EXOGENOUS GLUCAGON-LIKE PEPTIDE-2 IN NEONATAL PIGLET MODELS OF SHORT BOWEL SYNDROME: DOES THE INTESTINAL ADAPTIVE RESPONSE VARY WITH REMNANT INTESTINAL ANATOMY?

By

Megha Suri

A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Sciences

University of Toronto

© Copyright by Megha Suri (2013)
Exogenous glucagon-like peptide-2 in neonatal piglet models of short bowel syndrome: Does the intestinal adaptive response vary with remnant intestinal anatomy?

Megha Suri

Master of Science

Institute of Medical Sciences
University of Toronto

2013

ABSTRACT

Glucagon-like peptide-2 (GLP-2) augments intestinal adaptation in animal models of short bowel syndrome (SBS) and in adult patients with SBS. However, GLP-2 has not been used as a therapy for pediatric SBS. In this thesis, it is hypothesized that exogenous GLP-2 therapy will improve outcomes of intestinal adaptation in proximal intestinal resection (JI) and distal intestinal resection (JC) neonatal piglet models of SBS.

Improvements in morphological parameters (increased small intestinal length) and histological parameters (increased jejunal villus length or jejunal crypt depth) of intestinal adaptation in JI and JC neonatal piglets treated with GLP-2 were observed. However, improved clinical outcomes (fewer days of diarrhea, fewer days on parenteral nutrition, more days on enteral nutrition alone) were only observed in GLP-2 treated JC animals.

Since the JC anatomical subtype (no remnant ileum) represents the majority of clinical cases of neonatal SBS, these results support a potential role for GLP-2 therapy in pediatric SBS.
ACKNOWLEDGEMENTS

It is with great joy and sincere gratitude that I would like to acknowledge the people who have influenced this work and have inspired me along the way. This thesis is dedicated to three families and one sailor. The first family is my own, and most notably includes my dadi, Swarn Lata Suri, my father, Yogesh Suri, my mother, Renuka Suri, and my brother, Raghav Suri. The second family is the Wozniak family, and includes uncle Roman, aunty Bonnie, and my good friend Russell. The third family is often referred to as “camp Sigalet,” and runs under the leadership of Elaine and David Sigalet. Thank you for all the delicious food and engaging conversation over the years.

This thesis is also dedicated to the sailor, Ian Delong, who helped me to rekindle those desires that are necessary for a fulfilling and meaningful life, and during the final stages of this thesis, played a vital role in helping me to achieve that sense of fulfillment and meaning.

Firstly, I would like to acknowledge and thank my supervisor, Dr. Paul Wales, who believed in this project and granted me the opportunity to be a part of it. I am grateful to Dr. Wales for his guidance throughout my thesis, and for providing me with many learning opportunities. I am especially grateful for his surgical teaching during the animal operations, and for his mentorship and supportive advice for my career goals.

Secondly, I would like to thank the members of my committee: Dr. Benjamin Alman, Dr. Johane Allard, and Dr. Patricia Brubaker for their support and feedback during each stage of this thesis. Most notably, I would like to thank Dr. Brubaker who has been a tremendous inspiration to me. Dr. Brubaker’s enthusiasm for science, eye for perfection, relentless attention to detail, and uncompromising integrity makes her an invaluable part of the world of science, and I acknowledge my good fortune in having had the opportunity to be her student and to have her as a mentor.

Thirdly, I would like to thank Dr. Justine Turner and Dr. Patrick Nation from the University of Alberta. Dr. Turner was my supervisor during the laboratory component of my research in Edmonton, and I am grateful for her feedback and advice on several presentations, abstracts, papers, and my thesis text. Dr. Nation is a veterinary pathologist who performed all of the histological analyses for my thesis, and his work has been a tremendous contribution. I would also like to thank Pamela Wizzard, Zheng Hua, Christine Pendlebury, Rhian Ormond, and Charlane Gorsak for their help with all of the surgical procedures, daily and nightly animal care, and collection of samples. Without the commitment and hard work of these women, this project would not have been possible. I am also thankful to Joan Turchinsky who helped me to perform the fat extraction analyses in this thesis, and I am grateful for her teaching and assistance.

Lastly, I would like to thank Dr. Sigalet and Laurie Wallace from the University of Calgary who helped with all the molecular techniques and hormone assays in this thesis. I am grateful for their teaching and technical assistance, as well as for their coordination with Bolette Hartmann and Dr. Jens Holst from the University of Copenhagen who performed the plasma GLP-2 assays in this study. I am also thankful for the support and friendship I received from all the members of Dr. Sigalet’s laboratory, including Elaine de Heuvel and Estrella Bulloch.
# TABLE OF CONTENTS

**ACKNOWLEDGEMENTS** iii  
**TABLE OF CONTENTS** iv  
**LIST OF TABLES** x  
**LIST OF FIGURES** xi  
**LIST OF ABBREVIATIONS** xiii  

**Chapter 1: LITERATURE REVIEW** 1  

1.1 Short bowel syndrome 2  

1.1.i Definitions, epidemiology, and etiology 2  

1.1.ii Factors influencing outcomes in short bowel syndrome 3  

1.1.ii.a Intestinal length 3  

1.1.ii.b Small intestinal remnant anatomy 5  

1.1.ii.c Colonic intestinal remnant 6  

1.1.ii.d Presence or absence of the ileocecal valve 7  

1.1.iii Anatomical types of short bowel syndrome 8  

1.1.iv Intestinal adaptation 9  

1.1.iv.a Structural adaptation 10  

1.1.iv.b Motor adaptation 11  

1.1.iv.c Functional adaptation 11  

1.1.v Factors influencing intestinal adaptation 12  

1.1.v.a Pancreaticobiliary secretions 12  

1.1.v.b Nutrients 12  

1.1.v.b.i Carbohydrates 12  

1.1.v.b.ii Fiber and short-chain fatty acids 13
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1.v.b.iii</td>
<td>Protein</td>
<td>14</td>
</tr>
<tr>
<td>1.1.v.b.iv</td>
<td>Lipids</td>
<td>14</td>
</tr>
<tr>
<td>1.1.v.c</td>
<td>Hormones and growth factors</td>
<td>15</td>
</tr>
<tr>
<td>1.1.v.c.i</td>
<td>Growth hormone</td>
<td>15</td>
</tr>
<tr>
<td>1.1.v.c.ii</td>
<td>Insulin-like growth factor-1</td>
<td>16</td>
</tr>
<tr>
<td>1.1.v.c.iii</td>
<td>Epidermal growth factor</td>
<td>16</td>
</tr>
<tr>
<td>1.1.v.c.iv</td>
<td>Peptide YY</td>
<td>17</td>
</tr>
<tr>
<td>1.1.v.c.v</td>
<td>Glucagon-like peptide-2</td>
<td>18</td>
</tr>
<tr>
<td>1.1.vi</td>
<td>Management of short bowel syndrome</td>
<td>18</td>
</tr>
<tr>
<td>1.2</td>
<td>Animal models</td>
<td>20</td>
</tr>
<tr>
<td>1.2.i</td>
<td>Animal models for studying gastrointestinal disease and nutrition</td>
<td>20</td>
</tr>
<tr>
<td>1.2.ii</td>
<td>Justification for the use of a piglet model over a rodent model of gastrointestinal disease and nutrition</td>
<td>21</td>
</tr>
<tr>
<td>1.2.iii</td>
<td>Neonatal piglet model for the study of short bowel syndrome</td>
<td>23</td>
</tr>
<tr>
<td>1.2.iv</td>
<td>Assessing the quality of animal models for research on human disease</td>
<td>25</td>
</tr>
<tr>
<td>1.3</td>
<td>Glucagon-like peptide-2</td>
<td>26</td>
</tr>
<tr>
<td>1.3.i</td>
<td>Glucagon-like peptide-2 synthesis and metabolism</td>
<td>26</td>
</tr>
<tr>
<td>1.3.ii</td>
<td>Biological actions of endogenous and exogenous glucagon-like peptide-2 in the normal gastrointestinal tract</td>
<td>27</td>
</tr>
<tr>
<td>1.3.iii</td>
<td>Exogenous glucagon-like peptide-2 in the normal gastrointestinal tract of parenterally fed piglets</td>
<td>28</td>
</tr>
<tr>
<td>1.3.iv</td>
<td>Mechanisms of action of glucagon-like peptide-2 and its receptor</td>
<td>28</td>
</tr>
<tr>
<td>1.3.v</td>
<td>Endogenous glucagon-like peptide-2 secretion and the normal gastrointestinal tract</td>
<td>30</td>
</tr>
<tr>
<td>1.3.vi</td>
<td>Endogenous glucagon-like peptide-2 and the resected gastrointestinal tract</td>
<td>31</td>
</tr>
</tbody>
</table>
1.3.vii Exogenous glucagon-like peptide-2 in animal models of short bowel syndrome

1.3.viii Glucagon-like peptide-2 therapy for patients with short bowel syndrome

CHAPTER 2: HYPOTHESES AND RESEARCH AIMS

CHAPTER 3: METHODS

3.1 Research ethics approval

3.2 Sample size calculation

3.3 Animals and surgical procedures

3.3.i Induction and anesthesia

3.3.ii Central venous catheter insertion

3.3.iii Laparotomy and intestinal resection

3.3.iv Gastrostomy tube insertion and abdominal closure

3.4 Post-surgical piglet care

3.5 Nutrition

3.6 Glucagon-like peptide-2 or saline infusion

3.7 Daily piglet assessment

3.8 Clinical chemistry

3.9 Plasma glucagon-like peptide-2 collection and radioimmunoassay

3.10 Serum peptide YY collection and immunoabsorbance assay

3.11 Fecal sampling and enteral fat absorption

3.12 Terminal laparotomy and tissue specimens

3.13 Histology

3.14 Immunohistochemistry

3.15 Ki-67
3.16 Cleaved caspase-3

3.17 Quantitative real-time polymerase chain reaction (qRT-PCR): GLP-2 receptor mRNA quantification

3.18 Statistical analysis

CHAPTER 4: RESULTS

4.1 Animals

4.1.i Flow of animals

4.1.ii Morbidity and mortality

4.2 Baseline piglet characteristics

4.3 Trial characteristics

4.4 Bioactive plasma glucagon-like peptide-2 levels

4.5 Clinical and functional outcomes

4.5.i Exogenous glucagon-like peptide-2 improves outcomes relating to parenteral and enteral nutrition in piglets with a distal-intestinal resection

4.5.ii Exogenous glucagon-like peptide-2 improves fecal output but does not affect enteral fat absorption in piglets with a distal-intestinal resection

4.5.iii Exogenous glucagon-like peptide-2 does not affect number of septic episodes

4.5.iv Clinical chemistry

4.6 Morphological outcomes

4.6.i Exogenous glucagon-like peptide-2 does not affect piglet weight

4.6.ii Exogenous glucagon-like peptide-2 increases small intestinal length in piglets with a proximal and distal-intestinal resection

