy with gastrostomy insertion. Sham piglets did not receive intestinal transection because a previous study showed there was no significant increase in plasma GLP-2 in animals that underwent intestinal transection, when compared with animals had intestinal resection (147). Furthermore, our prior pilot experience demonstrated that sham piglets with transection were more prone to complications from adhesions. In the JI and JC piglets the mesentery was cauterized and cut using an electrocautery unit, for a bloodless resection, of bowel from mesentery. Then the small intestine was divided transversely, using scissors at the appropriate location, and the intestine was removed. After the bowel resection the two divided ends of the bowel were anastomosed with interrupted 4-0 monofilament absorbable sutures. Finally, abdominal musculature was closed with a continuous suture using 3-0 vicryl, penile ligaments were re-approximated using buried 3-0 vicryl interrupted sutures and skin was closed using a subcuticular suture of 5-0 vicryl.

3.2 Animal Daily Care

Postoperatively, piglets were placed in cotton jackets, housed in individual metabolic cages lined with Plexiglass and secured to a swivel system that allowed free movement. The piglet room had a stable temperature maintained at 25^0C, with a 12 hour light/dark cycle. Analgesia (bupinorphine) was given when the piglets

had recovered from anesthesia. In order to prevent line sepsis while on PN, cycled broad spectrum antibiotics (ampicillin, gentamicin and trimethoprim - sulfadoxin) were used. Similarly, ranitidine (4 $mg \cdot kg^{-1} \cdot day^{-1}$) was routinely added to PN bags by continuous infusion to prevent gastric hemorrhage. Hibitane was applied to the incision area to prevent potential incision infection.

Postoperatively, pain was monitored using a pain score and additional pain relief with buprinex was provided as required. Each morning, the piglets were weighed to evaluate growth and calculate the nutrient solution infusion rate. The urine output was measured and fluid balance was calculated according to fluid in and fluid out (urine plus diarrhea) volume. Stool condition was monitored every morning and categorized as normal, soft, diarrhea or no bowel movement.

3.3 Nutrition

Postoperatively, pyrogen free PN commenced immediately using an amino acid based solution formulated for piglets in our laboratory (305). Crystalline amino acids (Ajinomoto, Fort Lee, NJ) and glucose were dissolved with sterile water and minerals, including sodium, potassium, calcium, phosphate, zinc, and manganese. Under a laminar flow hood, the solution was filtered into sterile bags using a 0.22-µm filter (Milipore, Etobicoke, Ontario, Canada). Before delivery to the piglets, multivitamins (Multi-12/K_1 Pediatric, Sabex, Boucherville, Quebec, Canada), Vitamin B_{12} (Abbott, Mississauga, Ontario, Canada), additional trace minerals, iron dextran (Ferroforte; Bimeda-MTC, Cambridge, Ontario, Canada), and lipid (Intralipid 20%, Fresenius Kabi) were added to each PN bag for infusion.

Immediately after surgery (day 0), PN infusion started at 50% of final rate (6.75 $ml \cdot kg^{-1} \cdot hr^{-1}$), 8 hours later, 75% of the final rate (10.125 $ml \cdot kg^{-1} \cdot hr^{-1}$) was given until the next day (day 1) when the final infusion rate commenced. Target nutrient intake was 1.1

MJ·kg^{-1}·day^{-1} (270 cal·kg^{-1}·day^{-1}) and 13.5 ml·kg^{-1}·hr^{-1} with amino acids 16 g·kg^{-1}·day^{-1}, lipid 10 g·kg^{-1}·day^{-1}, and fluid 324 mL·kg^{-1}·day^{-1}. Two days postoperatively (day 2), EN was stared at 10% of final rate, increased by 10% every 12-24 hours, as PN concomitantly decreased. Within each surgical group, piglets were further randomly assigned to the two dietary groups, elemental formula or polymeric formula. Both enteral diets and the PN solutions were isonitrogenous and isocaloric. Elemental formula and PN differed only with respect to the source of carbohydrate. Glucose was replaced by Polycose® (Ross Products Division Abbott Laboratories, Columbus, Ohio, U.S.A.), which consists of long glucose polymers, to reduce osmolality and prevent osmotic diarrhea. The contents of the macronutrients in the elemental formula are listed in **Table 3.3-1**. The polymeric formula was made from a commercial infant formula (Similac regular, Abbott, Mississauga, Ontario, Canada), with added protein powder (Beneprotein, Nestle), polycose and minerals. The contents of polymeric formula are listed in **Table 3.3-2**. The difference of lipid and essential amino acids content was list in **Table 3.3-3** and **3.3-4** respectively.

Both PN and EN solution were delivered continuously by controlled pressure sensitive infusion pumps (IVAC 560, San Diego, CA). A standard feeding protocol determined the decision to increase EN, depending on the condition of the piglet and weight gain experienced every day. Expected weight gain was 50g/day during the first week or 100g/day during the second week and only in piglets without diarrhea was the infusion rate increased twice daily.

Table 3.3-1 Macronutrient Compositions of Elemental Formula

Ingredients	g/L	Energy (Cal)
Amino Acids		
L-Alanine	5.8824	
L-Arginine	4.4118	
L-Aspartate	3.3456	
L-Glutamate	5.8088	
Glycine	0.4411	
L-Histidine	1.7279	
L-Isoleucine	2.5368	
L-Leucine	5.7721	
L-Lysine-HCl	5.7353	
L-Methionine	1.0662	
L-Phenylalanine	2.24	
L-Proline	4.5956	
L-Serine	1.7849	
Taurine	0.2574	
L-Threonine	2.9412	
L-Tryptophan	1.1765	
L-Tyrosine	0.4301	
L-Valine	2.9412	
Glycyl-Tyrosine dihydrate	1.53	
L-Cysteine	0.8088	
Total Protein (based on exact nitrogen content)	55.43	
Total Carbohydrate		
Polycose (glucose polymers, 3.8Cal/g)	80.19	304.72
Total Lipid		
Intralipid (10Cal/g)	31.90	319.03
Total Non-protein Energy (Cal)		623.75

Table 3.3-2 Macronutrient Compositions of Polymeric Formula

Ingredients	g/L	Energy (Cal)
Similac (5.26 Cal/g)	120.0	631.2
-provides protein (8% of total energy) ▪ Source: non-fat milk & whey protein concentrate	13.08	50.50
-provides carbohydrate (43% of total energy) ▪ Source: lactose	66.6	271.42
-provides lipid (49% of total energy)	34.68	309.29
Beneprotein -provides protein	43.0	
Polycose (glucose polymers, 3.8Cal/g)	14.0	53.2
Total Protein	56.08	
Total Carbohydrate	80.6	324.62
Total Lipid	34.68	309.29
Total Non-protein Energy (Cal)		633.91

Table 3.3-3 Lipid Compositions of Elemental and Polymeric Formula

Fatty Acids	Elemental Formula (g/L)	Polymeric Formula (g/L)
Polyunsaturated	15.31-23.29	7.368
Monounsaturated	6.06-9.57	14.448
Saturated	2.68-6.22	12.432

Table 3.3-4 Essential Amino Acid in Elemental and Polymeric Formula

Amino Acids	Elemental Formula (g/L)	Polymeric Formula (g/L)
L-Histidine	1.7279	1.005
L-Isoleucine	2.5368	3.281
L-Leucine	5.7721	5.381
L-Lysine-HCl	5.7353	5.024
L-Methionine	1.0662	1.201
L-Phenylalanine	2.24	1.689
Taurine	0.2574	0.042
L-Threonine	2.9412	3.319
L-Tryptophan	1.1765	0.741
L-Valine	2.9412	2.956
Cystine	-	1.035

3.4 Sample Collection

3.4.1 Clinical Assessment and Growth

Piglet weight was measured each morning and stool and urine were collected to calculate fluid balance. Blood was taken at baseline and the end of the trial to measure hematology (complete blood count) and chemistry (urea, electrolytes and liver chemistry), analyzed at a veterinary laboratory (IDEXX, Canada) by automated procedures.

3.4.2 Plasma Sample Collection

Plasma GLP-2 samples were taken three times during the trial: at baseline, when the EN infusion rate increased to 50% of final rate, and at the end of the trial. Blood samples were drawn into chilled tubes containing EDTA, gently shaken, and centrifuged at 2000 G for 10 min immediately. The plasma collected was stored at -80℃ until further analysis.

3.4.3 Fecal Fat Collections

Fecal samples were collected on days 5-7 and days 12-14 during the trail. Fecal effluent was collected into fitted stoma appliances (Hollister, Canada). For each piglet, the total possible collection time was 48 to 72 hours, with individual collection periods every 6 to 8 hours. In this way only complete collections, where no leakage occurred, were included. EN bags were weighed for each collection period and the exact enteral lipid infused for the piglet was calculated.

3.4.4 Morphometry and Tissue Specimens

On day 14, all piglets underwent general anesthesia, the abdomen was opened, and the small bowel length was once again measured from the Ligament of Treitz to the ICV. Piglets were then euthanized using pentobarbital sodium (Schering, Canada). The entire small intestine distal to the ligament of Treitz was removed and immediately emptied of fecal matter and the dry weight was measured. The spiral colon and liver were also removed from the body and weighed.

In each segment of small intestine, cross-sectional samples were collected: 10 cm distal to the Ligament of Treitz, 10 cm proximal to the ileocecal valve (JI and sham). Liver specimens were also collected at a size of approximately 2cm×2cm×0.5cm. All these samples were preserved in 10% neutral-buffered formaldehyde and subsequently embedded in paraffin for at least 48-hour, for analysis of histology. Mid-section samples of each segment of intestine were also collected: jejunum, colon, and ileum (not for JC), and stored at -80℃ for mRNA analysis for GLP-2R, and immunohistochemistry analysis for GLP-1, L cell and crypt cell proliferation and apoptosis.

3.5 Plasma GLP-2 Analysis

Measurements of plasma GLP-2 concentration were performed as previously described (319). In short, the plasma samples were extracted in 75% ethanol (final concentration) to remove unspecific cross-reacting substances. GLP-2 immunoreactivity was measured with a specific NH_2 terminal radioimmunoassay using an antiserum raised

in rabbits (synthesized in University of Copenhagen, Copenhagen, Denmark). This antiserum cross-reacts with the N-terminal region of porcine GLP-2. The experimental detection limit was 5 pmol/L, and the intra-assay coefficient of variation was 2.3% at a concentration of 40 pmol/L.

3.6 Fat Absorption Analysis

The collected fecal samples were freeze-dried for 6 days before analysis. Each sample was analyzed in duplicate, with 5g as the standard sample size, and 2g the minimum sample size. Fat extraction was undertaken for 6 hours by petroleum ether distillation using a Goldfish apparatus (method Aa 4-38, AOAC 2000) (322). Fat extracted from each fecal sample was weighed after all ether solvent evaporated. The fat absorbed from the enteral diet was calculated by subtracting the fecal fat weight from the total amount of lipid infused during the total collection period. Fat absorption was adjusted for the total duration of fecal collection and expressed as grams per kilogram per day.

3.7 Histological Specimens and Analysis

The prepared paraffin blocks for the small intestine and liver samples were trimmed and placed in cassettes using standard techniques. From each intestinal site, 5 μm sections were stained with hematoxylin and eosin using a standard technique. A micrometer eyepiece (Nikon Eclipse 80i) was used to measure villus height and crypt depth. Only villi observed in longitudinal section were chosen to measure the height. The crypt depth measurement was taken in the same area as the chosen villus as often as possible. The section was required to be a true cross-section, with no angle. For each section, the first 10 measurements were used to calculate a mean and standard deviation for both villus height and crypt depth.

3.7.1 GLP-1 Measurement

Since anti-GLP-2 antibodies available cross react with too many other proteins, the anti-GLP-1 antibodies was used to get a clean picture. All sample sections were put in alcohol (final concentration was 70%) to remove paraffin. Subsequently, sections were incubated with primary antibody and secondary antibody in order. Then, the slides were visualized using 3, 3'-diaminobenzidine (DAB), and were counterstained by hematoxylin. Finally, a microscope was used to measure the number of cells per villus-crypt axis (for jejunum and ileum) or the number of cells per crypt (for colon).

3.7.2 L Cell Measurement

Preparation for L cell measurement was similar to the method for GLP-1 measurement. All slides were deparaffinized in alcohol, and incubated in primary antibody, secondary antibody, and Strept-HRP one by one. Then the sections were visualized with DAB, counterstained with hematoxylin, and finally watched under the microscope to count the number of cells per villus-crypt axis (for jejunum and ileum) or the number of cells per crypt (for colon).

3.7.3 Crypt Cell Proliferation and Apoptosis Measurements

The paraffin blocks used before was also used to cut slides for immunohistochemistry analysis. Ki67 immunohistochemistry was used to measure crypt cell proliferation. Deparaffinized 5 μm sections were rehydrated in ethanol baths. Following antigen retrieval, monoclonal mouse anti-Ki67 primary antibody was incubated with sides overnight at 4℃. An ultra streptavidin detection system was used to view the staining. Only crypts in longitudinal section were used to assess. The proliferation index was calculated as the percentage of Ki67 stained cells as a proportion of total cells within the ten entire crypts column.

Caspase-3 was used to measure apoptosis in the crypts and villi. The 5 μm paraffin sections were Deparaffinized and blocked with 3% hydrogen peroxide for 30 minutes to reduce endogenous peroxidase activity. Following one hour incubation in blocking buffer,

the slides were treated overnight at 4℃ with diluted anti-active caspase-3 antibody. After several washings, the slides were incubated with a diluted horseradish peroxidase-conjugated anti-rabbit IgG for 2 hours at room temperature. The diaminobenzidine was added to visualize activated casepase-3. Finally, the slides were counterstained with hematoxylin and analyzed. Only villi and crypts in longitudinal were used to exam. Crypt cell apoptosis was expressed as a percentage of the apoptosis cells (stained positive) out of the total number of cells examine in the ten crypts. Villi cell apoptosis was measured at the tips of villi, and 50 cells were counted on each side of the villus starting from the tip. The total count of each villous was 100 and the result was expressed as a percentage.

3.8 GLP-2 Receptor Measurements

GLP-2R was measured using reverse transcription polymerase chain reaction (RT-PCR) as described (236, 266). In short, total RNA was extracted from individual samples, and then the RNA was reverse transcribed. PCR amplification of GLP-2 receptor cDNA was performed on each individual sample, the product electrophoresed onto 1% agarose gel (BDH laboratory, Poole, UK) in Tris-borate-EDTA buffer and visualized by staining with 0.15% ethidium bromide. Cycle threshold (Ct), defined as the number of cycles required for the fluorescent signal to exceed background level, was obtained for each sample.

In each intestinal segment (jejunum, ileum and colon), the amount of GLP-2R in the sham piglets fed elemental formula was set as control. Therefore, the mean Ct values of GLP-2R mRNA in the three segments of the sham piglets fed elemental formula were calculated. Difference between test samples and control was calculated through: Ct (mRNA)-Ct (mRNA control). There is a linear relation between Ct and the logarithm of RNA amount (i.e., $Ct=k\log_2 RNA$), so the final result was the fold changes of the control

and expressed as $2^{-[Ct\ (mRNA)-Ct\ (mRNA\ control)]}$. Therefore, in each intestinal segment, the control was $2^{-[Ct\ (mRNA\ control)-Ct\ (mRNA\ control)]} = 2^0 = 1$.

3.9 Data Analysis and Statistics

All data were analyzed using SPSS (version 19), and the results were expressed as mean and standard deviation of the mean. Comparisons among the three surgical groups were analyzed by one-way analysis of variance (ANOVA). Comparison between the two formula groups was using independent-samples student's *t*-test. Within groups, paired-samples *t*-test was used to compare values collected at different timed or different segments of intestine. Comparison the ranking data between groups was using nonparametric test. Plasma GLP-2 levels were correlated with intestinal adaptation and fat absorption using Pearson Correlation Coefficient. An alpha value of $p<0.05$ was considered significant.

4 RESULTS

4.1 Piglets Performance & Nutrition

Overall, regardless of formula type the piglets in the JI and sham group were active and healthy, whereas piglets in the JC group were often lethargic. Within each surgical group, there was no difference in activity between piglets on the two types of formula. Sick piglets were removed from the trial and humanely euthanized. In total 30/78 piglets (25.6%) did not complete the trial. Amongst these, mortality rate in the JI group was 36.0% (9/25), and was 32.0% (8/25) in the JC group, and 46.4% (13/28) in the sham group with no significant difference (nonparametric test, p=0.538). Within the JI group, mortality rate of piglets fed elemental formula was 33.3% (4/12) and was 38.5% (5/13) in the piglets fed polymeric formula. In the JC group, mortality rate of piglets fed elemental formula was 25.0% (3/12) and was 38.5% (5/13) of polymeric fed piglets. In the sham group, the mortality rate was 56.3% (9/16) and 33.3% (4/12) in elemental fed and polymeric fed piglets, respectively. These mortality rates did not different between the two formula types within each surgical group, with p=0.794, p=0.480, and p=0.237, respectively.

On the basis of fever and/or persistent vomiting, catheter sepsis was suspected and blood cultures were taken. Positive blood culture results were found in two JI piglets (2/16), five JC piglets (5/17), and two Sham piglets (2/15), but these were not significantly different (chi-square test, p=0.382).

Weight gain was similar in each surgical group (JI, 143.0±40.2 g/day; JC, 128.7±47.9 g/day; Sham, 166.9±43.0 g/day; p=0.057). However, comparison between each two groups showed JC piglets had less weight gain than Sham (p=0.025), although not different to the JI (**Table 4.1-1**). JI had similar weight gain as Sham (p=0.120). Within each surgical group, between the formula types, weight gain also did not differ (JI, p=0.74; JC, p=0.63; sham, p=0.06) (**Table 4.1-2**).

The duration of PN support was longer in the JI and JC group than Sham group (JI, 10.3±2.7 days; JC, 13.4±1.5 days; Sham, 6.1±1.1 days; $p<0.001$). Duration of PN support was longer in the JC group than JI ($p=0.001$) (**Table 4.1-1**). All fifteen Sham piglets (100%) and eleven JI piglets (68.8%) were able to discontinue PN while only one JC piglet (5.9%) was able to wean off PN support (chi-square test, $p<0.001$). Simultaneously as expected, diarrhea was observed more often in JI and JC piglets compared to sham piglets (JI, 10.3±3.2 days; JC, 11.0±2.2 days; Sham, 2.5±2.9 days; $p<0.001$).

Within each surgical group, formula type had no effect on duration of PN support and diarrhea in the JI and sham group. In the JC group the duration of diarrhea and PN support were significantly longer given polymeric formula (days of diarrhea: elemental, 9.9±2.4 days, polymeric, 12.3±1.2 days, $p=0.02$; days of PN support: elemental, 12.7±1.9 days, polymeric, 14.1±0.4 days, $p=0.05$) (see **Table 4.1-2**). It was noticed that in the sham group, polymeric formula tended to be related to a higher weight gain than elemental formula and the difference approached significance ($p=0.059$). Sample power analysis showed the power for this was 0.543.

4.2 Piglets Hematology and Blood Biochemistry

The hematology results for all piglet groups are shown in **Table 4.2-1**. Both at baseline and at the end of the trial, all parameters among the three surgical groups were statistically the same. Differences between the baseline and terminal values were only found in the sham group, who increased hemoglobin and platelet levels throughout the trial ($p=0.005$, $p<0.001$; respectively).

At baseline all the hematology values were the same between the two formula types in each surgical group (see **Table 4.2-2**). In the JI piglets, formula type had no effect on the hematology results at the end of the trial, all parameters between the two formulas were the same. No difference between the baseline and terminal values was found. In the

JC group, comparison between the baseline and terminal value showed polymeric formula led to increased hemoglobin level (p=0.04) and decreased white cell count (p=0.03), but no difference was found when comparing these hematology values between the two diets at the end of trial. In the sham group the only differences were found in platelets levels through the trial, both elemental and polymeric formulas were associated with increased platelet levels (p=0.001, p=0.01; respectively), though no difference was found between the two diets at the end of trial (p=0.21).

Table 4.1-1 Piglet clinical observations (comparison among surgical groups)

	JI (n=16)	JC (n=17)	Sham (n=15)	*p*
Initial age (days)	5.2±1.1	5.1±0.9	4.6±1.1	0.221
Initial weight (kg)	2.3±0.3	2.3±0.3	2.2±0.3	0.481
Final age (days)	13.7±0.7	13.7±0.7	13.9±0.5	0.699
Final weight (kg)	4.2±0.7	4.1±0.9	4.5±0.7	0.332
Weight gain (g/day)	143.0±40.2	128.7±47.9[c]	166.9±43.0[b]	0.057
Days of PN support	10.3±2.7[bc]	13.4±1.5[ac]	6.1±1.1[ab]	<0.001
Days of diarrhea	10.3±3.2[c]	11.0±2.2[c]	2.5±2.9[ab]	<0.001
Highest EN tolerated (% total energy)	88.1±18.3[bc]	60.0±12.2[ac]	100.0±0[ab]	<0.001
EN rate 12 hr prior to termination (ml/hr)	40.9±18.6[bc]	19.8±14.0 [ac]	53.8±15.9[ab]	<0.001

Values are expressed as mean±SD

p values refer to comparison among the three groups using ANOVA

a, b, and c superscripts refer to comparison between each group using independent samples student T-test, p<0.05

Table 4.1-2 Piglet clinical observations (comparison within each surgical group)

		E (n=8)	P (n=8)	*p*
Final weight (kg)	JI	4.2±0.8	4.3±0.5	0.794
	JC	4.0±0.7	4.3±1.1	0.545
	Sham	4.2±0.3	4.8±0.9	0.141
Weight gain (g/day)	JI	139.4±53.7	146.6±23.5	0.735
	JC	123.1±37.8	134.9±59.3	0.627
	Sham	144.8±15.3	186.2±50.7	0.059
Days of diarrhea	JI	9.6±3.4	11.0±2.9	0.402
	JC	9.9±2.4	12.3±1.2	0. 023
	Sham	2.4±2.4	2.5±3.5	0.965
Days of PN support	JI	10.3±2.2	10.3±3.4	1.000
	JC	12.7±1.9	14.1±0.4	0.047
	Sham	6.0±0.0	6.3±1.6	0.668
Highest enteral tolerance rate (%)	JI	95.0±14.1	81.3±20.3	0.141
	JC	61.1±16.2	58.8±6.4	0.705
	Sham	100.0	100.0	-

Values are expressed as mean±SD

p, comparison between two formula types using independent samples student T-test, $p<0.05$

Table 4.2-3 summarizes the biochemistry results for all the piglets at baseline and end of trial. At baseline, there were no difference among the three surgical groups, except for galactosylhydroxylysyl glucosyltransferase (GGT), which was higher in the JC group (p=0.02). Throughout the trial, albumin and GGT levels increased, while alkaline phosphatase (ALP) levels decreased in all groups (albumin: JI, JC and Sham, $p<0.001$;

GGT: JI, JC, $p<0.001$, sham, $p=0.02$; ALP: JI, JC and Sham, $p<0.001$). Alanine aminotransferase (ALT) levels decreased in the JI and JC piglets, but not sham piglets (JI, $p=0.003$; JC, $p=0.009$). Increased total bilirubin was only observed in the JC piglets ($p=0.028$) and bile acids levels were not changed throughout the trial in all the three groups. Comparison of biochemistry values among the three groups showed that at the end of the trial the JC piglets had the highest total bilirubin and GGT levels (total bilirubin, $p=0.04$; GGT, $p<0.001$).

Table 4.2-4 summarizes the comparison of blood biochemistry results between the two formulas at baseline and end of trial. At baseline, all the blood biochemistry values were the same between the two formula types in each group.

In the JI group, both elemental and polymeric formulae were associated with decreased ALP levels and increased GGT level from baseline to the end of the trial. Decreased ALT level was only found in polymeric formula fed piglets, while increased albumin level, as well as decreased total bilirubin and bile acid levels, were only found in elemental formula fed piglets. When we compared each value at the end of trial between the two diets, the only difference observed was total bilirubin with a higher level in polymeric formula fed piglets ($p=0.039$).

In the JC group, both elemental and polymeric formula were associated with decreased ALP levels and increased GGT and albumin levels from baseline to the end of the trial. Decreased ALT level was only found in elemental formula fed piglets. When comparing each value at the end of trial between the diets, no difference was observed.

In the sham group, both elemental and polymeric formulae were associated with decreased ALP levels and increased albumin level from baseline to the end of the trial. Decreased bile acid levels were found in elemental formula fed piglets, while increased GGT level was only found in polymeric formula fed piglets. When comparing each value

at the end of trial between the two diets, no difference was found. The reference values of hematology and biochemistry in the normal piglets are listed in **Appendix 1**.

Table 4.2-1 Piglets hematology test (comparison among surgical groups)

		JI (n=16)	JC (n=17)	Sham (n=15)	*p*
Hemoglobin (g/L)	Baseline	82.3±6.7	83.1±10.1	79.9±10.7	0.619
	End	88.1±16.8	84.5±17.0	92.7±14.0	0.380
	p^*	0.179	0.576	0.005	
White cell count ($\times 10^9$/L)	Baseline	10.2±5.8	10.6±2.2	9.8±4.7	0.895
	End	7.7±2.9	9.2±5.6	7.8±3.4	0.541
	p^*	0.090	0.113	0.145	
Platelets ($\times 10^9$/L)	Baseline	389.3±103.5	432.6±98.3	403.4±70.0	0.431
	End	474.5±186.8	416.7±330.1	565.6±142.0	0.222
	p^*	0.136	0.776	<0.001	

Values are expressed as mean±SD

p values refer to comparison among the three groups using ANOVA

p^* values refer to comparison between baseline and terminal values using paired-samples t-test

Table 4.2-2 Piglets hematology test (comparison between two formulas)

			E	P	*p*
Hemoglobin (g/L)	JI	Baseline	83.6±9.3	81.3±3.7	0.524
		End	88.0±19.4	88.3±15.2	0.978
		p	0.444	0.297	
	JC	Baseline	85.1±8.2	81.4±11.8	0.492
		End	77.6±14.8	90.6±17.2	0.142
		p	0.374	0.040	
	Sham	Baseline	76.4±11.7	83.3±9.0	0.244
		End	89.6±16.0	95.5±12.3	0.432
		p	0.056	0.067	
White cell count ($\times 10^9$/L)	JI	Baseline	12.2±6.2	8.6±5.3	0.247
		End	7.8±1.7	7.6±3.9	0.864
		p	0.153	0.424	
	JC	Baseline	10.7±1.2	10.4±2.9	0.808
		End	12.2±6.6	6.5±2.7	0.069
		p	0.895	0.033	
	Sham	Baseline	11.5±5.0	8.1±3.8	0.181
		End	7.3±1.5	8.3±4.5	0.574
		p	0.069	0.973	
Platelets ($\times 10^9$/L)	JI	Baseline	383.1±57.5	394.6±136.1	0.839
		End	478.6±94.9	470.4±256.4	0.934
		p	0.074	0.486	
	JC	Baseline	433.9±132.2	431.5±65.9	0.965
		End	260.0±232.2	553.9±354.5	0.085
		p	0.079	0.385	
	Sham	Baseline	392.4±79.6	414.3±63.3	0.580
		End	515.3±118.6	609.6±153.5	0.211
		p	0.001	0.013	

Values are expressed as mean±SD; E, elemental formula; P, polymeric formula

p values refer to comparison between formula types using independent samples student T-test;

p^* values refer to comparison between baseline and terminal values using paired-samples t-tes*t*

Table 4.2-3 Piglet blood biochemistry (comparison among surgical groups)

		JI (n=16)	JC (n=17)	Sham (n=15)	*p*
Albumin (g/L)	Baseline	16.7±3.3	16.7±3.9	16.1±3.7	0.867
	End	23.4±5.0	22.2±4.4	23.2±2.7	0.671
	p^*	<0.001	<0.001	<0.001	
Total bilirubin (μmol/L)	Baseline	3.5±1.3	3.9±1.2	3.4±1.9	0.697
	End	6.6±8.0	13.6±13.5	4.8±3.5	0.041
	p^*	0.292	0.028	0.351	
Bile acids (μmol/L)	Baseline	7.3±3.4	6.4±2.5	6.4±2.4	0.612
	End	9.7±10.1	9.3±10.3	5.2±3.6	0.310
	p^*	0.306	0.372	0.281	
ALT (IU/L)	Baseline	35.8±7.3	32.7±8.3	32.1±10.8	0.485
	End	25.5±6.1	25.0±6.1	28.3±5.8	0.281
	p^*	0.003	0.009	0. 125	
ALP (IU/L)	Baseline	1441.5±266.8	1568.6±451.5	1646.6±436.3	0.375
	End	789.3±205.8	910.4±421.6	981.3±308.9	0.259
	p^*	<0.001	<0.001	<0.001	
GGT (IU/L)	Baseline	38.7±10.1	53.3±20.6	41.7±9.6	0.020
	End	50.4±13.5	125.6±60.5	59.1±27.4	<0.001
	p^*	<0.001	<0.001	0.020	

Values are expressed as mean±SD. ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; GGT, Galactosylhydroxylysyl glucosyltransferase

p values refer to comparison among the three groups using ANOVA

p^* values refer to comparison between baseline and terminal values using paired-samples t-test

Table 4.2-4 Piglets blood biochemistry (comparison between two formulas)

			E	P	*p*
Albumin (g/L)	JI	Baseline	16.0±4.7	17.4±1.5	0.484
		End	25.6±5.0	21.3±4.2	0.081
		p[¨]	<0.001	0.066	
	JC	Baseline	16.9±4.9	16.5±3.1	0.867
		End	23.0±5.2	21.4±3.5	0.476
		p[*]	0.036	0.003	
	Sham	Baseline	16.0±2.6	16.1±4.7	0.945
		End	22.1±3.4	24.1±1.5	0.160
		p[*]	0.006	0.003	
Total bilirubin (μmol/L)	JI	Baseline	3.4±1.0	3.6±1.6	0.845
		End	2.1±2.7	10.5±9.2	0.039
		p[*]	0.001	0.111	
	JC	Baseline	3.4±1.0	4.3±1.3	0.191
		End	13.9±15.8	13.4±12.3	0.948
		p[*]	0.289	0.071	
	Sham	Baseline	4.0±2.0	2.9±1.8	0.280
		End	5.0±4.7	4.6±2.4	0.836
		p[¨]	0.714	0.304	
Bile acids (μmol/L)	JI	Baseline	6.2±1.7	8.3±4.2	0.244
		End	4.6±2.3	14.2±12.3	0.064
		p[*]	0.045	0.180	
	JC	Baseline	5.8±1.8	7.0±3.1	0.379
		End	10.8±13.3	7.7±7.2	0.633
		p[*]	0.434	0.765	
	Sham	Baseline	6.8±2.7	6.0±2.3	0.559
		End	3.3±1.8	7.1±4.0	0.052
		p[¨]	<0.001	0.585	
ALT	JI	Baseline	36.7±10.3	35.0±3.5	0.665

(IU/L)		End	27.3±5.2	23.8±6.7	0.264
		p^*	0.140	0.006	
		Baseline	34.9±11.0	30.8±4.9	0.355
	JC	End	24.3±7.2	25.8±5.3	0.641
		p^*	0.047	0.081	
		Baseline	31.9±11.1	32.4±11.4	0.926
	Sham	End	26.9±3.4	29.5±7.4	0.403
		p^*	0.339	0.159	
		Baseline	1405.7±322.9	1472.9±224.9	0.644
	JI	End	882.6±219.8	696.0±150.2	0.067
		p^*	0.006	<0.001	
		Baseline	1678.1±534.8	1459.1±351.2	0.349
ALP (IU/L)	JC	End	1047.5±515.6	773.4±268.4	0.210
		p^*	0.031	0.005	
		Baseline	1639.7±454.0	1653.4±454.2	0.956
	Sham	End	1029.6±344.3	939.0±291.4	0.590
		p^*	0.027	0.007	
		Baseline	38.6±12.2	38.8±8.7	0.974
	JI	End	51.5±14.9	49.3±13.0	0.752
		p^*	0.003	0.016	
		Baseline	56.4±28.4	50.1±9.1	0.569
GGT (IU/L)	JC	End	124.5±64.7	126.6±60.4	0.947
		p^*	0.045	0.005	
		Baseline	44.6±11.0	38.9±7.8	0.283
	Sham	End	56.4±29.4	61.5±27.3	0.735
		p^*	0.268	0.043	

Values are expressed as mean±SD. E, elemental formula; P, polymeric formula; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; GGT, Galactosylhydroxylysyl glucosyltransferase;

p values refer to comparison between formula types using independent samples student T-test

p^* values refer to comparison between baseline and terminal values using paired-samples t-test

4.3 Fat Absorption

Table 4.3-1 shows enteral fat absorption in each surgical group. Results are expressed as fat absorbed per day, fat absorbed per day per kilogram, percent fat absorption and fat absorption per day per centimeter of small intestine length at day 14. During both the first (day 5) and second (day 12) fecal collections, enteral fat infusion in JI and Sham piglets was the same, but higher than in JC piglets ($p<0.001$). The total amount of enteral fat absorbed, in both collection periods, were different among groups, with Sham having the highest, and JC the lowest ($p<0.001$). During the first stool collection period, Sham piglets had the highest percent fat absorption and JC the lowest ($p<0.001$). During the second collection, JI piglets had the same fat absorption as JC piglets; lower than in Sham piglets ($p<0.001$). When fat absorption was adjusted for absorption per length of small intestine and expressed as gram of lipid per centimeter per day, the JI piglets had the highest and Sham piglets the lowest fat absorption ($p<0.001$). Between day 5 and day 12, enteral fat absorption was increased in JI piglets when expressed as grams of lipid per day or adjusted for the small intestine length ($g \cdot day^{-1} \cdot cm^{-1}$) ($p<0.05$). However, no difference in fat absorption between the periods was found in JC piglets. Within each surgical group, the amount of lipid delivered was the same between the two formulas, while the amount of fat that absorbed was also the same between the diets, both at day 5 and day 12 (see **Table 4.3-2**).