4.6.iii Exogenous glucagon-like peptide-2 increases colonic weight in piglets with distal-intestinal resection, but does not affect small intestinal or liver weight

4.7 Endogenous bioactive plasma glucagon-like peptide-2 levels do not correlate with small intestinal length and enteral nutrition feed levels at termination
4.8 Histological outcomes

4.8.i Exogenous glucagon-like peptide-2 increases jejunal villus height and crypt depth in piglets with a proximal-intestinal resection and increases jejunal crypt depth in piglets with a distal-intestinal resection

4.8.ii Exogenous glucagon-like peptide-2 does not affect jejunal crypt cellular proliferation and villus apoptosis but decreases the relative fold increase in apoptosis for JI and JC groups

4.9 Exogenous glucagon-like peptide-2 (GLP-2) increases GLP-2 receptor mRNA expression in jejunal, ileal, and colonic tissue

4.10 Total endogenous serum peptide YY levels

4.11 Total endogenous serum PYY levels do not correlate with small intestinal length and enteral feed levels at termination

CHAPTER 5: DISCUSSION

5.1 Quality of animal study

5.1.i Sample size calculation

5.1.ii Inclusion and exclusion criteria

5.1.iii Treatment allocation

5.1.iv Blinding to treatment allocation

5.1.v Flow of animals

5.1.vi Control of physiological variable

5.1.vii Control of study conduct

5.1.viii Statistical methods

5.2 Endogenous bioactive glucagon-like peptide-2 levels in proximal-intestinal and distal-intestinal resection neonatal piglet models of SBS

5.3 Exogenous glucagon-like peptide-2 and intestinal adaptation in proximal-intestinal and distal-intestinal resection neonatal piglet models of SBS

5.3.i Clinical and functional outcomes of intestinal adaptation

5.3.ii Morphological outcomes
5.3.iii Endogenous bioactive glucagon-like peptide-2 levels, small intestinal length, and enteral nutrients 126

5.3.iv Histological outcomes 127

5.4 Mechanisms of intestinal adaptation 129

5.4.i Crypt cellular proliferation and villus apoptosis 129

5.4.ii Exogenous GLP-2 and intestinal GLP-2 receptor mRNA expression 132

5.4.iii Total endogenous PYY levels and in proximal-intestinal and distal-intestinal resection neonatal piglet models of SBS 134

5.4.iv Total endogenous PYY levels, small intestinal length, and enteral nutrients 135

5.4.v Glucagon-like peptide-2 and peptide YY secretion and metabolism 136

CHAPTER 6: CONCLUSIONS 138

CHAPTER 7: FUTURE DIRECTIONS 140

REFERENCES 144
LIST OF TABLES

Chapter 1: INTRODUCTION

Table 1.1. Structural and functional parameters of intestinal adaptation in proximal-intestinal resection animal models of SBS 35

Table 1.2. Structural and functional parameters of intestinal adaptation in distal-intestinal resection animal models of SBS 36

Chapter 3: METHODS

Table 3.1. Amino acid content for 1L PN mixture 49

Table 3.2. Mineral content for 1L PN mixture 49

Table 3.3. Trace mineral solution 49

Chapter 4: RESULTS

Table 4.1. Clinical chemistry prior to surgery and termination 82
# LIST OF FIGURES

## Chapter 1: INTRODUCTION

- Figure 1.1. Anatomical subtypes of short bowel syndrome 9
- Figure 1.2. GLP-2 peptide sequence homology in the human and pig 27

## Chapter 3: METHODS

- Figure 3.1. Surgical groups 45

## Chapter 4: RESULTS

- Figure 4.1. Flow of animals 63
- Figure 4.2. Number of cases with no complications and morbidity/mortality 64
- Figure 4.3. Baseline piglet characteristics at surgery (day 0) 66
- Figure 4.4. Trial characteristics 68
- Figure 4.5. Plasma GLP-2 concentration over the course of the trial 71
- Figure 4.6. Plasma GLP-2 concentrations for sham, JI, and JC surgical groups at selected points in time during the trial 72
- Figure 4.7. Plasma GLP-2 concentrations at selected points in time during the trial 73
- Figure 4.8. Clinical outcomes relating to PN and EN 75
- Figure 4.9. Clinical outcomes relating to fecal output 76
- Figure 4.10. Enteral Fat Absorption for Days 12-14 77
- Figure 4.11. Change in enteral fat absorption from days 5-7 and days-12-14 78
- Figure 4.12. Number of positive (+), negative (-), and presumed (?) septic episodes 80
- Figure 4.13. Daily weight gain during trial 84
- Figure 4.14. Piglet weight at termination (day 14) 84
- Figure 4.15. Small intestinal length at termination (day 14) 85
- Figure 4.16. Tissue weight per piglet body weight 86
Figure 4.17. Small intestinal length vs. endogenous GLP-2 levels for all saline treated piglets

Figure 4.18. Small intestinal length vs. endogenous GLP-2 levels

Figure 4.19. Enteral feed levels vs. endogenous GLP-2 levels at termination for all saline treated piglets

Figure 4.20. Enteral feed levels vs. endogenous GLP-2 levels at termination

Figure 4.21. Small intestinal mucosal morphometry

Figure 4.22. H&E-stained intestinal sections (100x magnification) from jejunal tissue

Figure 4.23. H&E-stained intestinal sections (100x magnification) from ileal tissue

Figure 4.24. Ki67 immunoreactivity for jejunal half-crypt zones

Figure 4.25. Jejunal villus cleaved-caspase 3 immunoreactivity

Figure 4.26. Jejunal villus cleaved caspase-3 immunoreactivity for GLP-2 treated piglets relative to saline treated control piglets in the same surgical group

Figure 4.27. Relative GLP-2 Receptor mRNA expression in intestinal tissue at surgery (day 0)

Figure 4.28. Relative GLP-2 Receptor mRNA expression in intestinal tissue at termination (day 14)

Figure 4.29. Serum PYY concentration over the course of the trial

Figure 4.30. Serum PYY concentrations for sham, JI, and JC surgical groups at selected points in time during the trial

Figure 4.31. Serum PYY concentrations at selected points in time during the trial

Figure 4.32. Small intestinal length vs. endogenous PYY levels

Figure 4.33. Small intestinal length vs. endogenous PYY levels

Figure 4.34. Enteral feed levels vs. endogenous PYY levels at termination

Figure 4.35. Enteral feed levels vs. endogenous PYY levels at termination
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPP-IV</td>
<td>Dipeptidyl peptidase-IV</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>EN</td>
<td>Enteral nutrition</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>GH</td>
<td>Growth hormone</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>GLP-2</td>
<td>Glucagon-like peptide-2</td>
</tr>
<tr>
<td>GLP-2R</td>
<td>Glucagon-like peptide-2 receptor</td>
</tr>
<tr>
<td>Gly</td>
<td>Glycine</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and eosin</td>
</tr>
<tr>
<td>H₂</td>
<td>Histamine-2</td>
</tr>
<tr>
<td>I</td>
<td>Ileum</td>
</tr>
<tr>
<td>ICV</td>
<td>Ileocecal valve</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor-1</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>J</td>
<td>Jejunum</td>
</tr>
<tr>
<td>JC</td>
<td>75% distal small intestinal resection with jejunocolic anastomosis</td>
</tr>
<tr>
<td>JI</td>
<td>75% proximal small intestinal resection with jejunoileal anastomosis</td>
</tr>
<tr>
<td>KGF</td>
<td>Keratinocyte growth factor</td>
</tr>
<tr>
<td>LCPUFA</td>
<td>Long-chain polyunsaturated fatty acids</td>
</tr>
<tr>
<td>LCT</td>
<td>Long-chain triglycerides</td>
</tr>
<tr>
<td>MCT</td>
<td>Medium-chain triglycerides</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>NEC</td>
<td>Necrotizing enterocolitis</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>PBS</td>
<td>Pancreaticobiliary secretions</td>
</tr>
<tr>
<td>PDS</td>
<td>Polydioxanone suture</td>
</tr>
<tr>
<td>PPI</td>
<td>Proton pump inhibitor</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide YY</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Quantitative real time polymerase chain reaction</td>
</tr>
<tr>
<td>RPM</td>
<td>Rounds per minute</td>
</tr>
<tr>
<td>SA-HRP</td>
<td>Streptavidin-conjugated horseradish peroxidase</td>
</tr>
<tr>
<td>SBS</td>
<td>Short bowel syndrome</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short chain fatty acids</td>
</tr>
<tr>
<td>Sham</td>
<td>No small intestinal resection</td>
</tr>
<tr>
<td>STEP</td>
<td>Serial transverse enteroplasty</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetramethylbenzidine</td>
</tr>
<tr>
<td>TPN</td>
<td>Total parenteral nutrition</td>
</tr>
<tr>
<td>VIP</td>
<td>Vasoactive intestinal peptide</td>
</tr>
</tbody>
</table>
Methodological Abbreviations

% percent
° C degrees Celcius
d day(s)
g gram
hr hour(s)
J Joules
l litres
m metre
M molar (moles/l)
min minute(s)
mol moles
p statistical p-value
s second(s)
SE standard error of the mean
wk week
x times

Prefixes

k kilo- (x 10^3)
c centi- (x 10^-2)
m milli- (x 10^-3)
µ micro- (x 10^-6)
n nano- (x 10^-9)
p pico- (x 10^-12)
CHAPTER 1

LITERATURE REVIEW
1 Literature review

1.1 Short bowel syndrome

1.1.i Definitions, epidemiology, and etiology

Intestinal failure is a state of malabsorption in which there is inadequate intestinal function necessary for health and growth [1]. The causes of intestinal failure include mucosal enteropathies, dysmotility syndromes, and short bowel syndrome (SBS) [1]. Short bowel syndrome results from massive resection of the intestine that leads to a shortened and functionally inadequate intestinal remnant required for survival, growth, and hydration [1, 2]. In pediatric patients with intestinal failure, more than fifty percent of cases are caused by SBS [2]. The incidence and prevalence of SBS are often extrapolated from data on home parenteral nutrition (PN), and in children, the incidence is estimated to be 0.02-0.49/100,000 and the prevalence to be 0.03-0.89/100,000 [1, 3]. In a recent population-based study, Wales et al. reported that the incidence of neonatal SBS is 24.5/100,000 live births in term infants (infants born after 37-weeks of gestation) and 353.7/100,000 live births in pre-term infants (infants born prior to 37-weeks of gestation) [4]. The most common etiologies of SBS in children include necrotizing enterocolitis (NEC) (27%), jejunoileal atresia (23%), intestinal volvulus (23%), gastroschisis (14%), and Hirschsprung disease (4%) [3].