Table 4.3-1 Enteral fat absorption (comparison among surgical groups)

	JI (n=16)	JC (n=17)	Sham (n=15)	*p*
Enteral fat delivery, day 5 (g/day)	16.1±4.3^{b}	11.3±3.6^{ac}	18.0±4.6^{b}	<0.001
Fat absorbed, day 5 (g/day)	13.8±4.0^{bc}	8.2±2.9^{ac}	17.2±5.0^{ab}	<0.001
Enteral fat delivery, day 12 (g/day)	25.9±10.0^{b}	15.2±6.0^{ac}	30.6±6.7^{b}	<0.001
Fat absorbed, day 12 (g/day)	19.6±8.4^{bc}	10.7±7.1^{ac}	30.0±7.1^{b}	<0.001
Enteral fat delivery, day 5 ($g \cdot kg^{-1} \cdot day^{-1}$)	5.3±1.5^{b}	3.9±1.3^{ac}	6.0±1.2^{b}	<0.001
Fat absorbed, day 5 ($g \cdot kg^{-1} \cdot day^{-1}$)	4.6±1.3^{bc}	2.9±1.1^{ac}	5.7±1.4^{ab}	<0.001
Enteral fat delivery, day 12 ($g \cdot kg^{-1} \cdot day^{-1}$)	6.5±2.8^{b}	3.9±1.2^{ac}	7.6±2.0^{b}	<0.001
Fat absorbed, day 12 ($g \cdot kg^{-1} \cdot day^{-1}$)	4.8±1.9^{bc}	2.6±1.5^{ac}	7.4±2.1^{ab}	<0.001
Fat absorbability, day 5 (%)	85.6±8.4^{bc}	73.3±14.1^{ac}	94.8±10.5^{ab}	<0.001
Fat absorbability, day 12 (%)	76.3±22.9^{c}	65.2±27.4^{c}	97.5±6.1^{ab}	<0.001
Fat absorption, day 5 ($g \cdot day^{-1} \cdot cm^{-1}$)	0.07±0.02^{bc}	0.05±0.02^{ac}	0.02±0.01^{ab}	<0.001
Fat absorption, day 12 ($g \cdot day^{-1} \cdot cm^{-1}$)	0.10±0.04^{bc}	0.07±0.04^{ac}	0.04±0.01^{ab}	<0.001
Fat absorption, day 5 ($g \cdot kg^{-1} \cdot day^{-1} \cdot cm^{-1}$)	0.024±0.008^{c}	0.019±0.008^{c}	0.007±0.001^{ab}	<0.001
Fat absorption, day 12 ($g \cdot kg^{-1} \cdot day^{-1} \cdot cm^{-1}$)	0.025±0.010^{bc}	0.017±0.010 ^{ac}	0.010±0.003^{ab}	<0.001

Values are expressed as mean±SD

p values refer to comparison among the three groups using ANOVA

a, b, and c superscripts refer to comparison between each group using independent samples student T-test, $p<0.05$

Table 4.3-2 Enteral fat absorption (comparison between two formulas)

		E	P	*p*
Enteral fat delivery, day 5 ($g \cdot kg^{-1} \cdot day^{-1}$)	JI	5.4±1.1	5.3±1.9	0.940
	JC	3.9±1.3	4.1±1.4	0.760
	Sham	6.1±0.8	5.9±1.6	0.776
Fat absorbed, day 5 ($g \cdot kg^{-1} \cdot day^{-1}$)	JI	4.6±1.1	4.5±1.5	0.956
	JC	2.8±1.3	3.0±0.9	0.750
	Sham	5.5±1.3	5.8±1.6	0.649
Fat absorbability, day 5 (%)	JI	85.0±7.8	86.2±9.4	0.774
	JC	71.5±13.8	75.3±15.2	0.598
	Sham	90.1±14.3	98.9±2.0	0.152
Enteral fat delivery, day 12 ($g \cdot kg^{-1} \cdot day^{-1}$)	JI	7.4±2.9	5.6±2.5	0.199
	JC	3.8±1.2	3.9±1.2	0.764
	Sham	7.3±2.0	7.8±2.2	0.637
Fat absorbed, day 12 ($g \cdot kg^{-1} \cdot day^{-1}$)	JI	5.3±1.5	4.3±2.3	0.336
	JC	2.7±1.6	2.5±1.5	0.783
	Sham	7.0±2.1	7.8±2.2	0.491
Fat absorbability, day 12 (%)	JI	76.3±18.3	76.4±28.0	0.997
	JC	67.4±30.1	62.9±26.3	0.755
	Sham	95.3±8.7	99.5±0.6	0.245
Fat absorption, day 12 ($g \cdot day^{-1} \cdot cm^{-1}$)	JI	0.11±0.03	0.10±0.05	0.609
	JC	0.07±0.04	0.07±0.05	0.842
	Sham	0.04±0.01	0.04±0.01	0.245
Fat absorption, day 12 ($g \cdot kg^{-1} \cdot day^{-1} \cdot cm^{-1}$)	JI	0.027±0.008	0.023±0.011	0.461
	JC	0.019±0.011	0.015±0.008	0.447
	Sham	0.009±0.004	0.010±0.003	0.563

Values are expressed as mean±SD. E, elemental formula; P, polymeric formula

p, comparison between formula types using independent samples student T-test, $p<0.05$.

4.4 Small Intestine Growth and Adaptation

Absolute small intestine length over the course of the trial increased the most in sham piglets, compared to JI piglets, but did not increase in JC piglets (JI, 33.6±24.0 cm; JC, -0.8±15.7 cm; Sham, 191.7±110.6 cm; p<0.001). Dry small bowel weight was the highest in the sham piglets and lowest in the JC piglets (JI, 89.7±33.6 g; JC, 39.5±13.2 g; sham, 191.8±42.3 g; p<0.001). Expressed as a percentage of post-resection length, JI piglets and sham piglets had similar intestinal lengthening, while JC piglets did not lengthen (JI, 21.7±15.4%; sham, 32.5±20.2%; JC, -0.7±9.5%; p<0. 001). Similarly, the weight of the small intestine per unit length was greater in JI than JC and sham (JI, 0.46±0.16 g/cm; JC and sham, 0.25±0.07 g/cm; p<0.001). Colon weight was higher in the JI piglets than in the sham piglets, while was the same as in the JC piglets (JI, 102.9± 34.9 g; JC, 82.0±54.9 g; sham, 61.4±21.1g; p=0.023) (see **Table 4.4-1**).

Histological evidence of small intestinal adaptation was notable in JI piglets, with higher villi and deeper crypts in the ileum, than sham piglets. Histological adaptation of the jejunum was not observed in JC piglets during this study period. In fact the villus height was lower than the other two groups, which may represent atrophy. See **Table 4.4-1** and **Appendix 2**

Jejunum crypt cell proliferation measurement showed there was no difference among the three surgical groups (JI, 27.4±12.7%; JC, 27.6±9.1%; sham, 30.6±7.4%; p=0.639). The possible expansion could be the proportional increase of absolute proliferative cell number and total crypt cell number in the JI piglets (proliferative cell number: JI, 333.8±75.3; JC, 260.3±73.0; sham, 242.1±67.4; p=0.006; total crypt cell number: JI, 1044.1±148.8; JC, 973.0±204.7; sham, 794.4±139.7; p=0.002). Therefore, the proliferation index, expressed as the percentage of proliferative cell over total crypt cell number remained unchanged. Apoptosis measurement also showed no difference among the three groups. Jejunum crypt apoptosis was the same in the three groups (JI, 1.1 ±2.8%, JC, 0.9±0.5%; sham, 0.5±0.3%; p=0. 624). Similarly, there was also no

difference of jejunum villi apoptosis (JI, 5.2±5.0%, JC, 4.9±8.2%; sham, 1.8±1.6%; p=0.234) (see Table **4.4-2**).

Within each surgical group, there was no significant difference for all small intestinal growth and adaptation measures as well as colon weight between the elemental formula fed and polymeric formula fed (see **Table 4.4-3**). However, in the JC group, small bowel length change and small bowel length change expressed as the percentage were noticed to be higher in the polymeric formula fed piglets than elemental formula fed piglets, though no significant difference was found (p=0.066, p=0.072; respectively). Sample power analysis showed the powers for these two was 0.473 and 0.462 respectively.

Table 4.4-1 Morphological adaptation of small intestine (comparison among surgical groups)

	JI (n=16)	JC (n=17)	Sham (n=15)	*p*
Initial SBL (cm)	619.0±81.0	611.0±87.8	605.2±66.6	0.889
Post-resection SBL (cm)	158.6±14.2^{c}	157.1±16.5^{c}	605.2±66.6^{ab}	<0.001
SBL at terminal surgery (cm)	192.2±24.3^{bc}	156.3±24.5^{ac}	796.8±117.6^{ab}	<0.001
SBL change (cm)	33.6±24.0^{bc}	-0.8±15.7^{ac}	191.7±110.6^{ab}	<0.001
SBL change (%)	21.7±15.4^{b}	-0.7±9.5^{ac}	32.5±20.2^{b}	<0.001
SBDW (g)	89.7±33.6^{bc}	39.5±13.2^{ac}	191.8±42.3^{ab}	<0.001
SBDW/SBL (g/cm)	0.46±0.16^{bc}	0.25±0.07^{a}	0.25±0.07^{a}	<0.001
Colon weight (g)	102.9±34.9^{c}	82.0±54.9	61.4±21.1^{a}	0.023
Jejunum villi height (x10^{-1}mm)	7.2±1.7^{b}	5.3±1.0^{ac}	7.3±2.7^{b}	0.009
Jejunum crypts depth (x10^{-1}mm)	2.0±0.3^{bc}	1.7±0.3^{a}	1.6±0.4^{a}	0.002
Ileum villi height (x10^{-1}mm)	7.8±2.6	-	5.6±1.3	0.008
Ileum crypts depth (x10^{-1}mm)	1.8±0.3	-	1.4±0.3	<0.001

Values are expressed as mean±SD. SBL, small bowel length, SBDW, small bowel dry weigh; small bowel length change % is expressed as the percentage of small bowel length post resection

p values refer to comparison among the three groups using ANOVA

a, b, and c superscripts refer to comparison between each group using independent samples student T-test, p<0.05

Table 4.4-2 Jejunum crypt cell proliferation and apoptosis (comparison among surgical groups)

	JI (n=14)	JC (n=15)	Sham (n=14)	*p*
Ki67 cell count	333.8±75.3[bc]	260.3±73.0[a]	242.1±67.4[a]	0.006
Total cell count for Ki67	1044.1±148.8[c]	973.2±204.7[c]	794.4±139.7[ab]	0.002
Crypt cell proliferation (%)	27.4±12.7	27.6±9.1	30.6±7.4	0.639
Casepase-3 cell count	10.1±19.4	7.1±5.3	4.9±3.4	0.495
Total cell count for Case-3	1013.3±197.1	876.2±262.0	943.5±256.5	0.374
Crypt cell apoptosis (%)	1.1±2.8	0.9±0.5	0.5±0.3	0.624
Villous cell apoptosis (%)	5.2±5.0	4.9±8.2	1.8±1.6	0.234

Values are expressed as mean±SD

p values refer to comparison among the three groups using ANOVA

a, b, and c superscripts refer to comparison between each group using independent samples student T-test, $p<0.05$

Table 4.4-3 Morphological adaptation of small intestine (comparison between two formulas)

		E	P	*p*
Post-resection SBL (cm)	JI	161.3±13.0	156.0±15.6	0.478
	JC	156.4±16.1	157.8±18.1	0.877
	Sham	616.1±71.2	595.6±65.5	0.571
SBL end (cm)	JI	199.4±20.4	185.0±27.1	0.249
	JC	149.1±17.4	164.4±29.7	0.209
	Sham	814.9±156.5	781.1±78.0	0.618
SBL change (cm)	JI	38.2±20.7	29.0±27.6	0.464
	JC	-7.3±11.5	6.6±17.3	0.066
	Sham	198.8±110.9	185.5±117.6	0.826
SBL change (%)	JI	24.1±13.1	19.3±18.0	0.548
	JC	-4.6±6.8	3.7±10.6	0.072
	Sham	31.8±17.2	33.1±23.8	0.901
SBDW (g)	JI	100.8±34.6	78.6±30.8	0.197
	JC	36.1±8.5	43.3±16.9	0.305

	Sham	190.8±27.7	192.7±54.0	0.937
SBDW/SBL (g/cm)	JI	0.51±0.17	0.42±0.15	0.324
	JC	0.24±0.06	0.26±0.09	0.620
	Sham	0.24±0.06	0.25±0.08	0.846
Colon weight (g)	JI	99.1±32.5	106.6±39.1	0.684
	JC	73.9±41.1	91.1±69.1	0.537
	Sham	50.9±10.7	70.6±24.1	0.069
Jejunum villi height (10-1mm)	JI	7.0±1.6	7.3±2.0	0.792
	JC	5.1±1.0	5.6±1.1	0.297
	Sham	8.1±3.6	6.5±1.3	0.331
Jejunum crypts depth (10-1mm)	JI	2.1±0.3	1.9±0.4	0.212
	JC	1.7±0.4	1.7±0.1	0.917
	Sham	1.6±0.5	1.5±0.3	0.652
Ileum villi height (10-1mm)	JI	8.9±2.5	6.7±2.3	0.105
	JC	-	-	-
	Sham	5.8±0.5	5.5±1.9	0.652
Ileum crypts depth (10-1mm)	JI	1.9±0.3	1.7±0.3	0.301
	JC	-	-	-
	Sham	1.4±0.3	1.3±0.2	0.790

Values are expressed as mean±SD. SBL, small bowel length, SBDW, small bowel dry weigh; small bowel length change % is expressed as the percentage of small bowel length post resection; E, elemental formula; P, polymeric formula

p, comparison between two formula types using independent samples student T-test, $p<0.05$.

4.5 Plasma GLP-2 Levels

GLP-2 levels increased from baseline to the end of the trial in JI piglets (baseline, 21.3±11.4 pM; 50:50, 40.2±13.6 pM; day 14, 73.0±33.9 pM; $p<0.01$) (see **Figure 4.5-1**). In JC piglets, plasma GLP-2 only increased from the baseline to the time point when piglets were receiving 50% total energy enterally, no increase was found between this time point to the end of trial (day 14) (baseline, 17.1±11.7 pM; 50:50, 30.1±18.1 pM; end 28.4±21.0 pM). Plasma GLP-2 level did not change throughout the trial in sham piglets.

Comparing among groups, JI piglets showed the highest levels of plasma GLP-2 at the second collection, when all groups were on 50% enteral nutrition (JI, 40.2±13.6 pM; JC 30.1±18.1 pM; sham, 24.1±14.6 pM; p=0.024). Similarly at the end of trial, JI piglets had highest GLP-2 levels (JI, 73.0±33.9 pM; JC, 28.4±21.0 pM; sham, 31.8±21.7 pM, p<0.001). The amount of plasma GLP-2 levels changed from the baseline to the end of the trial was also significantly higher in the JI piglets than in the JC and sham piglets, the comparison can be seen in the **Figure 4.5-2**.

Within surgical groups, differences in plasma GLP-2 level were only found in JC piglets with polymeric formula, which induced a higher GLP-2 level than elemental formula (p=0.04). In both JI and sham piglets, formula type had no effect on plasma GLP-2 levels (see **Table 4.5, Figure 4.5-3**). When the data was separated by formula groups (elemental vs polymeric) and comparison was made at the end of the trial among the three surgical groups, no difference in GLP-2 level was found in piglets fed with polymeric formula among the three groups (p=0.087). However, significant difference in GLP-2 levels were still observed in the JI group when fed with an elemental formula (p<0.001) (see **Table 4.5-1**). A post hoc multiple comparison test was used to compare GLP-2 level in piglets fed polymeric formula and a significant difference between JI and sham piglets was found (p=0.041), while there was still no difference between JI and JC or JC and sham.

Interestingly, when the level of plasma GLP-2 at the end of the trial was divided by the small intestinal length and expressed as the level of GLP-2 per centimeter, the JI piglets still had the highest GLP-2 level (JI, 0.38±0.18pM; JC, 0.20±0.16pM; sham, 0.04±0.03pM; p<0.001), but JC piglets also had a higher plasma GLP-2 than the sham piglets (p=0.002) (see **Figure 4.5-4**).

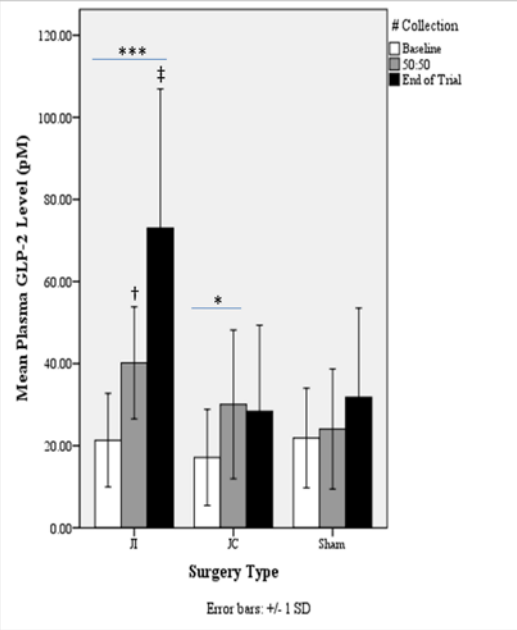

Figure 4.5-2 Comparison of plasma GLP-2 levels among and within three surgical groups

Comparison among groups: ‡ $p<0.001$, † $p<0.05$, both at the 50:50 and day 14, JI piglets had higher plasma GLP-2 level than JC and sham piglets. Comparison within group: *** $p<0.001$, in the JI group, plasma GLP-2 level increased from the baseline to the end of the trial, * $p<0.05$, in the JC group, increased plasma GLP-2 was only found from the baseline to the time point when piglets received 50% enteral nutrition; plasma GLP-2 level did not change throughout the trial in Sham piglets.

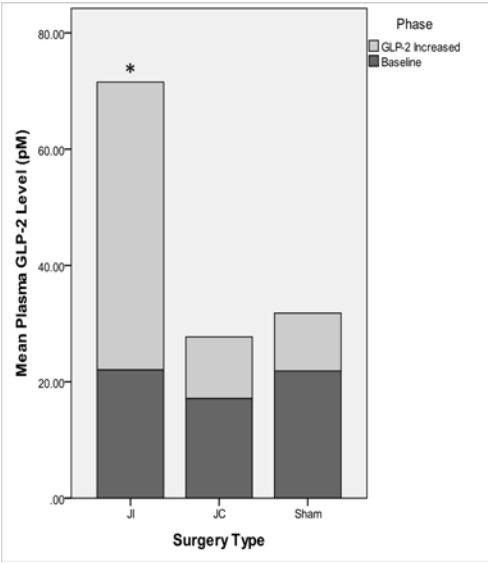

Figure 4.5-1 Comparison of the amount of plasma GLP-2 increased from baseline to the end of the trial among the three surgical groups

Dark grey bars represent plasma GLP-2 level at the baseline, and light grey bars represent the amount of GLP-2 increased from the baseline to day 14, therefore the dark and light grey bars together represent the plasma GLP-2 levels at the end of the trial (day 14). *$p<0.01$, the amount of increased plasma GLP-2 was higher in the JI piglets, while no difference were found between JC and sham piglets.

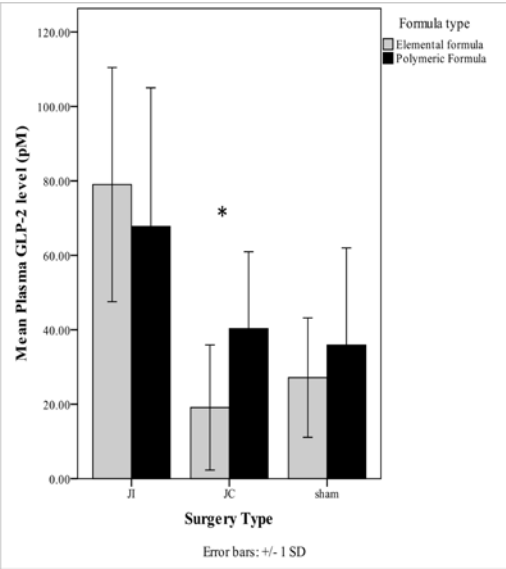

Figure 4.5-4 Comparison of plasma GLP-2 levels at the end of trial between the two formulas within each surgical group

*p=0.04, differences in plasma GLP-2 level were only found in JC piglets with polymeric formula induced a higher GLP-2 level than elemental formula; In both JI and sham piglets, formula type had no effect on plasma GLP-2 levels.

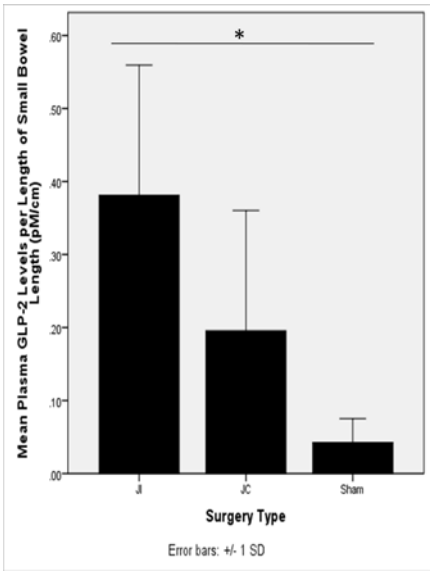

Figure 4.5- 3 Comparison of plasma GLP-2 levels per small bowel length (at the end of the trial) among the three surgical groups.

* p<0.001, JI piglets had the highest GLP-2 level per small bowel length while sham piglets had the lowest.

Table 4.5-1 Comparison of plasma GLP-2 level at day 14 among and within surgical groups

		Elemental Formula	Polymeric Formula	p
Plasma GLP-2 Level (pM)	JI	79.0±31.5	67.8±37.2	0.537
	JC	19.1±16.8	40.3±20.7	0.04
	sham	27.1±16.0	35.9±26.1	0.458
p^*		<0.001	0.087	

Values are expressed as mean±SD

p, comparison between two formula types within each surgical group using independent samples student T-test; p^*, comparison among the three surgical groups within each formula type

4.5.1 Plasma GLP-2 and Intestinal Adaptation

Correlations between plasma GLP-2 level at the end of trial and histological measurements of intestinal adaptation were calculated in short bowel piglets (JI and JC). Plasma GLP-2 levels were significantly correlated with small intestinal length, r =0.461, p=0.009 (**Figure 4.5.1-1a**); with the change or increase in small intestinal length, r =0.65, p<0.001; and with the remaining small bowel weight, r =0.67, p<0.001 (**Figure 4.5.1-1b**). A significant correlation was also observed between plasma GLP-2 level and total remaining colon weight, r =0.398, p=0.027.

In short bowel piglets (JI and JC), correlations between jejunum histological adaptation and plasma GLP-2 are significant (see **Figure 4.5.1-2**). Correlations between plasma GLP-2 levels and ileal adaptation are available for JI piglets. There was no correlation between GLP-2 level and ileal villus height, but there was a significant correlation with ileal crypt depth (**Figure 4.5.1-2**).

4.5.2 Plasma GLP-2 Levels and Nutrition

As enteral fat is known to be a potent stimulant of GLP-2 release, correlations between fat absorption and GLP-2 levels were studied. The amount of enteral lipid infused each day was significantly correlated with GLP-2 levels at the end of trial, r=0.684, p<0.001 (**Figure 4.5.2-1**). Plasma GLP-2 levels were significantly correlated with the amount of fat absorbed, r=0.592, p=0.001 (**Figure 4.5.2-2**), and fat absorbed per unit length of intestine, r=0.503, p=0.005 (**Figure 4.5.2-3**).

To examine the relationship between GLP-2 and enteral nutritional tolerance, the correlation between plasma GLP-2 and the highest enteral nutrition rate (percentage of total calories) was studied. Plasma GLP-2 levels were significantly correlated with enteral feeding rate, r =0.638, p<0.001 (**Figure 4.5.2-4**). Similarly, plasma GLP-2 had a significant inverse correlation with duration of PN support, r =-0.559, p=0.001 (**Figure 4.5.2-5**).

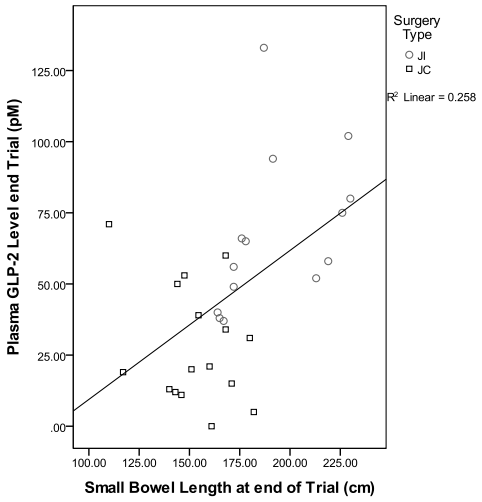

Figure 4.5.1-1a Plasma GLP-2 levels (pM) at day 14 vs. small bowel length (cm) in the short bowel piglets, linear regression analysis, p=0.009.

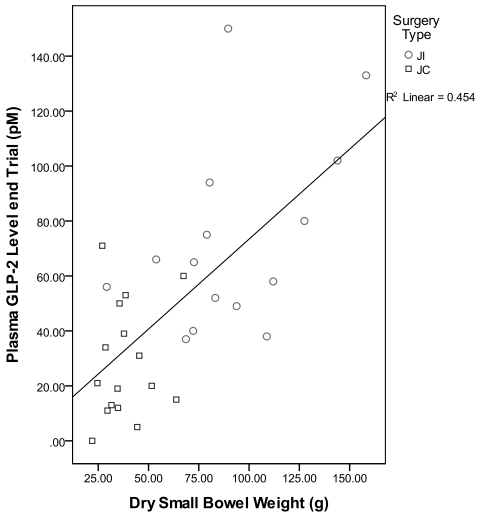

Figure 4.5.1-1b Plasma GLP-2 levels (pM) at day 14 vs. remnant small bowel weight (g) in the short bowel piglets, linear regression analysis, p<0.001.

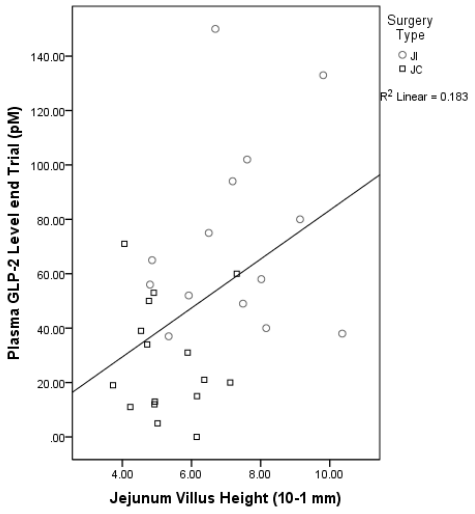

Figure 4.5.1-2a Plasma GLP-2 levels (pM) at day 14 vs. jejunum villus height (10^{-1}mm) in the short bowel piglets, linear regression analysis, p=0.018.

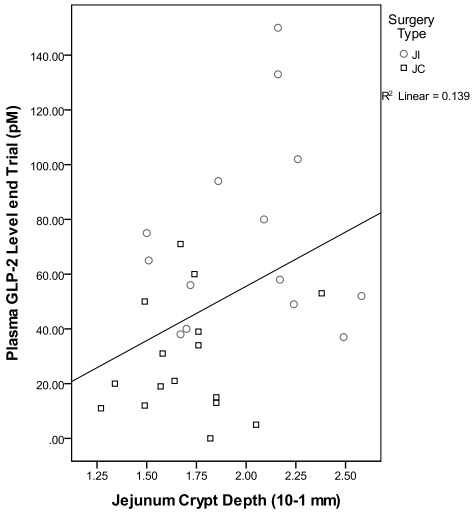

Figure 4.5.1-2b Plasma GLP-2 levels (pM) at day 14 vs. jejunum crypt depth (10^{-1}mm) in the short bowel piglets, linear regression analysis, p=0.042.

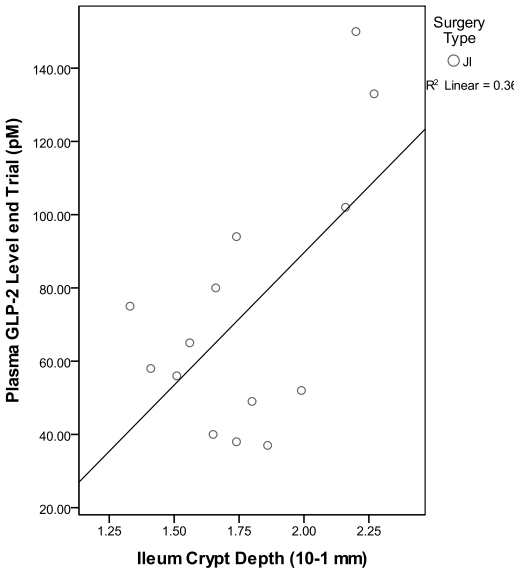

Figure 4.5.1-2c Plasma GLP-2 levels (pM) at day 14 vs. ileum crypt depth (10^{-1}mm) in the JI piglets, linear regression analysis, p=0.023.

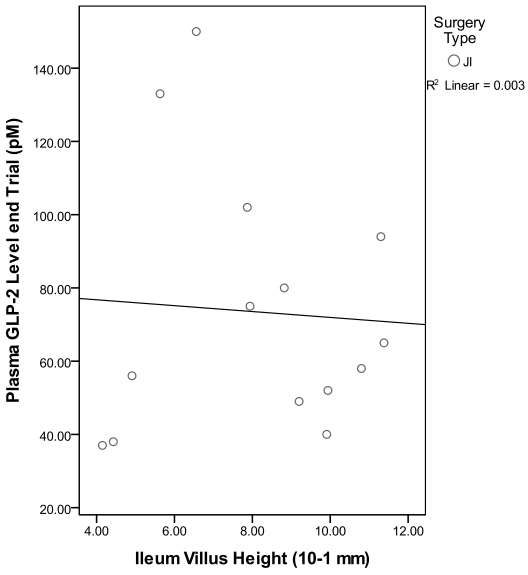

Figure 4.5.1-2d Plasma GLP-2 levels (pM) vs. ileum villus height (10^{-1}mm) in the JI piglets, linear regression analysis, p=0.843.