Parenteral nutrition is the mainstay of therapy for patients with SBS, and in children with SBS, PN is necessary for normal growth of the individual while the small intestinal remnant undergoes adaptation [5]. Although most children with SBS will undergo successful intestinal adaptation and be weaned from PN, this process may take up to several months or years [6]. The prolonged use of PN and long-term hospitalization
are associated with frequent central venous catheter complications, sepsis, cholestatic liver disease, liver failure, and failure to thrive [1, 7]. The complications of SBS are significant, and account for 37.5% of deaths in neonates with SBS, and 1.4% of all deaths in children younger than 4 years of age [4]. The significant morbidity and mortality associated with SBS, the considerable costs to society, and the poor quality of life for patients and their families warrants the need to wean patients off of PN and to achieve enteral independence as quickly as possible [2, 8].

1.1.ii  Factors influencing outcomes in short bowel syndrome

In the setting of SBS, there are several factors that influence successful adaptation of the remnant intestine, weaning of PN, and improved overall outcomes. These factors include the length, anatomy, and function of the small and large intestinal remnant; the adaptive capacity of the intestinal remnant; the presence or absence of the ileocecal valve; the age of the patient; the initial diagnosis and disease burden; and exposure to enteral nutrients, pancreaticobiliary secretions (PBS), and hormones and growth factors [9, 10].

1.1.ii.a  Intestinal length

The length of the remnant intestine is an important determinant of outcome in SBS [11-13]. Although absolute intestinal length is strongly related to outcome in adult patients with SBS [3], remnant intestinal length as a percentage relative to the gestational norm is a better predictor of outcome in neonates and infants [14-16]. Based on postmortem measurements, normal intestinal length in infants with a gestational age of 27 to 35 weeks is approximately $217 \pm 24$ cm, and $304 \pm 44$ cm in infants with a gestational age $\geq 35$ weeks. Term infants have a small intestinal length between 250 and
300 cm, and a large intestinal length between 30-40 cm [9]. Prospectively collected data from infants undergoing laparotomy suggest that infants with a gestational age of 27 to 35 weeks have a mean small intestinal length of $100.0 \pm 6.5$ to $120.8 \pm 8.8$ cm, and term to 6 month-old infants have a mean intestinal length between $142.6 \pm 12.0$ and $239.2 \pm 18.3$ cm. Colonic length from prospectively collected data in term to 6 month-old infants is $32.7 – 56.8$ cm [17]. Small intestinal length doubles in children by two-three years, and grows to a length of 275-850 cm in adulthood [18]. Hence, infants have significant intrinsic gut growth potential, and this may contribute to the capacity to wean PN in pediatric patients with SBS.

In pediatric patients, a shortened intestinal segment refers to an absolute length less than 75 cm, or a length shorter than 30% of the predicted length for a given gestational age [19]. Quiros-Tejeira et al. further classify short bowel according to the length of the intestinal remnant: short intestinal remnant (>38 cm), very short intestinal remnant (15-38 cm), and ultrashort intestinal remnant (<15 cm), and have demonstrated that these categories predict mortality and the successful weaning of PN in pediatric patients [20]. Spencer et al. also found that absolute small bowel length is slightly predictive of mortality and weaning of PN, however they demonstrated that remnant intestinal length expressed as a percentage of the expected length for a given gestational age is a stronger predictor of mortality and PN-independence. Specifically, Spencer et al. report that the mortality of patients with a small bowel length <10% of the expected intestinal length is 5.57 times greater than those with ≥10% of the expected intestinal length, and that the latter are 11.8 times more likely to successfully wean off PN relative to the former [16].
1.1.ii.b  Small intestinal remnant anatomy

The anatomical region of resected intestine is an important factor in the clinical course of SBS. The small intestine has unique morphological features that increase the luminal surface area for nutrient absorption, including the mucosal folds of Kerckring or valvulae coniventes, villi, and microvilli [21]. Small intestinal villi project into the intestinal lumen and are approximately 0.5-1.0 cm in length [22]. The jejunum has longer villi than the ileum, more absorptive surface area, and a higher concentration of brush-border disaccharidases and peptidases, as well as amino acid- and glucose-transporters [23]. Even though the absorption of carbohydrates, proteins, fats, minerals, water-soluble vitamins (C and B vitamins), and fat-soluble vitamins (vitamins A, D, E, K) primarily occurs in the jejunum, resection of the jejunum only results in a transient reduction in nutrient absorption [2, 9, 19, 24-26]. This is due to the highly adaptive capacity of the ileum to increase nutrient absorption and take-on the function of the jejunum [10, 18, 27].

In addition to its capacity for adaptation, the ileum is specialized for vitamin \( B_{12} \) and bile salt absorption, and has slower gastrointestinal transit than the jejunum – that permits more luminal contact time and absorption of nutrients. The mucosa of the ileum has “tight” intracellular junctions compared to the “leaky” mucosal intracellular junctions in the jejunum, and water that is secreted into the lumen of the jejunum following a hypertonic meal is absorbed by the ileum. The terminal ileum also contains intestinal L cells that secrete several hormones involved in the regulation of appetite, gastrointestinal motility, absorption, and adaption. These hormones include peptide YY (PYY),
glucagon-like peptide 1 (GLP-1), and glucagon-like peptide 2 (GLP-2) [2, 9, 10, 18, 19, 26].

Outcomes in patients with an ileal, rather than a jejunal, intestinal remnant are generally more favourable. Resection of the ileum results in vitamin B\textsubscript{12} deficiency, and patients may require life-long replacement therapy. In the absence of ileum, water secreted into the lumen of the proximal intestine after a meal is not absorbed, and this may result in considerable diarrhea, electrolyte and fluid disturbances. The ileum is also the site of bile acid absorption, and loss of the ileum leads to a spillage of bile salts into the colon. This spillage diminishes the overall bile salt pool, which may worsen symptoms of diarrhea, and cause malabsorption of fat and fat-soluble vitamins.

Malabsorption of bile acids also alters hepatic metabolism and secretion of bile, and this predisposes patients with SBS to gallstone formation. Furthermore, malabsorbed fat binds to enteric calcium and reduces calcium absorption. Hypocalcemia diminishes fecal oxalate excretion, which may lead to oxaluria and renal stone formation [2, 9, 19].

Intestinal motility in the ileum is slower than in jejunum and duodenum; hence, resection of the ileum decreases intestinal transit and decreases luminal contact and absorption of nutrients. Resection of the terminal ileum also diminishes L cell secretion of ileal-brake hormones (hormones that reduce proximal gastrointestinal motility), such as PYY and GLP-1, which further decreases intestinal transit time [10, 18, 27].

1.1.ii.c **Colonic intestinal remnant**

The colon absorbs fluids and electrolytes, increases gastrointestinal transit time, and improves energy absorption by bacterial fermentation of unabsorbed carbohydrates to short-chain fatty acids [1, 2, 18, 28]. Intestinal L cells are also present in the colon,
secreting intestinotrophic hormones that regulate intestinal adaptation (GLP-2) and transit (PYY) [29, 30]. In adult patients with SBS, the presence of the colon facilitates weaning of PN and improves enteral energy intake [28, 31, 32]. The importance of the colon in pediatric patients is less clear. Quiros-Tejeira et al. demonstrated improved weaning of PN in patients with a colonic remnant >50% of its original length [20], while Diamond et al. found no difference in weaning of PN between patients with or without a remnant colon [33].

1.1.ii.d Presence or absence of the ileocecal valve

The ileocecal valve (ICV) prolongs intestinal transit and prevents colonic enteric contents from entering the small intestine [9]. In adult patients with SBS, resection of the ICV does not affect outcomes [18]. In pediatric patients, Spencer et al. showed that the presence of the ICV is strongly predictive of PN weaning, however its presence does not improve overall patient survival [16]. Quiros-Tejeira et al. demonstrated no differences in weaning of PN between pediatric patients with an intact or resected ICV, however they found that patients with a small intestinal remnant < 15 cm were more likely to wean from PN in the presence of an ICV [20]. Wilmore suggested that in the absence of an ICV, 40 cm of small intestine is necessary for survival, while only 15 cm is necessary in the presence of an ICV [34]. If the presence of an ICV improves outcomes in SBS, this is less likely related to its barrier function and effect on intestinal transit, and more likely related to the specialized intestinotrophic properties of the terminal ileum, which is usually lost with a resection of the ICV [2].
1.1.iii Anatomical types of short bowel syndrome

There are three anatomical subtypes of SBS (Figure 1.1). Type 1: mid-intestinal, predominantly jejunal resection; jeuno-ileal anastomosis; preservation of some ileum; and an intact ICV and colon. Type 2: distal-intestinal, predominantly ileal and partial colonic resection; jeuno-colonic anastomosis; and preservation of some colon. Type 3: partial resection of the jejunum; complete resection of the ileum, ICV, and colon; and an end-jejunostomy [18]. With large fluid and electrolyte losses, patients with the type 3 subtype are the most difficult to manage, and generally have the poorest outcomes.

Patients with a type 1 subtype have the best outcomes, as the ileum is able to increase nutrient absorption and take-on the function of the jejunum, and both the ileum and colon are important to the adaptation process [2, 10, 18, 27]. Type 2 is the subtype most often encountered in the setting of pediatric SBS, as the commonest etiologies (NEC and atresia) affect the terminal ileum and proximal colon frequently [1]. Nevertheless, the type 1 subtype, rather than the type 2, is most often represented in animal model of SBS.
1.1.iv Intestinal adaptation

The small intestine has the ability to compensate for the loss of absorptive surface area following resection [35], and this adaptive response involves changes in intestinal structure, motility, and function [2]. In patients with SBS, adaptation of the remnant intestine results in an increase in enteral nutrient absorption, decreased dependence on PN, diminished fluid and electrolyte losses, and improved overall nutritional status. This in turn leads to successful clinical outcomes. Intestinal adaptation begins 12-24 hours after massive resection, and in adults continues for 2 years [2]. In pediatric patients, younger children should have a greater potential for adaptation as both the child and intestine continue to grow with age [9].
1.1.iv.a  **Structural adaptation**

The structural adaptive response following intestinal resection has been well studied in animal models in which remnant ileum is present (representing the type 1 anatomical subtype of SBS). In rodents, the compensatory response includes all layers of the small intestinal wall and is predominantly hyperplastic [35]. There is a marked increase in villous height and crypt depth, and enterocyte proliferation is increased as indicated by an increase in mucosal DNA, protein synthesis and crypt cell proliferation. Although the rate of apoptosis in villi and crypts is also increased following intestinal resection, enterocyte proliferation predominates over apoptosis so that the overall response favours adaptation. The longitudinal and circular layers of the small intestinal wall also undergo hypertrophy after resection, and this is associated with an increase in intestinal length and calibre, respectively [35-38]. In rodents, the magnitude of the hyperplastic adaptive response is also proportional to the length of the intestinal resection [39]. In contrast to rodent models, one study in neonatal piglets demonstrated that massive intestinal resection is associated with deeper crypts in the ileum but no changes in jejunal or ileal villus height [40]. Thomson et al. describe lengthening and dilatation of the small intestine in adult patients who underwent surgical resection [41]; however, other studies on adult patients have failed to demonstrate the increase in villus height and crypt depth that are typically observed in rodents after intestinal resection [42-44]. In a recent study on human infants, longer intestinal villi and deeper crypts were found in infants with an ostomy as compared to infants with a small bowel resection and restored intestinal continuity. A significant correlation was also found between the amount of intestine resected and the percent change in villous height, suggesting a similar structural
adaptive response between human infants who undergo intestinal resection and representative animal models [45].

1.1.iv.b Motor adaptation

Intestinal resection is associated with an increase in gastrointestinal motility, and this disruption in motility is dependent on the extent and location of the intestinal resection [9]. In cases of limited intestinal resection, progressive motor adaptation occurs with slowing of intestinal transit; this is less likely in cases of massive intestinal resection [2]. Motor adaptation is also less likely in cases of ileal and colonic resection, since resident L cells secrete ileal-brake hormones, PYY, GLP-1, and GLP-2, which delay gastric emptying and decrease gastrointestinal transit [2, 18].

1.1.iv.c Functional adaptation

Functional adaptation after intestinal resection leads to an improvement in the nutritional status of a patient, and this is characterized by an increase in enteral nutrient absorption, decreased dependence on PN, and diminished diarrhea [2, 18, 27]. Structural and motor adaptation contribute to the functional adaptation process, as the former increases intestinal surface area for absorption of nutrients, and the latter increases intestinal transit time, which increases luminal contact of nutrients for absorption. Functional changes also include an increase in the uptake of carbohydrate, protein, water, and electrolytes by enterocytes, as well as an up regulation of digestive enzymes and nutrient transporters [6, 35, 42, 46, 47].
1.1.v  Factors influencing intestinal adaptation

The factors that influence successful intestinal adaptation in patients with SBS are the same factors that affect overall outcomes. These include the length, anatomy, and function of the small and large intestinal remnant; the presence or absence of the ileocecal valve; the age of the patient; the initial diagnosis and disease burden; and exposure to enteral nutrients, pancreaticobiliary secretions, and hormones and growth factors. Factors that were not described previously are discussed below.

1.1.v.a  Pancreaticobiliary secretions

Pancreaticobiliary secretions are trophic to both jejunal and ileal mucosa [48, 49]. In rats, direct application of PBS to isolated jejunal and ileal segments induces a hyperplastic response in villi [50]. Diversion of bile and PBS to the ileum corresponds with an increase in villus height in ileal tissue [48]. Furthermore, jejunal transposition between the pylorus and duodenum leads to jejunal hyperplasia, which is absent if PBS are diverted distally to the jejunum [51].

1.1.v.b  Nutrients

1.1.v.b.i  Carbohydrates

Complex carbohydrates and polymeric diets appear to promote intestinal adaptation more than elemental diets by providing a functional workload to intestinal mucosa [10]. In rats, mucosal growth after small bowel resection is greater in animals administered an intestinal infusion of disaccharides compared to an infusion of monosaccharides [52]. Furthermore, complex carbohydrates promote intestinal water and sodium absorption leading to a decrease in stool volume, while monosaccharides tend to have an osmotic effect that promotes diarrheal losses.
Fiber and short-chain fatty acids

Short-chain fatty acids (SCFA) are produced by fermentation of fiber and unabsorbed carbohydrates by colonic bacteria. Patients with SBS who retain a colon benefit from soluble fiber in their enteral diet, as SCFA particularly butyrate, act as an energy source for colonocytes [53]. Furthermore, administration of SCFA with TPN to rodents with no intestinal resection, and in models of SBS, results in improved morphologic and functional outcomes of intestinal adaptation. In TPN-fed rats, Korduat et al. demonstrated that intravenous (IV) or intracolonic administrated of SCFA reduced the mucosal atrophy observed with administration of TPN alone. Mucosal weight, DNA and protein content were higher in rats that received IV or intracolonic SCFA as compared to TPN-only animals [54]. In a TPN-fed rat model of SBS (80% proximal-intestinal resection with type 2 anatomy), Tappenden et al. found that TPN supplementation with a mixture SCFA improved functional outcomes of intestinal adaptation, as D-glucose uptake and ileal mRNA expression of GLUT2 and sodium-dependent glucose transporter 1 were higher in rats that received TPN with SCFA as compared to animals that received TPN alone [55]. Similarly, in a 80% proximal-intestinal resection neonatal piglet model of SBS, Bartholome et al. demonstrated an increase in villus height, an increase in crypt cellular proliferation, and a decrease in cellular apoptosis in animals that received TPN supplemented with SCFA as compared to TPN alone. The intestinotrophic effects of TPN supplemented with SCFA were specifically attributed to butyrate in this study. Moreover, endogenous GLP-2 levels were higher throughout the study period in piglets that received SCFA supplementation,
suggesting that the intestinotrophic actions of butyrate may have been mediated through GLP-2 [56].

1.1.v.b.iii Protein

The amino acids arginine and glutamine have been shown to augment intestinal adaptation in several rodent studies of SBS. Wakabayashi et al. demonstrated that rats fed a diet deficient in arginine had more weight loss than rats fed a normal diet after small bowel resection [57], and Welters et al. showed that arginine supplementation decreased intestinal permeability in rat model of SBS [58]. TPN supplemented with glutamine and administered to rats after massive small bowel resection has shown to improve intestinal adaptation in these animals [59]. In humans with SBS however, the benefits of glutamine supplementation (typically with growth hormone) on intestinal adaptation have been less convincing. In a recent systematic review of clinical trials investigating the role of glutamine and human growth hormone (GH) in patients with SBS, Wales et al. found that administration of glutamine with GH improves energy absorption and weight gain in SBS patients. However, the authors noted that these benefits are not sustained upon cessation of therapy, and conclude that currently there is not sufficient evidence to treat SBS patients with glutamine and GH [60].

1.1.v.b.iv Lipids

Triglycerides and free fatty acids promote intestinal adaptation more than polysaccharides and proteins in rat models of SBS [10]. In one study, the addition of long-chain polyunsaturated fatty acids (LCPUFA) to the diet of rats that underwent small bowel resection resulted in higher intestinal mucosal mass as compared to control-fed animals [61]. Although both long-chain triglycerides (LCT) and medium-chain
triglycerides (MCT) promote intestinal adaptation, the effects are more pronounced with LCT. Vanderhoof et al. demonstrated decreased intestinal mucosal weight, decreased sucrase activity, and decreased leucine uptake in rats fed a diet containing MCT compared to a diet with LCT [62].

1.1.v.c  Hormones and growth factors

1.1.v.c.i  Growth hormone

Growth hormone is produced by the anterior pituitary gland, and binding of GH to the GH receptor stimulates hepatic and intestinal insulin-like growth factor-1 (IGF-1) production [63]. In rodent models of SBS, the effects of GH administration have been mixed. Some studies have failed to demonstrate an increase in mucosal hyperplasia beyond what is typically seen after intestinal resection [64, 65], while others have demonstrated increases in jejunal and ileal epithelial cell proliferation and a decrease in cellular apoptosis with GH therapy [66, 67]. In one study, GH administration was associated with an increase in ileal sucrase and maltase activity in rats that underwent intestinal resection [65]. In humans, studies investigating GH therapy with or without concomitant glutamine administration have had mixed results, with some studies suggesting a benefit in absorption of nutrients, stool output, and weight gain, and others conferring no additional benefits to placebo [6]. No improvements in morphological outcomes of intestinal adaptation have been observed in adult human studies of GH and glutamine therapy. As mentioned in a previous section, a recent systematic review of clinical trials investigating the role human growth hormone and glutamine in patients with SBS demonstrated that administration of human GH with glutamine improves energy absorption and weight gain in SBS patients. However, since these benefits are not
sustained upon cessation of therapy, the authors of this study concluded that currently there is not sufficient evidence to treat SBS patients with glutamine and GH [60].

1.1.v.c.ii **Insulin-like growth factor-1**

Insulin-like growth factor-1 is produced in the liver and gastrointestinal tract, and it is postulated to be the downstream mediator of the intestinotrophic actions of GH and GLP-2 [63, 68]. Vanderhoof *et al.* found that IGF-1 administered to rodents after small intestinal resection increased duodenal and jejunal DNA and protein content, and increased sucrase, maltase, and leucine aminopeptidase activity [69]. In a PN and SBS rodent model, administration of IGF-1 was associated with improved weaning from PN [70]. Even though IGF-1 administration in rodent models of SBS enhances morphological and functional parameters of intestinal adaptation, there have been no clinical trials of IGF-1 in humans.

1.1.v.c.iii **Epidermal growth factor**

Epidermal growth factor (EGF) is found in plasma, platelets, macrophages, breast milk, saliva, and urine, and it belongs to a family of growth factors that share several EGF receptors [63]. The effects of EGF on intestinal adaptation in animal models of SBS are controversial. Chaet *et al.* demonstrated that EGF administration was associated with an increase in animal weight, small bowel weight, small intestinal length, mucosal thickness, and DNA protein content in rats after small intestinal resection [71]. Similarly, Fiore *et al.* found an increase in animal weight and mucosal thickness, as well as an increase in crypt cell proliferation, in rodents that underwent intestinal resection and were administered EGF [72]. In contrast to these findings, Lukish *et al.* and Iskit *et al.* failed to demonstrate morphological or proliferative adaptive changes in rodents administered
EGF after intestinal resection [73, 74]. Sigalet et al. have investigated the effect of EGF therapy in pediatric patients with SBS. In this pilot study, five patients (aged 6 months to 4 years) were treated with enteral EGF for six weeks, and EGF therapy was associated with an increase in carbohydrate nutrient absorption, and tolerance of enteral feeds [75].

Peptide YY

Peptide YY is a 36-amino acid peptide released by the intestinal L cells [76]. PYY immunoreactive cells are abundant in the distal small intestine and colon, and relatively fewer cells are present in the proximal intestine [77]. PYY release occurs in response to nutrient ingestion, and meals with higher caloric loads cause higher post-prandial levels of PYY. Fasting plasma PYY concentrations in healthy human adults are 8.5-11 pmol/L, and these levels are increased by 16.2 pmol/L following a 870-kcal meal, and 45.0 pmol/L following a 4500-kcal meal [76]. In addition to caloric intake, nutrient composition also influences the magnitude of PYY release. Post-prandial PYY release is higher in isocaloric meals composed of fat compared to protein or carbohydrate [78].

Circulating levels of PYY consist of two bioactive forms. Peptide YY (1-36) is secreted by the intestinal L cell, and truncated to PYY (3-36) by the enzyme dipeptidyl-peptidase IV (DPP-IV). The half-life of PYY (1-36) is 9 minutes in humans [76]. Peptide YY (1-36) is a mediator of the ileal brake response, as it decreases gastric emptying and gastric acid secretion, and increases intestinal transit time, while PYY (3-36) is mediator of the anorectic response, as it specifically increases satiety following a meal [79].