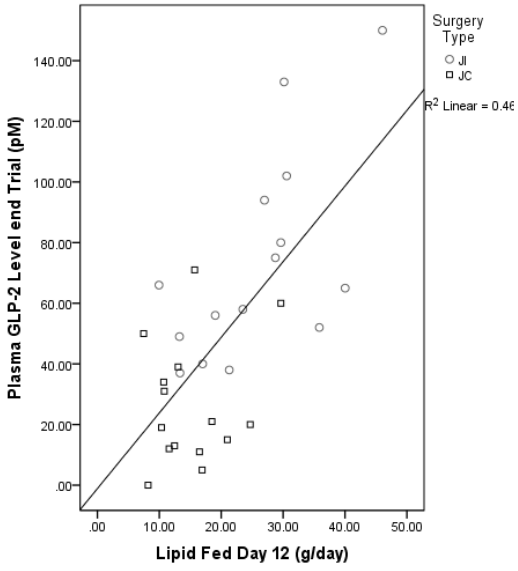

Figure 4.5.2-1 Plasma GLP-2 levels (pM) at day 14 vs. the amount of enteral lipid infused (g/day) in the short bowel piglets, linear regression analysis, p<0.001.

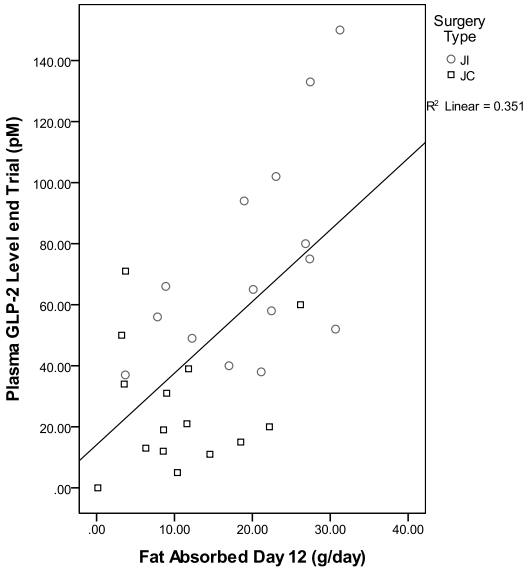

Figure 4.5.2-2 Plasma GLP-2 levels (pM) at day 14 vs. that amount of enteral lipid absorbed (g/day) in the short bowel piglets, linear regression analysis, p=0.001.

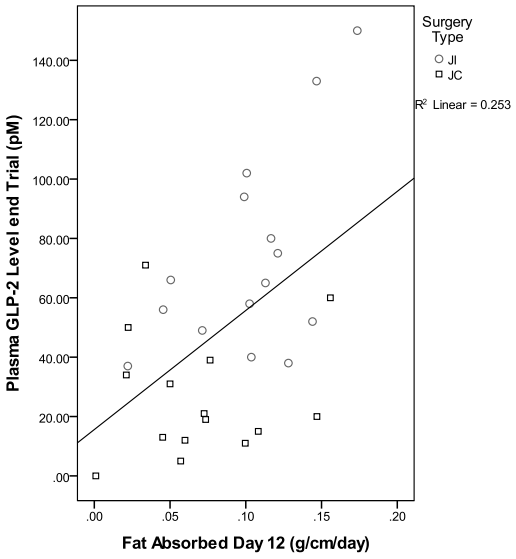

Figure 4.5.2-3 Plasma GLP-2 levels (pM) at day 14 vs. the amount of enteral lipid absorbed per unit small bowel length ($g \cdot day^{-1} \cdot cm^{-1}$), linear regression analysis, p=0.005.

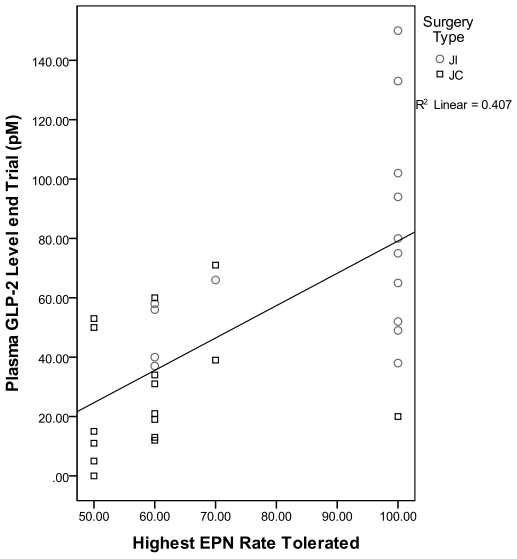

Figure 4.5.2-4 Plasma GLP-2 levels (pM) at day 14 vs. the highest enteral infusion rate (percentage of total calories) in the short bowel piglets, linear regression analysis, p<0.001.

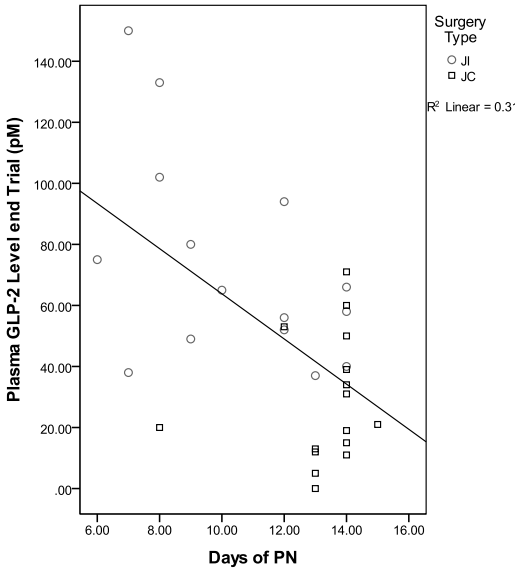

Figure 4.5.2-5 Plasma GLP-2 levels (pM) at day 14 vs. the duration of PN support (days) in short bowel piglets, linear regression analysis, p=0.005.

4.6 GLP-2 Receptor (GLP-2R) Expression

Some extremely out-lying values were noted and excluded from the analysis (it is noted that inclusion of the extreme out-lying values reduced the statistical significance of the results). Within each surgical group, we compared the GLP-2R expression in jejunum, ileum and colon between each intestinal segment. Within the JI and JC piglet groups, the jejunum had the highest GLP-2R expression, but this difference was not found in the sham piglets (see **Table 4.6-1**). Comparisons of GLP-2R expression among different surgical groups per each intestinal segment are listed in **Table 4.6-2** (baseline and termination)**,** and shown graphically in **Figure 4.6-1**. At the end of the trial (day 14), GLP-2R expressions in the jejunum was lower in the sham piglets than in the JI and JC piglets (p=0.001). In the ileum, no difference of GLP-2R expression was found between JI and sham piglets (p=0.120). In the colon, the GLP-2R expression was the highest in the JC piglets and the lowest in the sham piglets. Comparison of GLP-2 expression between baseline and termination showed the expression was increased in the ileum of JI piglets and in the colon of JC piglets (**Table 4.6-3**).

When correlations between tissue GLP-2R expression and plasma GLP-2 (expressed as pM or pM/cm) were calculated, the only positive correlation was found in the jejunum of the JI piglets (r=0.689, p=0.028; r=0.710, p=0.021, respectively) (see **Figure 4.6-2**).

Table 4.6-1 Comparison of GLP-2R expression between different segments (jejunum, ileum, and colon) within each surgical group

	Jejunum	Ileum	Colon
JI	3.0 ± 2.2^{b}	0.3 ± 0.3^{a}	1.2±1.5
JC	6.0 ± 4.6^{c}	-	1.6 ± 1.0^{a}
Sham	0.7±0.5	0.6±0.5	0.8±0.5

Values are expressed as mean±SD

a, b, and c superscripts refer to comparison between different segments within each surgical group using paired samples T-test, p<0.05.

Table 4.6-2 GLP-2 receptor expression in the jejunum, ileum and colon (baseline and termination)

		JI	JC	Sham	*p*
Baseline (fold change)	Jejunum	8.6±9.4	6.7±5.4	-	0.584
	Ileum	0.2±0.2	0.6±0.6	-	0.013
	Colon	-	0.03±0.05	-	-
Termination (fold change)	Jejunum	3.0±2.2	6.0±4.6	0.7±0. 5	0.001
	Ileum	0.3±0.3	-	0.6±0.5	0.120
	Colon	1.2±1.5	1.6±1.0	0.8±0.5	0.264

Values are expressed as mean±SD

p values refer to comparison between the two groups at base line using independent samples student T-test or comparison among the three groups using ANOVA

Table 4.6-3 Comparison of GLP-2R expression in each segment between baseline and termination within each surgical group

		Base	Term	*p*
JI	Jejunum	8.9±10.0	3.5±2.1	0.165
	Ileum	0.13±0.18	0.34±0.28	0.024
JC	Jejunum	6.7±5.4	6.2±4.8	0.761
	Colon	0.04±0.06	1.8±1.0	0.007

Values are expressed as mean±SD

p values refer to comparison between baseline and termination of each segment using paired samples T-test

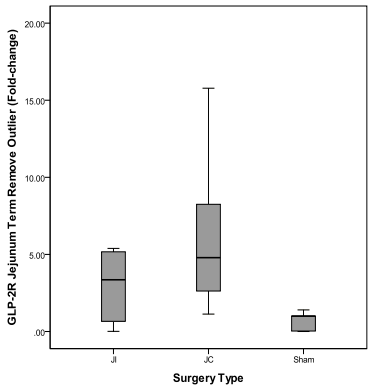

Figure 4.6-1a GLP-2R expression in the jejunum at day 14

Jejunum GLP-2R expression was higher in the sham piglets than in the JI and JC piglets, while no difference were found between JI and JC piglets, *p*=0.110.

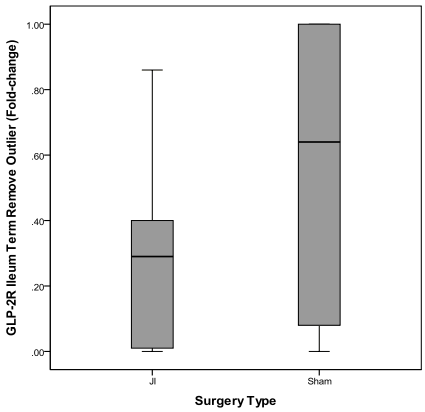

Figure 4.6-1b GLP-2R expression in the ileum at day 14

GLP-2R expressions in the ileum were higher in the sham piglets than in the JI piglets, $p<0.001$.

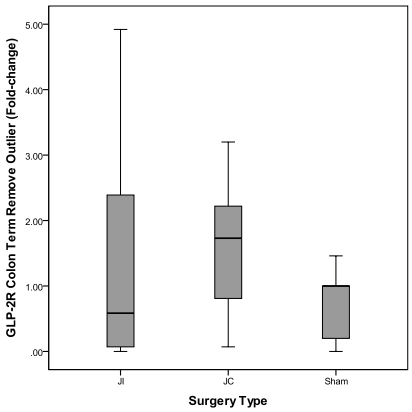

Figure 4.6-1c GLP-2R expression in the jejunum at day 14

GLP-2R expressions in the colon were the higher in the sham piglets than in the JI and JC piglets, $p<0.001$.

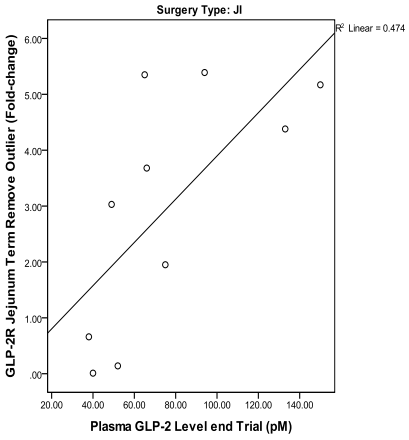

Figure 4.6-2a GLP-2R expression in the jejunum vs. plasma GLP-2 levels at day 14 in the JI piglets, linear regression analysis, $p=0.028$.

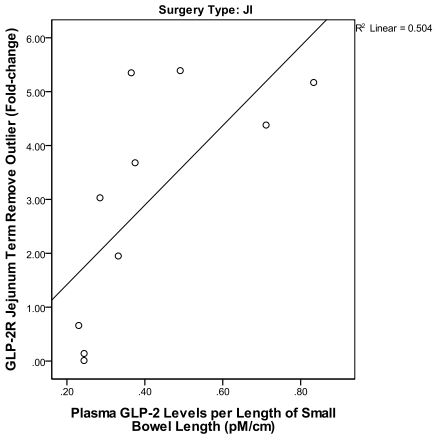

Figure 4.6-2b GLP-2R expression in the jejunum vs. plasma GLP-2 levels per small bowel length at day 14 in the JI piglets, linear regression, $p=0.021$.

4.7 GLP-1 Expression and L Cell Staining

The expression of GLP-1 at all anatomical sites was not different among the three surgical groups (jejunum, p=0.819; ileum, p=0.861; colon, p=0.481). However, there were within group differences. In the JI piglets GLP-1 expression in all three segments was the same (p=0.405). However, in the JC piglets the colon showed greater GLP-1 expression compared to the jejunum (paired-samples t test, p=0.002). In the sham piglets the GLP-1 expression was also greatest in the colon compared to both the jejunum and ileum (paired-samples t test, p=0.045, p=0.046; respectively). Given this trend the data was pooled and the GLP-1 expression in the colon of all three groups was compared to small bowel GLP-1 expression. Increased expression of GLP-1 in the colon relative to jejunum and ileum was demonstrated ($p<0.001$, $p=0.017$; respectively). In contrast, there were no differences among or within groups for L cells immunohistochemical staining at all anatomical sites for all groups (jejunum, p=0.314; ileum, p=0.278; colon, p=0.932). All these results are summarized in **Table 4.7-1**.

Correlations between GLP-1 and L cells were significant in all the three anatomical segments (**Table 4.7-2**). Within each surgical group, the correlations were also significant (see **Figure 4.7-1, 2, 3**). Since the result of L cells and GLP-1 measurement was expressed as the number per villus/crypt axis for the jejunum and ileum or per crypt for the colon, it is more reasonable to assess the correlation between L cells or GLP-1 with GLP-2 levels per unit length of small bowel (instead of total levels). The correlation is shown in **Table 4.7-3**, significant correlation was found between GLP-2 and L cells in the colon only for the JC piglets.

Table 4.7-1 GLP-1 and L-cells expression in the jejunum, ileum and colon

			JI (n=16)	JC (n=17)	Sham (n=15)	*p*
GLP-1	(#cells/villus-crypt axis)	Jejunum	0.2±0.3	0.2±0.4	0.3±0.4	0.819
		Ileum	0.3±0.4	-	0.3±0.4	0.861
	(#cells/crypt)	Colon	0.4±0.3	0.7±0.9	0.6±0.9	0.481
p			0.405	0.032	0.191	
L cells	(#cells/villus-crypt axis)	Jejunum	0.1±0.2	0.2±0.3	0.3±0.6	0.314
		Ileum	0.2±0.3	-	0.4±0.6	0.278
	(#cells/crypt)	Colon	0.4±0.6	0.4±1.1	0.5±0.7	0.932
p			0.213	0.364	0.799	

Values are expressed as mean±SD

p values refer to comparison among the three groups using ANOVA; *p** value refer to comparison within each surgical group using ANOVA

Table 4.7-2 Correlation between L cells and GLP-1

		r	*p*
L cells vs. GLP-1	Jejunum	0.658	<0.001
	Ileum	0.805	<0.001
	Colon	0.447	0.001

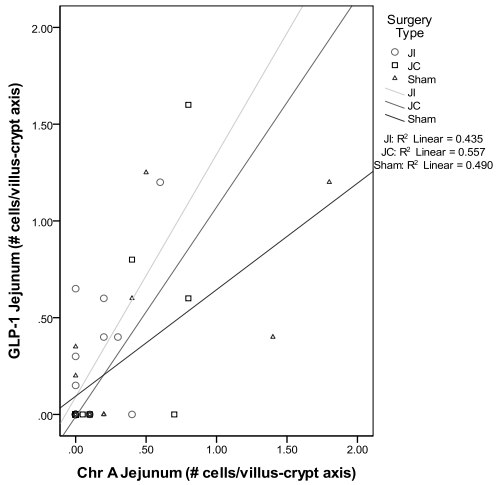

Figure 4.7-1 GLP-1 expression vs. L-cells expression in the jejunum. Linear regression analysis, JI, *p*=0.005; JC, *p*=0.001; sham, *p*=0.004.