Human PYY profiles in patients with SBS vary according to remnant intestinal anatomy. Andrews et al. found that fasting levels of PYY were significantly higher (approximately 50 pmol/L) in SBS patients with remnant colon in continuity with
jejunum (type 2 anatomical subtype of SBS) than in healthy controls (approximately 10 pmol/L). Two hours following a meal, PYY levels peaked at approximately 130 pmol/L and 15 pmol/L in SBS patients and healthy controls, respectively [80]. Nightengale et al. demonstrated similar results: fasting levels of PYY were found to be 71 pmol/L in patients with a type 2 anatomical subtype of SBS compared to 11 pmol/L in control patients, and post-prandial levels increased to 110 pmol/L and 33 pmol/L in each respective group. In patients with an end jejunostomy (type 3 anatomical subtype of SBS), fasting PYY levels were 7 pmol/L and post-prandial levels peaked at 19 pmol/L [81]. Based on the results of these studies, fasting PYY levels are significantly higher in SBS patients with a remnant colon relative to control subjects, while there are no differences between fasting PYY levels in patients with an end jejunostomy compared to controls. Furthermore, relative to healthy controls, postprandial PYY release is not as high in SBS patients with an end jejunostomy while its release is significantly higher in SBS patients with remnant colon. These studies highlight the importance of the colon in the release of PYY after intestinal resection. No studies to date have examined the effect of exogenous PYY in animal models or patients with SBS.

1.1.v.c.v Glucagon-like peptide 2

Glucagon-like peptide 2 and its therapeutic role in SBS are discussed in detail in a subsequent section.

1.1.vi Management of short bowel syndrome

The management of patients with SBS differs according to the clinical phase of the disease. After intestinal resection, the acute phase (1-3 months) is characterized by diarrhea, diminished enteral absorption, dysmotility, and gastric hypersecretion, and the
goals of therapy are directed at maintaining electrolyte and fluid balances, correcting acid-base imbalances, and controlling gastric hyperacidity with histamine-2 (H₂) receptor blockers or proton pump inhibitors (PPIs) [19].

The acute phase of SBS is followed by the adaptive phase, which lasts up to 2 years in adult patients with SBS, and possibly longer in pediatric patients. During the adaptive phase, the goals of therapy are to wean patients from PN and promote enteral independence by enhancing intestinal growth, absorption, and function [9]. Medical management includes optimizing enteral and parenteral diet; slowing intestinal transit and decreasing diarrhea with anti-diarrheal medications (loperamide, narcotics); reducing gastrointestinal secretions with the use of anti-secretory agents (H₂-receptor antagonists, PPIs, octreotide, and clonidine); treating bacterial overgrowth with antibiotics, prokinetics and probiotics; and promoting intestinal adaptation with hormones and growth factors [2].

Surgical management of SBS is directed at preserving the intestinal remnant, increasing intestinal absorptive surface area, and improving gastrointestinal motility [2]. Remnant intestinal length can be increased with the Bianchi or serial enteral transverse enteroplasty (STEP) procedure. The Bianchi procedure involves longitudinal dissection of the mesentery supplying a dilated segment of intestine, longitudinal division of the dilated intestinal segment, and an end-to-end anastomosis of the two parallel intestinal segments [82]. The STEP procedure involves the sequential application of a linear stapler to a dilated intestinal segment in the plane perpendicular to the mesenteric axis to create a lengthened and tapered zigzag intestinal channel [83, 84]. This procedure works
by tapering and lengthening the dilated segment to improve peristalsis, while preserving mucosa and increasing absorptive surface area [85, 86].

Intestinal transplantation is indicated with or without a concomitant liver transplant in cases of growth failure, permanent PN dependence, loss of central venous access, recurrent sepsis, or irreversible liver disease from long-term PN use [87]. In children receiving long-term PN, 50% of deaths are related to PN-induced liver failure, and the high morbidity and mortality associated with transplantation makes it a last resort in the management of SBS [2, 87].

In addition to optimizing adaptation, management also focuses on preventing or minimizing complications associated with long-term hospitalization and PN therapy, including sepsis, complications related to central venous catheters, liver disease, and psychosocial stress, and hospital costs [2]. Moreover, if these complications are minimized, patients may have the opportunity for a prolonged period of adaptation.

1.2 Animal models

1.2.i Animal models for studying gastrointestinal disease and nutrition

Short bowel syndrome is a complex problem with multiple etiologies, varying remnant intestinal anatomy, a range of complications, and several therapeutic approaches. The heterogeneity of the disease process affects patient outcomes, and limits the ability to study SBS in the clinical setting. Furthermore, genetic diversity, methodological constraints, small sample size, and ethical considerations also hamper clinical research in human neonates [88]. Animal models are invaluable to the study of gastrointestinal disease and nutrition, as they offer a solution to the limitations encountered in research on human subjects. Controlled experimental conditions can be imposed in animal models
including a homogenous genetic population, equivalent disease burden (for instance, similar surgical anatomy in the case of a SBS model), and uniform delivery of therapy. The rapid growth and development of animals, the ability to obtain a large sample size, conduct invasive blood sampling with catheters and perform euthanasia to obtain tissue samples also make animal models an attractive alternative to clinical research [88, 89].

1.2.ii Justification for the use of a piglet model over a rodent model of gastrointestinal disease and nutrition

Rodent and piglet models are widely used to study human neonatal gastrointestinal disease and nutrition [88, 90, 91]. Although other animal models have been used, such as nonhuman primates, dogs, and rabbits, ethical considerations, lifespan, and/or expensive housing restrict their use [88]. Rodent models are appealing for research on human disease, since genomic sequences and biochemical pathways are similar between rodents and humans. Moreover, the low cost of maintaining rodents during experiments and the ability to genetically modify the animals facilitates research on mechanistic pathways [88]. However, humans and rodents have several physiological, anatomical, and developmental differences, and these limitations may preclude successful translation of gastrointestinal research from rodents to humans. For instance, humans and rodents have a significantly different lifespan and body size, different food intake (humans are “meal eaters” while rodents are “nibblers” [92]), different energy expenditure, and enteric flora and intestinal morphology, and rodents practice coprophagy while humans do not [89]. Developmentally, the gestational length of rodents (19-22 days) is significantly shorter than that of humans (37 weeks). Premature birth in humans occurs at 70-90% of gestation (28-36 weeks) and at 94-97% gestation in rats (21 days), and human neonates are viable after 70% gestation, while rats are viable
after 90% gestation [90]. The gastrointestinal tract of humans is significantly mature at birth, and term human neonates are able to digest non-milk carbohydrates and proteins as well as nutrients from milk. Rodents have very immature gastrointestinal tracts at birth, and they are unable to tolerate adult diets until late postnatal life [90]. Gradual gastrointestinal maturation takes place in rodents after birth during lactation (0-21 days after birth), while rapid gastrointestinal development takes place during the weaning period (transition from milk to solid foods) [90]. This pattern of gastrointestinal maturation in rodents is significantly different from what occurs in human neonates.

Compared with rodents, neonatal piglets have similar gastrointestinal anatomy, physiology, and metabolism to human neonates [88-90, 93, 94]. The gestational length of the piglet is 115 days, and although the gastrointestinal tract of a term piglet is less mature than that of a term human neonate, it is more mature at birth than that of a rodent [90]. Gastrointestinal maturity during lactation and weaning periods in neonatal piglets follows a similar pattern of development to human neonates [88, 90]. Anatomically, pigs and humans have similar gastrointestinal tracts with a few minor differences: pigs possess a gastric diverticulum; pigs have a spiral-shaped ascending colon while humans have a square-shaped colon; pigs do not have a vermiform appendix; and the pancreatic and bile ducts of the pig enter at distinct points into the duodenum [89, 94]. Intestinal transit time in pigs and humans is also similar [89]. Neonatal piglets are extensively used in the study of human nutrition and disease, as there are several advantages to using this animal model: a relatively short reproductive cycle; a large litter size; rapid growth rates; an omnivorous diet; an ability to be weaned at birth and bottle-fed; and an ability to be maintained in metabolic cages [94].
Human neonates are born with an average birth weight of 3 kg, while piglets are born with a weight of approximately 2 kg. The small and large intestinal lengths of human and piglet neonates are similar at birth, approximately 200 cm in vivo (small intestine) and 50 cm in vivo (large intestine) in humans, and 215-380 cm (small intestine) and 75-80 cm (large intestine) in piglets [94]. Despite a lower birth weight and a relatively immature gastrointestinal system at birth, protein deposition is rapid in piglets during the neonatal period and the piglet grows approximately 186 g/day or 95.4 g/kg/day. The human neonate on the other hand grows 20-30 g/day or 4.7 g/kg/day [93, 94]. The piglet is able to double its birth weight after 7 days and double its small intestinal length after ten days, while the human neonate doubles its birth weight after 6 months and double its small intestinal length after 2-3 years [93]. On account of the similarities in physiology and metabolism between neonatal piglets and humans, as well as the rapid postnatal growth of piglets, neonatal piglet models can be regarded as an accelerated model of human neonatal growth and development [88].

1.2.iii Neonatal piglet model for the study of short bowel syndrome

Neonatal piglet models have been widely used for studying enteral and parenteral nutrition [91]. Similarly, piglet models have been used to study SBS in humans. Piglet models of SBS have been validated against the clinical syndrome in humans by demonstrating similar clinical signs and symptoms (malnutrition, weight loss, diarrhea), morphological changes (increased small intestinal length and diameter), histological changes (increased crypt depth and villus height; increased enterocyte proliferation and decreased apoptosis), and functional changes (increased absorption and transport of
nutrients) to what is observed in humans [95-100]. These studies are discussed in detail in a subsequent section.