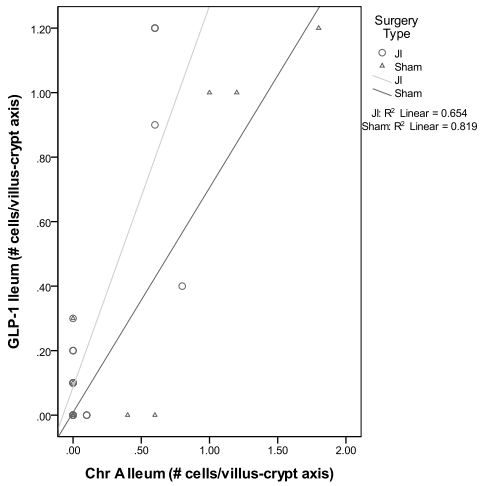

Figure 4.7-2 GLP-1 expression vs. L-cells expression in the ileum. Linear regression analysis, JI, p<0.001; sham, p<0.001.

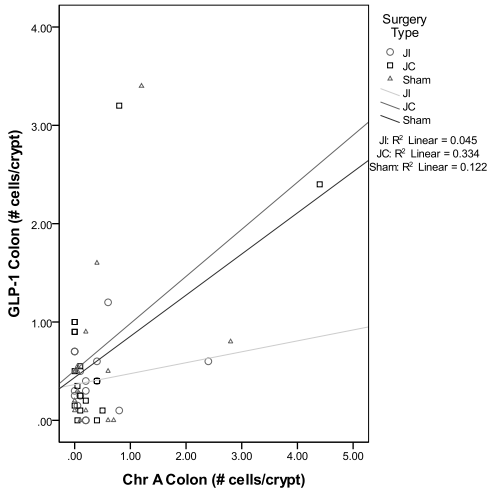

Figure 4.7-3 GLP-1 expression vs. L-cells expression in the colon. Linear regression analysis, JI, p=0.431; JC, p=0.015; sham, p=0.202

Table 4.7- 3 Correlation between L cells, GLP-1 and GLP-2

		r	*p*
JI	L cells jejunum vs. GLP-2	-0.121	0.668
	L cells ileum vs. GLP-2	0.026	0.927
	L cells colon vs. GLP-2	-0.131	0.641
	GLP-1 jejunum vs. GLP-2	-0.084	0.767
	GLP-1 ileum vs. GLP-2	0.264	0.342
	GLP-1 colon vs. GLP-2	0.469	0.078
JC	L cells jejunum vs. GLP-2	0.374	0.153
	L cells colon vs. GLP-2	0.710	0.002
	GLP-1 jejunum vs. GLP-2	0.129	0.634
	GLP-1 colon vs. GLP-2	0.312	0.240
Sham	L cells jejunum vs. GLP-2	0.045	0.875
	L cells ileum vs. GLP-2	0.017	0.954
	L cells colon vs. GLP-2	-0.024	0.934
	GLP-1 jejunum vs. GLP-2	0.243	0.384
	GLP-1 ileum vs. GLP-2	0.212	0.448
	GLP-1 colon vs. GLP-2	-0.022	0.937

GLP-2 here is expressed as pM/cm

4.8 Enteral Nutrition and Intestinal Adaptation

Irrespective of formula type, there was no correlation between small intestinal length and enteral lipid infusion or fat absorption. However, when comparing the amount of enteral lipid infused and fat absorption to the small intestinal weight, correlations were found in JC piglets and for fat absorption in the JI piglets, but not lipid infusion (p=0.277) (**Figures 4.8-1**). These were not observed in the sham piglets (p=0.089, p=0.1612; respectively). Histologically, a higher amount of total lipid infused was related to higher jejunum villus height in the JC piglets (r=0.645, p=0.007; see **Figure 4.8-2a**). On the other hand, the amount of lipid infusion was inversely correlated to ileal crypt depth in the sham piglets (r=-0.655, p=0.011) (see **Figures 4.8-2b**).

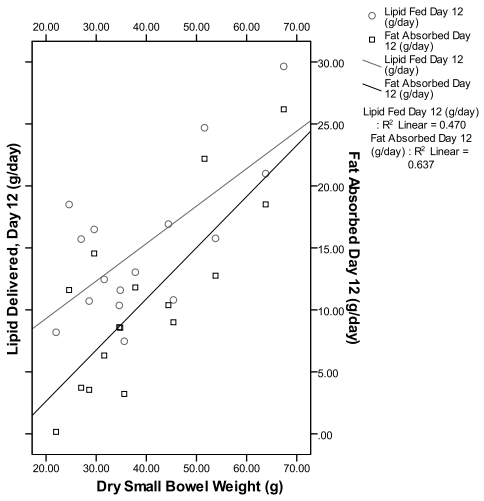

Figure 4.8-1a The amount of enteral lipid delivered and fat absorbed vs. small bowel weight in the JC piglets. Linear regression analysis, the amount of lipid delivered vs. small bowel weight, p=0.003; fat absorbed vs. small bowel weight, p<0.001.

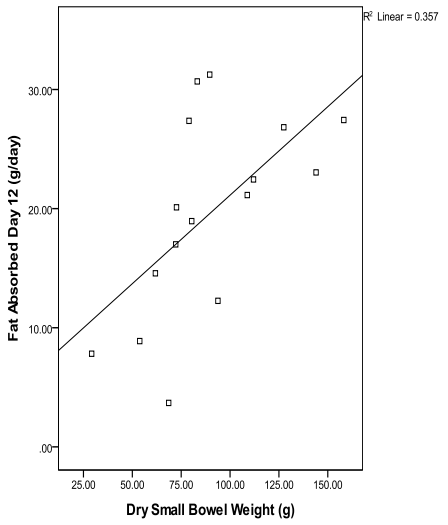

Figure 4.8-1 b The amount of enteral fat absorbed vs. small bowel weight in the JI piglets, linear regression analysis, p=0.014.

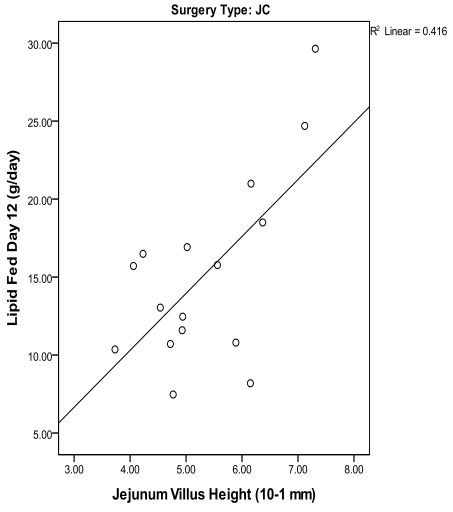

Figure 4.8-2a The amount of enteral lipid delivered vs. jejunum villus height in the JC piglets, linear regression analysis, p=0.007.

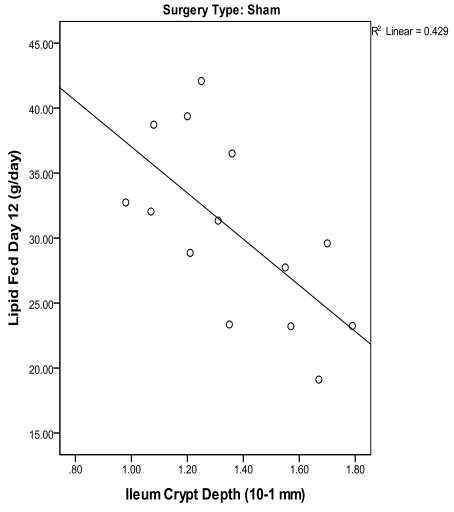

Figure 4.8-2b The amount of enteral lipid delivered and ileal crypt depth in the sham piglets, linear regression analysis, p=0.011.

5 DISCUSSION

5.1 Small Intestinal Adaptation

5.1.1 Gross morphological adaptation

The sham piglets had the longest small intestine length after surgery, as they did not have resection, so not surprisingly they also had the longest small intestine length at the end of the trial. The percent increase of small intestinal length was also the highest in the sham piglets, representing normal small intestinal growth. Although the JI piglets had less small intestine length increase, compared to sham piglets, when it was expressed as a percentage increase over the remnant length at initial surgery, the values were similar. Therefore, it is reasonable to propose that JI piglets have normal small intestinal growth, as do the sham piglets. Conversely, in the JC piglets, small intestine length was not increased at all, regardless of how it was expressed. Obviously, normal small intestinal growth did not occur in the JC piglets, within the time frame of our observation.

Similar to small intestine length, small intestine weight was also highest in the sham piglets and lowest in the JC piglets. Interestingly, when the weight was divided by the final length of small intestine, JI piglets had the highest weight per cm, while JC and sham piglets actually had very similar weights per cm. This finding could be explained by structural small intestinal adaptation with lager caliber or/and thicker small intestine in the JI piglets. This can be confirmed by our observations at the terminal surgery that the small intestine in the JI piglet looked wider and the intestinal wall felt thicker (see **Appendix 3**).

5.1. 2 Histological adaptation

Furthermore, histologically, as expected, JI piglets had higher villi and deeper crypts than the JC and sham piglets. This further supports that structural intestinal adaptation occurs in the JI piglets, explaining their thicker small intestinal wall. Comparison of jejunal or ileal villi height among the surgical groups showed the JI piglets had similar

jejunal villi height as sham piglets but higher ileal villi. This difference could be considered as a greater adaptation in the ileum than in the jejunum. This point is supported by a 14 weeks study in juvenile piglets that underwent 75% proximal small intestinal resection or transection (136). In this study increased villi height and surface area were found the most prominent in the middle and distal ileum and the crypt depth increased significantly in the mid-ileum. Therefore the authors concluded that mid-ileum had the greatest adaptation among the different small intestinal segments. Actually, as pointed out in Chapter 1, after jejunal resection the ileum has greater adaptation than the jejunum would, after ileal resection.

5.1.3 Crypt cell proliferation and apoptosis

No difference of jejunum crypt cell proliferation and apoptosis, as well as villous cell apoptosis, were found among the three surgical groups, despite the greatest intestinal adaptation in the JI piglets (crypt cell proliferation, p=0.639; crypt cell apoptosis; villous cell apoptosis). These findings are consistent with another study, in which young pigs were used (136). After 75% proximal small intestinal resection, piglets had increased villi and crypts of the remaining intestinal with only modest increased mid-ileal crypt cell proliferation index (number of proliferative crypt cell per total number of crypt cells) but without alteration in the jejunum. One reason for this could be the greater adaptive ability of the remaining ileum than remnant jejunum, as we found with histological adaptation. Another possibility could be the proportional increase of the proliferative cells and total crypt size, which means the percentage of crypt proliferative cells out of the total crypt cells remain unchanged, although the absolute number of proliferative cell increased. This is supported by the aforementioned Lauronen's study in which the number of crypt cells per unit crypt length was increased in the proximal jejunum and mid-ileum.

5. 2 Plasma GLP-2 Levels

In the JI piglets, a progressive increase in plasma GLP-2 level was found, both from baseline to the time point when piglets were receiving 50% enteral nutrition and to the end of the trial. On the other hand, JC piglets only had an increase in GLP-2 from the baseline to the time point at which they were receiving 50% EN. This increase was still less when compared with the level in JI piglets at the same time point. In fact, when we compare among surgical groups, both at the time when piglets were fed with 50% EN and at the end of the trial, plasma GLP-2 levels in JI piglets was the highest amongst the three groups. When the plasma GLP-2 at the end of the study was divided by the small bowel length (at termination), JI piglets still had the highest GLP-2 levels. In contrast the sham had the lowest GLP-2 levels; while the JC had intermediate levels. This difference may be consistent with an adaptive response post intestinal resection, with respect to production of GLP-2. However, such a conclusion should be balanced against recognition of the failure to have normal small intestinal growth in the JC piglets, leading to an artificial elevation in GLP-2 value when it is expressed per cm of final small intestinal length.

We know that the main sites for GLP-2 release, in both the pig and human, are the distal ileum and proximal colon (217, 323). This is one likely reason that JI piglets, with distal ileum remaining in remnant bowel, can have increased plasma GLP-2 post-resection (219). By contrast, JC piglets with the entire ileum and the proximal colon resected, had only part of the remaining colon as a potential site for release of GLP-2. Therefore, JC piglets could only have a partial increase in endogenous GLP-2 production post resection. The plasma GLP-2 level in the JC piglets was not high enough, compared to the level observed in the JI, to promote adaptation (or growth) of the intestine. A similar finding from Sigalet et al, in human babies, suggested the colon did not play a role in raising plasma GLP-2 levels post intestinal resection (231). In the present study, it appears GLP-2 level increased transiently and then decreased to the control level (sham)

at the end of trial (day 14). Therefore, the plasma GLP-2 level in the pediatric patients in the aforementioned study may also have had a transient increase, before the sample was taken post-resection. Another way to view these findings is to consider that the developing colon is unable to sustain a progressive increase in plasma GLP-2 release post resection, as can be seen to occur from the remnant ileum.

When compared with the short bowel piglets, the sham piglets with intact ileum and complete (100%) EN tolerance (by day 7 on average), did not have a significant plasma GLP-2 increase. This could be explained by the intestinal resection itself, being an important factor in regulating GLP-2 release, of course absent in the sham group. In our study we did not elect to do a small bowel transection as we had observed in pilot work a significant increased morbidity in sham animals, from adhesive small bowel obstructions, and we wanted to avoid increasing abdominal adhesions. However, in a previous study, comparing animals with intestinal resection to those that had only intestinal transaction, the latter was not associated with significantly increased plasma GLP-2 (147). Considering the early post resection increase in plasma GLP-2 level, at the time point when JC piglets received 50% EN, it may be simply the result of the intestinal resection.

Regardless, the effect of resection appears to be transient, and persistent endogenous GLP-2 release is affected by many other factors, in particular the presence and amount of luminal nutrition (220, 268). This may be a very important reason for the differences in the temporal increase in GLP-2 release between the JI and JC piglets in our study. In the JI piglets, the highest EN rate tolerated (expressed as a percentage of total calories) was 88.1%, while in JC piglets it was only 60.0%. This means from the time point when piglets were all fed with 50% EN, until the end of the trial, JI piglets increased the amount of luminal nutrition to an average of almost 90%, while by contrast, JC piglets only increased, on average, a further 10 percent. They were maintained on this amount until the end of the trial, or it may even have been decreased due to poor weight gain or

diarrhea. This is similar to previous study by Burrin et al, showing that 60% EN, as a percentage of total energy, is the minimal amount required to have an increase in plasma GLP-2 in neonatal piglets fed with differing proportions of EN and PN (320). In short bowel piglets, both the highest EN rate achieved and the duration of PN support required can be related to the circulating GLP-2 level. This is consistent with previous studies in piglets and humans that demonstrate the importance of the absolute amount of luminal nutrition (261, 279). In a neonatal piglets study by Petersen et al, newborn piglets fed with PN had plasma GLP-2 levels around 11 pM, but in the present study, plasma GLP-2 level at baseline was around 20 pM (266). One reason could be the different time we collect blood sample, in the study by Petersen the GLP-2 level was measure in newborn piglets, while in our study, we collect the sample when piglets are about 4 days old, and probably four days makes a difference. An alternate reason could be the presence of EN, since in Petersen's study piglets were fed parenterally only, but in our study these piglets were allowed to sow feed after birth, before they entered to the study, and again EN (and particularly colostrum) is a major factor in inducing GLP-2 release. Similarly, in Petersen's study, 23 pM plasma GLP-2 level was found in neonatal piglets fed with PN, while in our sham piglets the plasma GLP-2 level was about 31 pM at the end of the trial (day 14), the presence of luminal nutrients being the likely explanation.