Most of the early studies using juvenile or neonatal piglet models of SBS involved mid-intestinal resections. As mentioned previously, the most common etiologies of SBS in neonates, such as necrotizing enterocolitis and intestinal atresias, involve the ileum and colon [1]; hence, mid-intestinal resection models do not adequately represent what is observed in the clinical setting. Furthermore, since the presence of remnant ileum augments intestinal adaptation [10, 18, 27], mid-intestinal resection models have better outcomes than distal-intestinal resection models that lack remnant ileum. Recently, a distal-intestinal resection neonatal piglet model of SBS has been developed and validated [40]. In this study, neonatal piglets that underwent a 75% distal-intestinal resection with a jejuno-colic anastomosis were compared to piglets that underwent a 75% mid-intestinal resection with a jejuno-ileal anastomosis. Similar to what is observed clinically, mid-intestinal resection animals demonstrated successful intestinal adaptation as compared to distal-intestinal resection animals. The latter were observed to have lower body weights at the end of trial, more days on parenteral nutrition, lower enteral tolerance, and higher fat malabsorption, as compared to mid-intestinal resection piglets. Animal models of SBS in which the ileum is absent represent the majority of clinical cases of neonatal SBS, and this is one of the surgical models used in this study.
1.2.iv Assessing the quality of animal models for research on human disease

Successful translation of research conducted on animals to humans is the ultimate goal of any animal model. However, only one third of animal research is translated at the level of randomized trials in humans, and only one-tenth of these trials lead to interventions that are approved for use in patients [101]. Failure to translate research from animal models to humans may occur if the animal model does not express the clinical syndrome associated with the disease that is investigated [102], or from methodological limitations of the study [103]. Methodological limitations may include insufficient statistical power of the animal study, methodological shortcomings, overly optimistic conclusions, inadequate internal validity (the differences observed between study groups are due to error rather than due to true differences in intervention), and inadequate external validity (the animal model does not appropriately represent the disease in humans) [103, 104]. In order to address these potential methodological shortcomings and improve the quality of animal research, van der Worp et al. have suggested that the following information be reported within the manuscript of an animal study: (1) sample size calculation; (2) inclusion and exclusion criteria; (3) treatment allocation; (4) blinding to treatment allocation; (5) flow of animals during the study, particularly excluded animals; (6) control of physiological variables; (7) control of study conduct; and (8) statistical methods [103]. In order to evaluate the quality of the neonatal piglet models of SBS used in this study, these factors will be reported and discussed in the Discussion section of this thesis.
1.3 **Glucagon-like peptide-2**

1.3.i **Glucagon-like peptide-2 synthesis and metabolism**

Glucagon-like peptide-2 (GLP-2) is a 33-amino acid peptide released from enteroendocrine L-cells following nutrient ingestion [105]. GLP-2 is a product of the proglucagon gene, and is derived by tissue-specific post-translational processing of proglucagon by prohormone convertase-1/3 in the intestinal L-cell [106]. The proglucagon gene is also expressed in pancreatic A cells, hypothalamus, and brain stem, and each tissue type generates a unique set of peptide fragments after post-translational cleavage of the proglucagon peptide [107]. In the intestine, GLP-2 is co-secreted in a 1:1 manner with glicentin/oxyntomodulin, glucagon-like peptide-1, and intervening peptide-1 [108].

GLP-2 is secreted in its active form (1-33), and is inactivated to truncated GLP-2 (3-33) after N-terminal cleavage by dipeptidylpeptidase IV (DPP-IV). In humans, GLP-2 is metabolized by the kidney; the half-life of GLP-2 (1-33) is 7 minutes [109] and the half-life of truncated GLP-2(3-33) is 27 minutes [110]. Active GLP-2 (1-33) accounts for approximately 15% of total serum GLP-2 in humans [111]. The short half-life of active GLP-2 has lead to the widespread use of (Gly²)GLP-2 (ALX-0600, Teduglutide) in clinical trials and animal studies [112]. (Gly²)GLP-2 is a synthetic analog of human GLP-2 that contains an N-terminal glycine² substitution for alanine², and this substitution prevents degradation by DPP-IV. The half-life of human (Gly²) GLP-2 is significantly higher than that of human GLP-2 (1-33), and ranges from 3.2-5.5 hours [113].

Porcine GLP-2 is a 35-amino acid peptide with 88% sequence homology to human GLP-2 [110]. Unlike human GLP-2, porcine GLP-2 has two additional amino acids
(serine and leucine) at the C-terminus (Figure 1.2) [114]. In a similar manner to human GLP-2, bioactive porcine GLP-2 (1-35) is truncated to the inactive form, porcine GLP-2 (3-35), after N-terminus cleavage by DPP-IV. In pigs, GLP-2 is also cleared by the kidney, and the half-lives of bioactive porcine GLP-2 (1-35) and inactive GLP-2 (3-35) are 8.5 minutes and 34.0 minutes, respectively [114]. Bioactive porcine GLP-2 (1-35) and human GLP-2 (1-33) have similar rates of metabolic clearance, while truncated porcine GLP-2 (3-35) has a longer half-life than truncated human GLP-2 (3-33).

<table>
<thead>
<tr>
<th>Human</th>
<th>HA DGS FSDEM NTILD NLAAR DFINW LIQTK ITD 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>HA DGS FSDEM NTVKD NLATR DFINW LLHTK ITDSL 35</td>
</tr>
</tbody>
</table>

Figure 1.2  GLP-2 peptide sequence homology in the human and pig [110, 114].

1.3.ii Biological actions of endogenous and exogenous glucagon-like peptide-2 in the normal gastrointestinal tract

The earliest evidence for the intestinotrophic actions of GLP-2 came from a case report of a patient who developed intestinal mucosal hyperplasia secondary to a renal glucagonoma [115]. Drucker et al. confirmed this finding by demonstrating that the injection of proglucagon-producing tumors into nude mice resulted in intestinal mucosal hyperplasia, and subsequently isolated GLP-2 as the key peptide responsible for these intestinotrophic effects [116]. This led to early studies investigating the actions of exogenous GLP-2 on intestinal mucosa. In normal rodents, exogenous administration of native rat GLP-2 has been shown to increase small and large intestinal weight, lengthen villi, deepen crypts, increase epithelial cell proliferation and reduce apoptosis [116-119]. Functionally, native GLP-2 has been shown to increase carbohydrate, protein, and lipid
absorption [120-122], and up regulate the expression of epithelial nutrient transporters and digestive enzymes [120, 123, 124]. Furthermore, native GLP-2 reduces gastrointestinal motility, increases intestinal blood flow, improves epithelial barrier capacity, and hence confers anti-inflammatory effects [107, 125].

1.3.iii Exogenous glucagon-like peptide-2 in the normal gastrointestinal tract of parenterally fed piglets

The intestinotrophic effects of GLP-2 have also been observed in piglets administered exogenous human GLP-2. The majority of these studies have been conducted in piglet models of parenteral nutrition. In TPN-fed piglets, human GLP-2 has been shown to increase intestinal weight, lengthen villi, deepen crypts, and decrease apoptosis [126, 127]. At higher doses of human GLP-2 (10 nmol·kg⁻¹·day⁻¹ continuous IV infusion), intestinal epithelial proliferation is also increased [127]. Human GLP-2 has also been shown to increase carbohydrate and protein absorption, and up regulate the expression of epithelial nutrient transporters and digestive enzymes [128-130]. In PN piglet models, human GLP-2 decreases gastrointestinal motility [131] and restores the intestinal blood flow that is otherwise reduced by TPN [131, 132].

1.3.iv Mechanisms of action of glucagon-like peptide-2 and its receptor

Understanding the mechanisms of action of GLP-2 has been challenging. This is in part due to the multiple mediators involved in the actions of GLP-2 and the complexity of GLP-2 receptor (GLP-2R) expression. The trophic effects of GLP-2 on intestinal epithelium are likely exerted indirectly via downstream growth factors, since the GLP-2R is not identified on intestinal epithelial cells [112]. The GLP-2R has been localized to enteric neurons, subepithelial myofibroblasts, and rare enteroendocrine cells, with the
highest abundance in the jejunum relative to the other parts of the gastrointestinal tract [133, 134].

Insulin-like growth factor-1 is secreted from intestinal subepithelial myofibroblasts, and consequently it has been implicated as one of the downstream mediators of GLP-2. Dube et al. demonstrated that the intestinotrophic effects of GLP-2 are diminished in IGF-1 knockout mice, when compared to wild-type littermates [135]. Recently, Lee et al. found that small bowel weight, crypt cell proliferation, jejunal villus height, crypt depth, and DNA content were significantly reduced in GLP-2R knockout mice administered GLP-2, as compared to wild-type littermates. Furthermore, administration of IGF-1 to GLP-2R knockout mice was associated with an increase in small bowel weight (which was not seen in knockout mice administered human GLP-2), as compared to saline controls. These findings support the role of IGF-1 as a downstream mediator of the actions of GLP-2 [134]. Other downstream mediators of GLP-2 activity that have been studied include keratinocyte growth factor (KGF), vasoactive intestinal peptide (VIP), ErbB ligands, and endothelial nitric oxide synthase (eNOS) [136].

In rats, GLP-2R mRNA expression in the jejunum and ileum is significantly higher in fetal and neonatal animals as compared to adults, while colonic GLP-2R mRNA expression is highest at birth and tapers off with age [137]. In fetal piglets, GLP-2R mRNA expression increases during the fetal period and peaks at birth. There is a rapid decline in GLP-2R mRNA expression in piglets after birth, followed by a rise in GLP-2R mRNA expression during the neonatal period [138].

Studies evaluating the effects of exogenous GLP-2 administration on GLP-2R expression are limited. In one study, Dube et al. found no differences in distal jejunal
GLP-2R mRNA expression between mice administered exogenous human (Gly\textsuperscript{2})GLP-2 or saline [135]. In another study, de Heuvel et al. demonstrated an increase GLP-2R mRNA expression in rodent colonic submucosal enteric neurons exposed to human GLP-2 [139].

1.3.v Endogenous GLP-2 secretion and the normal gastrointestinal tract

The most potent stimulus for GLP-2 production is ingestion of nutrients. Post-prandial GLP-2 secretion follows a biphasic profile, with the first peak occurring approximately 30 minutes after ingestion of nutrients, and the second peak occurring at 60-120 minutes [140, 141]. Since intestinal L cells are predominantly found in the distal intestine and colon [29, 30], and carbohydrates and proteins rarely transit to the distal intestine under normal physiologic conditions, direct effects of carbohydrates and proteins on the L cell and the release of GLP-2 are probably limited. On the other hand, ingestion of fats in humans has been shown to increase GLP-2 secretion [141], and the direct application of long-chain monounsaturated fatty acids (≥C16, such as olive oil) to fetal rat intestinal cultures in vitro stimulates L cell secretion [142]. Lipids are probably the most important stimulus for GLP-2 secretion, since the products of lipid digestion reach the distal intestine under normal physiologic conditions.

Following a meal, GLP-2 levels in adult humans increases by a factor of two to threefold from a fasting level of 15-20 pmol/L [143]. In premature neonates, Amin et al. found fasting GLP-2 levels to be three times higher than adult levels (61.0 ± 38.0 vs. 17.0 ± 4.8 pmol/L). One hour after the ingestion of a meal, GLP-2 levels rose to 191.0 ± 96.0 pmol/L in premature infants and 49.0 ± 26.0 pmol/L in adults [144]. From this study, it appears that both fasting and post-prandial GLP-2 levels are significantly higher in
premature neonates as compared to adults. Like humans, GLP-2 levels are higher in neonatal rats as compared with adult animals. In particular, Lovshin et al. found fasting plasma GLP-2 levels to be 8.6 times higher in neonatal rats as compared to adult rats [137].

Neonatal piglets have similar fasting and post-prandial GLP-2 profiles to adult humans. Burrin et al. found fasting GLP-2 levels in neonatal piglets to be $18 \pm 8$ pmol/L [145], and post-prandial levels to be in $75.4 \pm 7.0$ pmol/L in formula-fed piglets [127]. Furthermore, there are no differences in post-prandial GLP-2 levels between piglets fed bolus or continuous feeds [145]. In the developing piglet, Petersen et al. demonstrated that endogenous GLP-2 release correlates with enteral feeding. During the neonatal period, GLP-2 levels were found to peak at birth (when feeds are initiated); decline over the suckling period; and dramatically decline after weaning (weaning anorexia - when enteral intake decreases) [138]. Not surprisingly, the weaning period in piglets is associated with a reduction in intestinal villous height, crypt depth, digestive enzyme activity, and nutrient absorption [138].

1.3.vi Endogenous glucagon-like peptide-2 and the resected gastrointestinal tract

Proximal-intestinal resection (type 1 anatomical subtype of SBS; remnant ileum and colon present) augments intestinal adaptation in animal models of SBS. This is characterized by an increase in villous height, crypt depth, and enterocyte proliferation (structural hyperplasia), and an up-regulation of nutrient transporters and digestive enzymes (functional adaptation) [35-37, 146]. Not surprisingly, plasma GLP-2 levels are higher in animals after proximal-intestinal resection relative to surgical controls. In rodents that underwent 70-90% proximal-intestinal resections with a jejunoileal
anastomosis, plasma GLP-2 levels (measured during a fed-state) were found to be
significantly higher than in surgical controls [147-150]. Recently, Hua found higher
plasma GLP-2 levels in the fed-state of neonatal piglets that underwent a 75% proximal-
intestinal resection relative to control animals [151]. Perez et al. demonstrated that GLP-
2 is an endogenous mediator of intestinal adaptation in a proximal-intestinal resection
rodent model of SBS. Specifically, they found that the hyperplastic adaptive changes
(increased villus height, crypt depth, and decreased apoptosis) that occurred in rats that
underwent a 75% proximal-intestinal resection were diminished in rats that underwent
intestinal resection and received GLP-2 immunoneutralization with anti-GLP-2
antibodies [152].

The literature on plasma GLP-2 levels after distal-intestinal resection (type 2
anatomical subtype of SBS; no remnant ileum, but intact colon) is more conflicting. Liu
et al. [153] and Koopmann et al. [154] found no differences in plasma GLP-2 levels
between rodents that underwent a 60% distal-intestinal resection with a jejunocolic
anastomosis and surgical controls. Koopman et al. also demonstrated the absence of
adaptation in the remnant jejunum of rats that underwent distal-intestinal resection [154].
Similarly, Hua found no differences in plasma GLP-2 levels in the fed-state of neonatal
piglets that underwent a 75% distal-intestinal resection relative to control animals, and
adaptation of the remnant jejunum was also absent in the surgically resected animals
[151]. These findings suggest that the presence of distal small bowel and colon is
necessary for GLP-2 secretion, since intestinal L cells are predominantly found in the
distal intestine and colon [29, 30].
In contrast to these studies, Topstad et al. observed higher plasma GLP-2 levels in rodents that underwent a distal-intestinal resection with either a 10 or 20 cm jejunal remnant, as compared to surgical controls [155]. Similarly, Jeppesen et al. found SBS patients with a jejunoileal anastomosis (68 pmol/L) had fasting GLP-2 levels that were similar to patients who retained 10 cm of ileum and had a jejunoileal anastomosis (72 pmol/L). Furthermore, all SBS patients in this study had significantly higher fasting GLP-2 levels compared to healthy, sex and age matched controls (23 pmol/L) [156]. In another study by Jeppesen et al., fasting median GLP-2 levels were 32 and 5 pmol/L for two groups of patients with an end jejunostomy and less than 150 cm of small bowel who received different caloric meals. Post-prandial GLP-2 levels did not increase for SBS patients with an end jejunostomy, as they did for healthy sex and age matched controls [157]. Lastly, Sigalet et al. demonstrated that neonatal SBS patients with a jejunooileal anastomosis (jejunum, ileum, and colon in continuity) had the highest plasma GLP-2 levels (in the ongoing fed state), followed by patients with an end ileostomy (jejunum and ileum in continuity), and patients with an end jejunostomy had the lowest plasma GLP-2 levels. In addition to remnant anatomy, GLP-2 levels were also correlated with residual small intestinal length [158].
1.3.vii Exogenous glucagon-like peptide-2 in animal models of short bowel syndrome

Exogenous administration of human GLP-2 or (Gly²)GLP-2 in proximal-intestinal rodent models of SBS has been shown to augment structural and functional parameters of intestinal adaptation in remnant jejunum and ileum [150, 159-163]. However, in one study conducted on juvenile piglets, intestinal adaptation was absent in piglets that underwent proximal-intestinal resection and were administered human GLP-2 [164]. The results of these studies are summarized in Table 1.1. Similarly, exogenous administration of native rat or human GLP-2 in distal-intestinal rodent models of SBS has been shown to augment structural and functional parameters of intestinal adaptation in remnant jejunum [153, 165, 166], and these results are summarized in Table 1.2. Although the intestinal adaptive response is observed in proximal-intestinal resection rodent and piglet models of SBS regardless of exogenous GLP-2 administration, augmentation of intestinal adaptation is only observed in distal-intestinal resection rodent models of SBS in the presence of exogenous GLP-2.
Table 1.1  Structural and functional parameters of intestinal adaptation in proximal-intestinal resection animal models of SBS

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Rodent</td>
<td>Rodent</td>
<td>Rodent</td>
<td>Rodent</td>
<td>Rodent</td>
<td>Rodent</td>
<td>Piglet</td>
</tr>
<tr>
<td>Proximal-intestinal Resection</td>
<td>75%</td>
<td>70%</td>
<td>90%</td>
<td>80%</td>
<td>90%</td>
<td>90%</td>
<td>75%</td>
</tr>
<tr>
<td>GLP-2</td>
<td>human (Gly2)</td>
<td>human</td>
<td>human (Gly2)</td>
<td>human (Gly2)</td>
<td>human</td>
<td>human</td>
<td>human</td>
</tr>
<tr>
<td>GLP-2 Dose</td>
<td>0.1µg/g SC BID</td>
<td>100µg/kg/d IV continuous</td>
<td>? SC</td>
<td>0.1 mg/kg SC BID</td>
<td>10µg/kg/hr IV continuous</td>
<td>10µg/kg/hr IV continuous</td>
<td>800/400µg SC BID</td>
</tr>
<tr>
<td>Nutrition</td>
<td>Ad libitum chow</td>
<td>Ad libitum chow</td>
<td>Pair-fed chow</td>
<td>Pair-fed chow</td>
<td>TPN</td>
<td>TPN</td>
<td>Ad libitum</td>
</tr>
<tr>
<td>Small bowel weight</td>
<td>↑</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>↑</td>
<td>↑ at 1&lt;sup&gt;st&lt;/sup&gt; wk</td>
<td>ø at 2&lt;sup&gt;nd&lt;/sup&gt; wk</td>
</tr>
<tr>
<td>Small bowel length</td>
<td>ø</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>↑</td>
<td>↑ at 1&lt;sup&gt;st&lt;/sup&gt; wk</td>
<td>ø at 2&lt;sup&gt;nd&lt;/sup&gt; wk</td>
</tr>
<tr>
<td>Protein Content</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>ø</td>
<td>--</td>
<td>--</td>
<td>ø</td>
</tr>
<tr>
<td>DNA Content</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>ø</td>
<td>--</td>
<td>--</td>
<td>↑</td>
</tr>
<tr>
<td>Villus height</td>
<td>↑</td>
<td>ø</td>
<td>↑</td>
<td>↑</td>
<td>--</td>
<td>ø</td>
<td>↑</td>
</tr>
<tr>
<td>Crypt Depth</td>
<td>↑</td>
<td>ø</td>
<td>ø</td>
<td>↑</td>
<td>--</td>
<td>ø</td>
<td>ø</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Proliferation</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>SGLT1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>GLUT5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Sucrase/Maltase/Lactase activity</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>ø</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>GLP-2R mRNA</td>
<td>--</td>
<td>--</td>
<td>ø</td>
<td>↑</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Carbohydrate absorption</td>
<td>↑</td>
<td>ø</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>ø</td>
</tr>
</tbody>
</table>

↑/↓ : significant increase or decrease relative to treatment-control for same surgical anatomy
ø : no difference between GLP-2 treated and control animals for same surgical anatomy
Table 1.2  Structural and functional parameters of intestinal adaptation in distal-intestinal resection animal models of SBS

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Distal-intestinal Resection</th>
<th>GLP-2</th>
<th>GLP-2 Dose</th>
<th>Nutrition</th>
<th>Small bowel weight</th>
<th>Small bowel length</th>
<th>Jejunum</th>
<th>Protein Content</th>
<th>DNA Content</th>
<th>Villus height</th>
<th>Crypt Depth</th>
<th>Apoptosis</th>
<th>Proliferation</th>
<th>SGLT1</th>
<th>GLUT5</th>
<th>Sucrase activity</th>
<th>GLP-2R mRNA</th>
<th>Carbohydrate absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirotani et al.</td>
<td>Rodent</td>
<td>70 cm of distal ileum</td>
<td>rat</td>
<td>20 µg/d</td>
<td>Ad libitum chow</td>
<td>Ø</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Sigalet et al.</td>
<td>Rodent</td>
<td>80%</td>
<td>human</td>
<td>10 µg/kg/hr IV continuous</td>
<td>TPN</td>
<td>↑</td>
<td>--</td>
<td>--</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Liu et al. [153]</td>
<td>Rodent</td>
<td>60%</td>
<td>human</td>
<td>100 µg/kg/d IV continuous</td>
<td>TPN</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Liu et al. [153]</td>
<td>Rodent</td>
<td>60%</td>
<td>human</td>
<td>100 µg/kg/d IV continuous</td>
<td>PN+EN</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

↑/↓: significant increase or decrease relative to treatment-control for same surgical anatomy  
Ø: no difference between GLP-2 treated and control animals for same surgical anatomy
1.3.viii Glucagon-like peptide-2 therapy for patients with short bowel syndrome

Early clinical trials of human GLP-2 therapy (400 µg SC BID for 35 days) or teduglutide (0.03-0.15 mg/kg/day OD for 21 days) for adult patients with SBS demonstrated improvements in enteral energy absorption and fluid balances, decreased gastric emptying, decreased fecal output, and increased small intestinal villus height and crypt depth in patients treated with GLP-2 compared to placebo [167, 168]. In a recent randomized control trial, patients treated with teduglutide (0.05 mg/kg/day OD for 24 weeks) had decreased parenteral requirements, improved urine output, decreased oral fluid intake, increased small intestinal villus height and crypt depth, and increased colonic crypt depth, compared to patients who received placebo [44]. Even though clinical trials of GLP-2 therapy have been promising in adult patients with SBS, the positive effects on intestinal adaptation that are seen in adult patients with short bowel syndrome tend to diminish with cessation of GLP-2 therapy [168]. To date, there are no published clinical trials of GLP-2 therapy in pediatric SBS patients despite their greater growth potential than adult SBS patients. A Phase 1 clinical trial investigating the safety and dosing of GLP-2 in infants and children with intestinal failure is currently underway (NCT01573286), and the results of this study will help to direct future clinical trials of GLP-2 therapy in pediatric patients with SBS.
CHAPTER 2