We know that fasting and re-feeding could significantly influence the plasma GLP-2 level, by a decrease and increase in GLP-2 release respectively (324). Although we attempted to ensure the EN delivered prior to the termination was consistent for all piglet groups, the variation in amount of nutrition being delivered remains a confounding variable for our study results. Notably a correlation between the EN rate prior to termination and the plasma GLP-2 level was significant (p=0.001).

In addition, coincidental with the different total amount of EN delivered was the different amount of lipid infused. During both the first (day 5) and the second (day 12)

fecal fat collections, JI piglets received more lipid than the JC piglets. Enteral lipid is a very important nutritional stimulus of GLP-2 release (282). In the present study, correlations between plasma GLP-2 levels and either the total amount of lipid infused or the amount absorbed were identified. This observation is also similar to the previously mentioned study in human infants, which noted a positive correlation between GLP-2 levels and nutrient absorptive capacity (231). Conversely, a study in rodents determined an inverse correlation between GLP-2 level and fat absorption (43). One reason for the varying results in these studies could be the age of animals studied. Adult rats, in the latter study, have already finished intestinal development and would of course be different from the neonatal human or piglet. That study had concluded that malabsorption increased the presence of undigested nutrients in the distal intestine and this was a stimulus for GLP-2 release and adaptation. However, in our study and the study in human neonates, both with a developing colon, this did not seem to play a role in producing GLP-2. Therefore, it is reasonable to say the presence of luminal nutrients in the distal intestine or colon, as a stimulus to GLP-2 release, may only apply to mature animals or certain species, such as rodents. The species differences are very relevant as rodents have significant difference in gastrointestinal physiology and nutrient usage, which limit translational findings to human neonates, as compared to the piglet (301, 317). Unlike humans and pigs, the production of butyric acid on high fiber diets is more significant in rodents (325). On the other hand, pigs can produce similar SCFAs on high fiber diets as can humans (325). Furthermore, the degree of resection could itself influence the correlation between nutrient absorption and GLP-2. In the study of Martin et al, rats underwent three different levels of intestinal resection (43). That study concluded that the resulting malabsorption was the stimulus for GLP-2 release and adaptation, but could not exclude that the different amount of intestinal resection itself was the factor. By contrast,

in the present study, the magnitude of intestinal resection was controlled (75%) and the only difference was the site of resection.

5. 3 Plasma GLP-2 and Intestinal Adaptation

In this study sham piglets without intestinal resection are considered as a normal control, and the plasma GLP-2 level in these piglets could be considered to be at a normal physiological level. Therefore, levels higher could be considered adaptive levels, as was found in the JI piglets. A positive correlation between the plasma GLP-2 level and structural small intestinal adaptation was identified in this present study. In the short bowel piglets, higher GLP-2 level was related to longer small intestinal length, heavier weight and to more noticeable histological adaptation. Besides, functional small intestinal adaptation was also present in the short bowel piglets. Significant positive correlation between plasma GLP-2 and fat absorption or enteral nutrition tolerance, as well as a negative correlation between plasma GLP-2 and duration of PN support was found.

5. 4 Tissue GLP-2 Receptor Expression

Comparison of tissue GLP-2R expression between each two intestinal segments, in all three surgical groups, showed that the jejunum had the highest GLP-2R expression. This result is consistent with studies in multiple species showing the highest GLP-2R expression is in the upper small intestine (265). As we expected, compared with short bowel piglets (JI and JC), sham piglets had the lowest level of tissue GLP-2R expressions in the jejunum and colon. This difference, for the SBS piglets, could be considered as an adaptive process after intestinal resection, absent in the sham piglets.

However, the GLP-2R expression in the jejunum and colon was lower in the JI piglets than in the JC piglets, despite higher circulating GLP-2 level in the JI piglets. It is possible that the presence of higher circulating GLP-2 level is associated with a suppression of GLP-2R expression. Interestingly this has been shown to occur during piglet development (260). It was described that after birth the GLP-2R mRNA was high,

when circulating GLP-2 level was relatively low, in newborn piglets. Subsequently small intestinal GLP-2R mRNA level decreased, while the plasma GLP-2 concentration increased. Furthermore, this situation is known to occur with other growth factors, like EGF and its receptor (326). However, against this hypothesis, a positive correlation between plasma GLP-2 and tissue GLP-2R expression was found in the jejunum of JI piglets. Therefore, it seems the ileum release of GLP-2 is related to altered tissue expression of GLP-2R in the jejunum. This could be a direct relationship, but may also require a second messenger other than GLP-2, but likely also from the remnant ileum, given the JC piglets without ileum did have the same relationship.

Although the GLP-2R expression in the jejunum and colon of the JC piglets were higher than in the JI piglets, the plasma GLP-2 levels were very low and so the increased GLP-2R expression has little useful contribution to intestinal adaptation in the JC piglets. The possibility that other signals, similarly absent because of loss of ileum, contribute to this outcome needs future exploration.

5. 5 L cells and GLP-1

It must also be understood that circulating and tissue concentrations of GLP-2 are not the same and have to be considered independently. In order to better understand local tissue expression of GLP-2 we can assess L cell number and tissue GLP-1 expression by immunohistochemistry stains. Both GLP-1 and GLP-2 are encoded by the proglucagon gene and are co-released from the intestinal L cells in a 1:1 ratio (327).

In our study the L cell stain result showed no difference among the three groups studied. This is similar to a study by Aiken et al, which showed no L cell change after jejunal-ileal transposition in the rat (270). Similarly, in another study rats underwent proximal small intestinal resection, distal small intestinal resection or ileal transposition, and did not show an increase in the L cell population (227). At first glance this may seem

counter-intuitive, as we know L cells are the site for GLP-2 release and the amount of GLP-2 was different among the three groups. We therefore conclude that the function of the L cells must be different among the groups, becoming more sensitive to the signaling that produces GLP-2 release, in order to bring about the measured increase in circulating GLP-2 observed in the JI piglets. It is reasonable to hypothesize that the function of the L cell was increased in the JI piglets. This hypothesis can be supported by a study of 80% small bowel resected rodents, measuring the amount of ileal proglucagon mRNA and L cell number, that found the proglucagon mRNA was increased, without a concomitant increase in L cells per given field or villus unit (328).

Similarly, in our study, tissue GLP-1, as a marker for tissue GLP-2 expression, was not different among the groups. Since we did not measure tissue GLP-1 level at the beginning of the trial, we could not assess the change in tissue GLP-2 expression during the trial, following intestinal resection. We compared the tissue GLP-2, by proxy staining for GLP-1, among the surgical groups only at the end of the trial and found no difference. However, we know GLP-2 release varies from different sites of the intestine, so accordingly, it is reasonable to hypothesize that tissue GLP-2 should also be different. This may in fact be an explanation for the large variations in the data seen in our results. Another reason may be that GLP-2 release into the circulation varies according to factors that stimulate release, but the total tissue level remains relatively constant. As we do not know exactly how GLP-2 functions to cause crypt proliferation, distal to its site of release in the L cell, this implies that circulating GLP-2 acts via a secondary messenger, perhaps not isolated in the tissue, but in the circulation (such as IGF-1 or IGF-II for example). In order to understand this better, it may be more important to measure the mRNA expression of tissue proglucagon gene, which is posttranslationally spliced into GLP-1 and GLP-2, both in the tissue and as released into the circulation. So for example, in the rodent study described by Dahly et al, post resection, there was a significant correlation

between bioactive GLP-2 and proglucagon mRNA levels in the ileum (147). In addition studying further the relationship between GLP-2 and the IGF-GH axis is necessary.

The only significant correlation between GLP-2 and L cells was the circulating level expressed per unit length of small bowel and the L cells number identified in the colon of the JC piglets. Since we know colon is the only presumed site for GLP-2 release in the JC piglets, it is reasonable to say that this data supports that for JC piglets, colonic L cells are responsible for the GLP-2 production.

5. 6 Nutritional Outcomes

In our study, no matter by which route they received nutrition (enteral or parenteral or combined), all piglets were fed the same total energy; as the PN component was adjusted upward if weight gain was inadequate, as with malabsorption. Therefore, as expected according to the experimental design, the overall weight gain in the three groups by the end of the trial, compared by ANOVA, was the same. However, short bowel piglets (JI and JC) had on average less EN infused and of course a longer duration of PN support, and longer duration of diarrhea, consistent with greater malabsorption. They were dependent on PN to achieve normal growth, and hence by definition they developed intestinal failure. This condition was by far the most serious in the JC piglets. This would be the reason why statistically the JC piglets gained significantly less weight when compared directly to the weight gain of the sham piglets. SBS is defined by inadequate nutrient absorption, due to malabsorption, the result of inadequate bowel length and or function. Such malabsorption of nutrients accounts in part for the longer duration of diarrhea observed in the short bowel piglets. Absorption was directly measured as fat absorption in this study and is a major factor that can explain the weight gain difference; as absorption of dietary fat is the major source of energy for growth in mammalian species. During both the first and the second fecal collection, JC piglets received fewer calories from enteral lipid than JI and sham piglets, which had similar amounts. Normally,

fat absorption (expressed as percentage of total lipid) in early weaning pigs (21 - 26 days old) is 92 to 100% (329). In this study, only sham piglets reach this level, while JI piglets absorbed less fat than the sham piglets and the JC piglets less again. Dietary fat can only be absorbed in the small intestine and is dependent on bile salt reuptake, from the ileum, its hepatic re-circulation and excretion. Therefore, the decrease of essential absorption surface area in SBS piglets will impair fat absorption, but this situation is worse in JC piglets with ileum resection.

In a previous study, fat malabsorption was only observed after 90% small intestinal resection in the rat, but in our study, in neonatal piglets, 75% small intestinal resection caused fat malabsorption (43). Again species difference is important here, compared with humans it is known that the rat has much higher bile fluid production, an important factor in regulating dietary lipids absorption (330). Similarly, it has been suggested that the rat is more efficient in diverting absorbed lipid into lymphatics (331). However, the difference also relates to maturity of the gut, with newborn piglets being even more immature from the gut development point of view than term babies, more akin to preterm babies (301, 305). Preterm human infants can excrete 20-30% of dietary fat because of gut immaturity, reduced bile salt uptake and circulation and decreased pancreatic lipase excretion, as well as dysmotility and a short transit time (197). How much more relevant such factors would be to the immature neonatal piglet with gut resection, especially of the ileum. Finally the amount of lipid that is infused is also a factor that may cause the difference, because fat excretion in SBS is dependent on intake (180). In the study of Martin et al, with 90% intestinal resection in rodents, the amount of lipid that the animals ingested was not known, so that study is not comparable to ours for many reasons.

Although structural small intestinal adaptation occurred in the JI piglets, the amount of fat absorbed was still less when compared to the sham piglet. However, when the amount of fat absorbed was divided by the length of the remnant small intestine,

measured at termination of the study, the absorption expressed per unit length, this was in fact highest in the JI piglets and lowest in the sham piglets. This difference was similar to GLP-2 release, when expressed as pM per unit length of small intestine; both could be considered as functional small intestinal adaptation in the short bowel piglets. It reflects both structural and true functional absorption, represented as dietary fat absorption. However, since the small intestine length in the JI piglets was only about a quarter of the sham piglets, even less for JC piglets, this compensation could not completely enable short bowel piglets to have the same amount of fat absorption as sham piglets. Therefore, adaptation in the short bowel piglets was inadequate to completely restore normal function, with regards to fat absorption.

By comparison, the amount of fat absorbed (expressed as gram per day) from the first to the second collection periods was only increased significantly in the JI piglets. Fat absorption, expressed as the percentage of total lipid infused, was not increased in either short bowel piglet groups. In regards to the JC piglets, this reflects lack of functional adaptation; however the finding is contradictory for the JI group. Perhaps achieving the peak small intestinal adaptation in the short bowel piglets is very slow post surgical resection, so no difference was seen during this study period. An alternate explanation is the increased amount of total enteral nutrition infused at the second time point may have exceeded the total capacity of the already completely adapted JI small intestine to absorb more fat, increasing the amount of fat excreted, while the same amount was absorbed.

5. 7 Enteral Formula Types with Growth and Adaptation

In this study two kinds of enteral formula were used within each surgical group, in order to determine if protein or amino acid would promote greater adaptation and GLP-2 release. Regardless of formula type, within each surgical group, intestinal lengthening, histological adaptation and fat absorption were all the same. However, the duration of PN support was longer in the JC piglets fed with polymeric formula. This reflected greater

diarrhea in this group. As fat absorption was actually determined to be the same, then the diarrhea must reflect either protein or carbohydrate malabsorption. In the polymeric formula, the predominant carbohydrate source is lactose and the nitrogen source is intact protein. In the elemental formula the carbohydrate source was glucose polymers and the nitrogen source was amino acids. One possibility therefore could be the presence of the lactose in the polymeric formula, compared with elemental formula, which only contains glucose polymers, caused greater diarrhea. In neonatal piglets, replacing colostrum with infant formula leads to reduced lactose digestive capacity, because of insufficient lactase activity (332). On the other hand, glucose polymers in premature infants are well tolerated as glycosidase enzymes for glucose polymers are active in small premature infants (333). Furthermore, the undigested lactose in the colon could be fermented to SCFAs, which related to NEC (189). Analysis of stool patterns of infants diagnosed with NEC showed a significantly increased number of stools (334). Therefore, it could also be possible that the increased diarrhea in polymeric fed JC piglets was a result of the development of inflammation and symptoms, similar to NEC. Diarrhea with blood in the stools is the classic symptom of NEC (335). In the present study, six short bowel piglets fed polymeric formula were found to have blood and pneumatosis, histologically with eosinophilia prominent in the submucosa, while only one short bowel piglets fed elemental formula had bloody stool (data not show). Another alternative possibility could be the osmotic fluid load caused by the presence of lactose, causing osmotic diarrhea. The osmotic activity per unit weight of glucose polymers is lower than lactose or monosaccharides (336). However, the osmotic load of amino acids is greater than protein, so on balance the difference between the formulae is not clear. Certainly, in certain commercial infant formulas, lactose is often partially replaced by glucose polymers precisely in order to decrease the osmolarity of the formula (337).

The role of the intact protein causing the increased diarrhea also has to be considered. A relationship between dietary intact protein and longer duration of diarrhea has been reported in rats after 80% small intestinal resection during a four weeks study (172). In a infant colitis study, all patients developed bloody diarrhea shortly after the introduction of intact protein, which may related to an allergic reaction (338). Allergy to protein diets may be common in infants with SBS, and possible reason could be the "leaky gut" in these infants increasing sensitization to milk or soy protein (339). In infant studies antibodies to milk protein have been found in 75% of health children under age one (340, 341). In children, the common gastrointestinal symptom of hypersensitivity to cow's milk includes bloody diarrhea, vomiting, failure to thrive, or abdominal colic (340). Therefore, it is reasonable to hypothesis the longer during of diarrhea in this study could be the result of either the source of carbohydrate or the intact protein in polymeric formula. A better study design would have been to ensure that a difference in only one macronutrient was present at a time.

As according to our protocol weaning from PN was dependent on both weight gain and duration of diarrhea, so it is not surprising that duration of PN support was longer in the JC piglets fed polymeric formula. Duration of PN support is a critical factor to consider in the clinical management of infants with SBS, as long term PN support is usually associated with liver disease, the leading cause of death in neonatal SBS (62). Therefore, considering the elemental formula was associated with shorter duration of PN support, regardless of no noticeable advantage for adaptation, it is reasonable to say elemental formula is a better choice for neonatal SBS. Elemental diet of amino acids, instead of intact protein, reduce the need for complex digestion, as well as avoiding potential protein sensitivity or allergy and thus have been recommended clinically (16, 82, 174). Considering all the components of enteral nutrients together, elemental formulas

containing simple amino acids and approximately 40% to 50% lactose and 50% to 60% glucose polymers appears to be superior for preterm infants (333).

The plasma GLP-2 levels were statistically the same among all three surgical groups given the polymeric formula. On the other hand, given elemental formula JI piglets had the highest GLP-2 level among the three groups. This contrasts with the result seen for the combined piglet groupings (elemental plus polymeric) where the JI had the highest plasma GLP-2 level at both time points measured after baseline. It is noticeable that the variations in plasma GLP-2 levels in these three groups are high, so one possible reason is that a type II error occurred, because of the small sample size. As a result, a post-hoc power analysis was done and it showed the minimal sample size to get 70% power was 13. However, in this study, considering the formula types in each surgical group, the sample size was eight or nine, which means the probability to have a type II error is higher than 30%.

The comparison of GLP-2 level between the two formula types, within each surgical group, found the JC piglets given polymeric formula had a higher level of GLP-2. This is plausible as literature exists to show that polymeric formula, specifically protein may be superior in promoting adaptation and in inducing GLP-2 release (216). Similarly, studies using juvenile pigs show a superior effect of polymeric formula in promoting structural and functional intestinal adaptation, as well as inferior effect of elemental formula on colonic cell number and proligeration, post resection (174, 342). However, if intact dietary protein does not cause increased release of GLP-2, expected to be the same for all groups, what could be the reason? One possible explanation could be that plasma GLP-2 is protected from degradation by the presence of intact dietary proteins, because they occupy active sites of the pancreatic proteases (177). However, again this should apply to both JI and JC, and we did not find better adaptation; in fact JC piglets on the polymeric formula had more diarrhea. That may be the key, as the differences may depend on the

extent to which malabsorbed nutrients can stimulate colonic GLP-2 production. The higher plasma GLP-2 level in the polymeric fed JC piglets may be the result of more malabsorbed nutrients present in the colon, rather than the formula type itself. As discussed, since fat absorption was the same between the two diets in the JC piglets, the increased formula content that stimulates GLP-2 release in the colon of polymeric fed JC piglets could be carbohydrate or protein. Many studies support that unabsorbed carbohydrate in the colon can be fermented by bacteria and convert to SCFAs (179, 180). SCFA, especially butyrate, is known to induce GLP-2 release from L cells in the colon (288). As the JI piglets had similar duration of diarrhea between the two formula types; therefore they may have had better absorption of the two sources of carbohydrate in the different formula and less SCFAs production, as a stimulus for GLP-2 release in the colon. Increased presence of unabsorbed lactose in the colon, the site of GLP-2 production in the absence of ileum, may have induced the GLP-2 release in the JC piglets.

Piglet performance, growth and intestinal adaptation were all compared within each surgical group, according to the formula fed. Between the two formulae, within each surgical group, weight gain was the same, intestinal length was the same, histological changes representing adaptation were the same, as was fat absorption. However, the power analysis of comparison of weight gain between the two formulas in the sham group showed the power was only 54%, which means the possibility to make a type II error is 46%. Therefore, it is possible that polymeric formula did induce a higher weight gain than the elemental formula. Similarly, power analysis of comparisons of small bowel lengthening and small bowel lengthening, expressed as the percentage of length post surgery, in the JC piglets showed the power were 47% and 46%. The possibility to make a type II error was 53% and 54% respectively. Therefore, it is also possible that polymeric could induce a greater small intestinal lengthening, than the elemental formula

in JC piglets. Interestingly, this is relevant considering the higher level of plasma GLP-2 in the polymeric formula fed JC piglets, it is possible that polymeric formula in JC piglets could induce increased plasma GLP-2 and better small intestinal adaptation; we did not study enough piglets to know.

5. 8 Hematology and Biochemistry of Piglets

Both at the baseline and the end of the trial, hemoglobin levels in the JI and JC piglets were lower than the reference values, while sham piglets had a normal value at the end of the trial. Compared with sham piglets, short bowel piglets in this study had less EN through the trial, even though the weight gain was the same. This could be the reason why the hemoglobin levels in the short bowel piglets did not reach normal values at the end of the trial. Although there was no difference of hemoglobin level at the end of the trial, between the two diets in the JC piglets, increased levels were found in the piglets fed polymeric formula throughout the trial. This could relate to the different iron contents of the formulae, the iron was about 4.392mg/L more in the polymeric formula compared with the elemental formula. Intramuscular injection of iron as a treatment for increased respiration rate, pale skin color, fatigue and weakness, is another possible factor. Two piglets fed elemental formula received iron treatment, while three polymeric formula fed piglets received iron injection; although it seems unlikely this made a difference. The platelets level was increased throughput the trial in the sham piglets, but is within the normal reference range.

In all the three surgical groups, the albumin levels were increased significantly to normal values at the end of the trial, while lower than normal at the beginning of the trial. This is likely to reflect improved protein energy nutrition over time post delivery and is consistent with what might be expected in piglets normally.

At baseline total bilirubin levels were normal in the three surgical groups, but the level exceeded normal in the JC piglets at the end of the trial. JC piglets had longer duration of PN support, than piglets in the other two groups, and longer PN duration is related to the development of cholestasis (343). Decreased ALT levels were found in the JI and JC piglet throughout the trial, however a normal range is not available for comparison. One possible reason for decreased aminotransferase is deficiency of zinc (344). This could be a problem for SBS piglets, and zinc levels were not measured. This may also relate to ALP levels, which were decreased from baseline to the end of the trial, although levels at the end were actually higher than the reference range in all groups. The higher levels can also reflect rapid bone growth, again expected in piglets. GGT levels were increased and exceeded the normal range in all three groups at the end of the trial, but especially in the JC piglets. Higher levels of ALP and GGT together may also indicate a possible presence of cholestatic liver disease (345). Again this condition would be expected to be most severe in the JC piglets, with the longest duration of PN support.

6 CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Summary & Implications of Findings

In this present study, JI piglets with remaining ileum had increased plasma GLP-2, associated with small intestinal adaptation, while JC piglets without ileum did not. In addition, JI piglets with higher plasma GLP-2 levels had normal small intestinal growth and noticeable small intestinal adaptation. By contrast, JC piglets, with no increase in plasma GLP-2 level, did not have normal small intestinal growth. This contrasts with the sham piglets that had normal small intestinal growth. Finally, in the JC piglets, classical intestinal failure developed, with prolonged duration of diarrhea and PN support, imitating the condition observed in human neonates with SBS.

The present study shows that ileum plays an important role in the intestinal adaptation and that the presence of ileum was associated with noticeable increased plasma GLP-2 levels. On the other hand, after resection, the absence of ileum was only related to a temporary GLP-2 increase. Plasma GLP-2 level, as expected, was associated with small intestinal adaptation, both structurally and functionally. Elemental formula, compared with polymeric formula, was associated with less diarrhea and shorter duration of PN support. Contrary to what was hypothesized, polymeric formula did not lead to better intestinal adaptation.

6.2 Limitations

It is noteworthy that plasma GLP-2 level did increase from baseline to the time point when the JC piglets receive 50% nutrition enterally. However, a limitation of this study is that we do not know what was occurring in terms of small intestinal histological adaptation at this time. Perhaps if we had collected tissue at this time point, when there was an increase in GLP-2 level, we would have observed adaptation. However, even if there is such early adaptation, it is either very limited or subsequently reversed, as no significant adaptation was observed in the JC piglets at the end of the trial. What is not

known is whether if we could have increased enteral nutrition, at the expense of high output diarrhea, would such potential early adaptation have been preserved. Understanding this further has clinical relevance for how we manage pediatric SBS.

It was very noticeable that the variation of plasma GLP-2 was large in this study. One reason could be the time of sample collection, since it is known that postprandial GLP-2 increases happen as early as 15 minutes after meal ingestion (43). Therefore, in addition to the different amounts of nutrition being delivered to every piglet as discussed, additional factors to consider for the observed variability include normal variation in motility between piglets and inconsistencies in nutrient delivery, related to pump failures. Therefore, a consistent sampling time, at the same enteral delivery rate, after a minimum of one full one hour of EN delivery, would be recommended for future research.

Another potential sampling problem present in this study is the tissue collection for histological analysis. For jejunum, we collected the tissue 10 cm after the ligament of Treitz, but we know the small intestinal growth was different among the groups, so the final small intestine length and site of tissue sampling was different for all groups. Specifically, for example, the segment 10 cm distal to the ligament of Treitz in the JI piglets cannot represent the same segment in the JC piglets, who did not have a similar lengthening of jejunum. Meanwhile, this problem is also applied to sham and JI piglets with the ileum samples collected 10 cm proximal to the ICV. Moreover, it is known that the degree of adaptation is different along the intestine, relative to the site of resection and anastomosis. The maximal adaptation, based on prior research, is at the anastomosis site and tapers distally (114). Therefore, collecting samples according to the percentage of the total length of the intestine or related specifically to the resection margins may be a better choice for future studies.

While this study intended to determine if intact protein, as compared to amino acids, altered adaptation and release of GLP-2, the formula actually varied from nitrogen delivery alone in a number of important ways. Firstly, when we look at the lipid sources in the elemental formula, long chain polyunsaturated fatty acid is predominant. Long chain fatty acids are thought to be a potent lipid stimulus for intestinal adaptation. However, to be fair this difference may not have had a significant impact, as there was no difference in adaptation. Perhaps more importantly, different types of carbohydrate were present in the two formulas, which clearly had a potential effect on diarrhea and plasma GLP-2 level in JC piglets. A better study design would have been to ensure that a difference in only one macronutrient was present at a time: such as by using PN solution mixture with amino acids or with isonitrogenous protein powder (rather than comparing to an infant formula).

6.3 Future Directions

In this study increased endogenous GLP-2 was accompanied by small intestinal adaptation after intestinal resection. Neonatal piglets without ileum appear to have a GLP-2 deficiency state, associated with lack of adaptation and normal growth of the intestine. As was discussed, the early rise in GLP-2 post resection in both JI and JC piglets may have reflected intestinal resection. Perhaps a certain exposure over time to elevated GLP-2 is required to see more marked and prolonged intestinal growth or adaptation, as was seen in the JI piglets. If so JC piglets, deficient in adequate GLP-2 exposure over time, may benefit from its replacement. This will be a very important focus for future research endeavours.

In a study of human neonates with SBS, GLP-2 level was correlated with tolerance of enteral nutrition and nutrient absorption, and this was suggested as a predictor of ability to wean from TPN (231). However, as in this present study, correlation does not prove causation. A trial of exogenous GLP-2 is warranted in short bowel piglets without

ileum. Similarly, to elucidate mechanisms further, delivering exogenous GLP-2 in the presence of a GLP-2 blocker, could be used to determine if the adaptation observed in JI piglets is entirely dependent on GLP-2.

In this present study only GLP-2 was considered, alternatively other factors may be important. Since the GLP-2R is not localized to proliferating crypt cells, the function of GLP-2 is related to a complex network of indirect mediators that induce signaling pathways, including IGF-1, IGF-2, and ErbB ligands, which link to small intestinal growth (258). IGF-1 and IGF-2 share biological actions in the intestine with GLP-2 and may be necessary for GLP-2 action (346). The ErbB ligands, including EGF, are potential factors in maintaining intestinal growth and function, and are found to be increased after GLP-2 administration (347, 348). PYY, co-released from the ileum with GLP-2, is increased after small bowel resection and is considered as a humoral mediator of the intestinal trophic response (349). Similarly, factors like KGF, endothelial nitric oxide synthase (eNOS) and IGF-2, are reported to participate in the actions of GLP-2 on the colon, which may need to be studied independently of small intestine adaptation (258). All these complex downstream signaling factors that may induce the action of GLP-2 in adaptation are not clearly understood, further studies of these factors will also elucidate the mechanisms of GLP-2 and help to augment its therapeutic role. GH has already been used in some SBS studies, and some positive effects has already been found (350, 351). Besides, hepatocyte growth factor, vascular endothelial growth factor, and fibroblast growth factor may also deserve consideration (348).

Further studies of GLP-2 and related growth factors is warranted and has important implications for babies with short bowel syndrome and intractable intestinal failure. In order to develop GLP-2 as a potential therapeutic adjuvant for SBS patients, it is necessary to better elucidate its mechanism, efficacy and safety, prior to clinical use. The varying treatment required at different ages and stages of development, the optimal

timing for GLP-2 treatment, the minimal length of treatment, the maximal amount of GLP-2 before toxic effects may occur and the benefit for combined treatments with other growth factors all needs to be explored, before GLP-2 be widely used in neonatal SBS. It is exciting to consider that GLP-2 may be a new therapy that can improve the prognosis of neonatal SBS and benefit the child, their family and our society.

6.4 Summary

Using neonatal piglets as an animal model for short bowel syndrome, with and without ileum, this thesis demonstrates that short bowel piglets with ileum, have small intestinal adaptation associated with progressive increase in plasma GLP-2. On the contrary, short bowel piglets without ileum do not have normal small intestinal growth, nor intestinal adaptation and do not have progressive increase in plasma GLP-2.

The absence of ileum is a common finding in human infants with SBS that fail to undergo adaptation and wean from PN. These babies either succumb to complications of this therapy or require intestinal transplantation. Based on the work in this thesis it is feasible to conclude that small intestinal adaptation may be improved in human neonates, with ileal resection, by given exogenous GLP-2 and this should be further explored.

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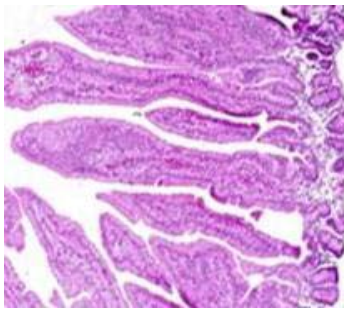
APPENDIX

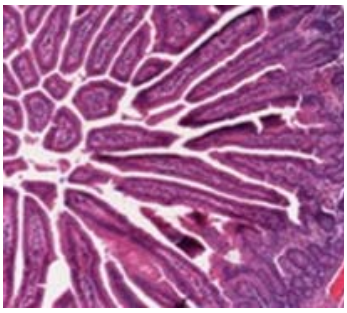
Appendix 1 Reference value of hematology and biochemistry in normal piglets

	Reference
Hemoglobin, g/L	90 - 150
White cell count, $\times 10^{9}$/L	11.0 – 21.0
Platelets, $\times 10^{9}$/L	100 - 900
Albumin, g/L	19 - 32
Total bilirubin, µmol/L	0 - 6
Alkaline phosphatase (ALP), IU/L	180 – 460
Galactosylhydroxylysyl glucosyltransferase (GGT), IU/L	8 - 40

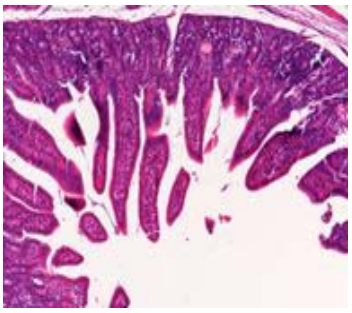
Appendix 2 Histological adaptation of small bowel



JI, villus hypertrophy with increased crypt depth

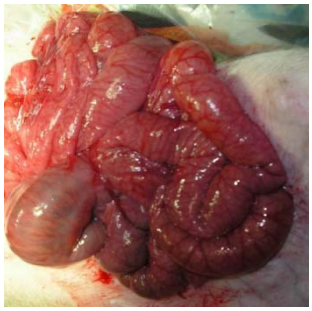


JC, minimal crowding of villi no increased length

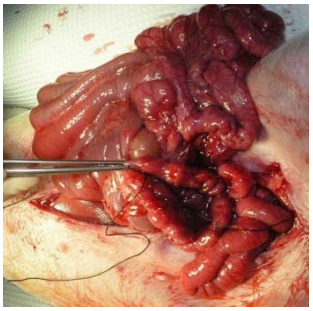


Sham, normal length

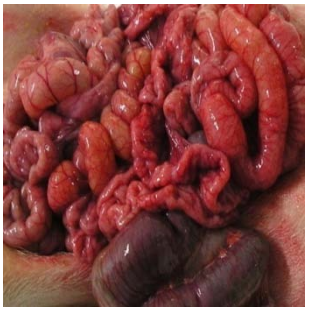
Appendix 3 Pictures of small bowel at termination



JI, dilation



JC, no dilation



Sham, normal