HYPOTHESES AND RESEARCH AIMS
2 Hypotheses and research aims

Currently, there are no published clinical trials of GLP-2 therapy in pediatric patients with SBS. The growth potential of the gastrointestinal tract of human infants differs from that of human adults, as the former have greater potential for growth and adaptation [9]. Neonatal piglets have similar gastrointestinal anatomy, physiology, and metabolism to human neonates [88-90, 93, 94], and the developmental capacity of the intestine in neonatal piglets may more closely resemble that of human neonates than adult humans. The effect of human GLP-2 therapy in a proximal-intestinal resection juvenile piglet model of SBS has been previously investigated [164]; however, one limitation of this study is that a distal-intestinal resection model of SBS, rather than a proximal-intestinal resection model of SBS, is more representative of the clinical condition found in human neonates [1]. Furthermore, it has previously been shown that endogenous bioactive GLP-2 levels are reduced in a distal-intestinal resection neonatal piglet model of SBS compared with a proximal-intestinal resection neonatal piglet model of SBS, and that animals with a distal-intestinal resection have blunted clinical and morphological outcomes of intestinal adaptation than animals that have undergone a proximal-intestinal resection [151]. Based on these findings, the following hypotheses and aims were developed in this thesis:

_Hypothesis 1:_ Endogenous bioactive GLP-2 levels will be lower in a distal-intestinal resection neonatal piglet model of SBS that lacks remnant ileum (type 2 remnant anatomy) compared with a proximal-intestinal resection neonatal piglet model of SBS in which the ileum is present (type 1 remnant anatomy).
**Hypothesis 2**: Exogenous human GLP-2 administration will improve clinical, functional, morphological, and histological outcomes of intestinal adaptation in a distal-intestinal resection neonatal piglet model of SBS that lacks remnant ileum and represents the type 2 anatomical subtype of SBS.

**Aim 1**: To determine endogenous bioactive GLP-2 levels in a distal-intestinal resection neonatal piglet model of SBS (type 2 remnant anatomy) as well as a proximal-intestinal resection neonatal piglet model of SBS (type 1 remnant anatomy), and to compare these results to previously established findings.

**Aim 2**: To determine the effect of human GLP-2 therapy on clinical, functional, morphological, and histological outcomes of intestinal adaptation in a clinically relevant distal-intestinal resection neonatal piglet model of SBS, and to compare these outcomes with outcomes of intestinal adaptation in a proximal-intestinal resection neonatal piglet model of SBS that is representative of most animal models used in current research on intestinal adaptation.

**Aim 3**: To understand in detail the mechanisms that underlie the two hypotheses by an examination of (i) the relationship between endogenous bioactive GLP-2 levels and absolute small intestinal length, (ii) the relationship between endogenous bioactive GLP-2 levels and enteral nutrients, (iii) crypt cellular proliferation and villus apoptosis, (iv) GLP-2 receptor mRNA expression, (v) total endogenous PYY levels in a proximal- and distal-intestinal resection neonatal piglet model of SBS, (vi) the relationship between endogenous PYY levels and absolute small intestinal length, and (vii) the relationship between endogenous PYY levels and enteral nutrients.
CHAPTER 3

METHODS
3 Methods

3.1 Research ethics approval

All procedures in this study were approved by the University of Alberta Animal Care and Use Committee for Livestock (#125/02/11).

3.2 Sample size calculation

The sample size of this experiment was determined in accordance with the study by Sangild et al., in which small intestinal length was 8.5% longer in glucagon-like peptide-2 (GLP-2)-treated piglets (397±14 cm) compared with saline controls (366±16 cm) [128]. Based on these findings, in order to observe a difference of 20% (effect size, δ) in small intestinal length between GLP-2 treated and control animals with a two-tailed statistical significance of 0.05 (α), a power of 80% (1-β), and a standard deviation of 15 cm (as reported by Sangild et al. [128]), a sample size of 8 animals in each experimental group was required.

3.3 Animals and surgical procedures

3.3.i Induction and anesthesia

Fifty-one male Large/Landrace White Cross piglets (Hypor, Regina, SK, Canada) of age 2-5 days and weighing 1.8 – 2.6 kg were obtained from the University of Alberta Swine Research and Technology Centre. The piglets were weaned from the sow prior to surgery.

Piglets were administered pre-operative intramuscular ampicillin (20 mg/kg; Novopharm Ltd., Toronto, ON, Canada) and atropine (0.04mg/kg; Sandoz Canada Inc., Quebec, Canada). Anaesthesia was induced with butorphanol (0.015mg/kg; Temgesic; Wyeth Canada, Guelph, ON, Canada), and isoflurane (2-3%; Bensen Medical Industries
Inc., Markham, ON, Canada) by mask. The animals were then intubated and maintained on mechanical ventilation with isoflurane as the sole anaesthetic agent. Body temperature was monitored with a rectal thermometer and regulated with an electric heating pad to maintain a temperature of 38.0-39.5 °C.

3.3.ii Central venous catheter insertion

All piglets received external jugular central venous catheters, gastric feeding tubes, and laparotomies in accordance with the surgical procedures reported previously [40, 91]. A 5-French central venous catheter (Silastic laboratory tubing; 0.76mm internal diameter x 1.65 mm outer diameter; Dow Corning, Midland, MI, USA) was inserted into the left external jugular vein using a venous cut-down technique in order to administer parenteral nutrition and intravenous GLP-2 or vehicle therapy during the trial. The tip of the catheter was measured to the sternal notch, in order to ensure adequate internal placement. The catheter was tunneled dorsally under the skin and secured internally to the external jugular vein with a 3-0 silk suture (Ethicon Inc., Soerville, NJ, USA), and secured externally to the fascia under the skin with a silicone tack and a 3-0 Prolene suture (Ethicon Inc., Soerville, NJ, USA). After insertion of the jugular central venous catheter, piglets received IV butorphanol (0.005mg/kg), trimethoprim-sulfadoxine 40/200 (0.5 mL; Borgal; Intervet Canada Ltd., Whitby, ON, Canada), and ketamine hydrochloride (2mg/kg; Ketalean; Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada).
3.3.iii Laparotomy and intestinal resection

The skin of the abdomen was prepped with chlorhexidine (Solu-IV; Solumed, Laval, QU, Canada). A laparotomy was then performed through a midline incision and the intestine was eviscerated. Using a 60 cm premeasured silk suture, the length of the small intestine was measured along its antimesenteric boarder in situ from the ligament of Treitz to the ileocecal junction. In the surgical control group (sham), no intestinal resection was performed. In the jejunoileal group (JI), piglets underwent a 75% mid-intestinal resection with a jejunoileal anastomosis. In the jejunocolic group (JC), piglets underwent a 75% distal-intestinal resection with a jejunocolic anastomosis. In the JC animals, the cecum was detached from its peritoneal attachments, and resected 5 cm from the ileocecal junction prior to the anastomosis. Intestinal resections were performed using electracautery (Valleylab, Boulder, CO, USA) to divide the mesentery, and intestinal continuity was restored with a hand-sewn interrupted anastomosis using 4-0 PDS (Ethicon Inc., Soerville, NJ, USA). Mesenteric defects were closed with interrupted 4-0 PDS sutures. A schematic of the three surgical groups are summarized in Figure 3.1.
3.3.iv  **Gastrostomy tube insertion and abdominal closure**

A 10-French gastric feeding tube (Silastic laboratory tubing; 1.98 m I.D. x 3.18 mm O.D.; Dow Corning, Midland, MI, USA) was inserted using a Stamm gastrostomy to administer enteral feeds during the trial. The gastrostomy tube was tunneled dorsally under the skin and secured externally with a 3-0 Prolene suture. It was brought into the peritoneal cavity through the muscle of the abdominal wall, and a purse-string suture using 4-0 PDS was placed on the body of the stomach. A gastrostomy was created with
electrocautery, and 5 cm of the silastic tube was inserted into the stomach. The tube was secured to the interior abdominal wall with a 4-0 PDS stitch prior to abdominal closure.

The abdominal incision was closed in layers with 3-0 Vicryl (Ethicon Inc., Somerville, NJ) to close the abdominal wall musculature, and 5-0 Vicryl subcuticular stitch (Ethicon Inc., Somerville, NJ) to close the skin.

3.4 Post-surgical piglet care

Post-operatively, piglets were administered IV narcotic, buprenorphine hydrochloride (0.02 mg/kg; Buprinex; Rekitt and Colman Pharmaceutical, Richmond, VA, USA), followed by buprenorphine hydrochloride (0.005 mg/kg) every 8 hours for two consecutive days. For analgesic support, piglets also received oral NSAID, meloxicam (0.1mg/kg; Metacam; Boehringer Ingelheim, Burlington, ON, Canada) for 3 consecutive days. Routine IV antibiotics were administered on study days 0-3 and days 7-9. These included ampicillin sodium (10 mg/kg) and trimethoprim-sulfadoxine 40/200 (0.5 mL; Borgal; Intervet Canada Ltd., Whitby, ON, Canada). Chlordexidine acetate cream (1% w/w; Hibitane; Wyeth Animal Health, Wyeth Canada, Guelph, ON, Canada) was applied to all incisions twice daily. In cases of suspected sepsis, piglets were empirically treated with ampicillin sodium (10 mg/kg) and trimethoprim-sulfadoxine 40/200 (0.5 mL), if these antibiotics were not already being administered. After two days of therapy, if the piglets did not respond to ampicillin sodium or trimethoprim-sulfadoxine, the antibiotic regimen was broadened to include IV clindamycin (3mg/kg; Sandoz Canada Inc., Quebec, Canada) and enrofloxacin (Baytril; 5mg/kg; Bayer Inc., Animal Health, Toronto, ON, Canada). All antibiotics were maintained until either an improvement in the piglet’s clinical condition was observed or until the end of the 14-day
Aerobic and anaerobic blood cultures were obtained from the external jugular central venous catheter prior to starting antibiotics or broadening the antibiotic regimen.

Piglets were housed in individual Plexiglass cages to which they were secured by a tether-swivel system (Alice King Chatman Medical Arts; Los Angeles, CA, USA). Piglets were placed in cotton jackets and the swivel was attached to both the jacket and the superior segment of the cage. This allowed piglets to move freely, while protecting the external portions of the central venous and gastric catheters. Catheters were channeled through the internal lumen of the swivel and connected to a dual-channel infusion pump (IVAC Signature Gold Infusion Pump; ALARIS Medical Systems, Inc.; San Diego, CA, USA). The patency of the jugular venous catheter was evaluated twice daily by aspirating from the line to ensure withdrawal of blood or a vacuum seal, and then by flushing the line with 1.5 cc of heparinized saline. The temperature of the room was maintained at 25°C with the aid of heat lamp, and lighting was established with a 12-hour light/dark cycle. Plastic toys were provided to each piglet for entertainment.

### 3.5 Nutrition

Elemental parenteral nutrition (PN) commenced immediately after surgery. It was infused through the external jugular central venous catheter and delivered by an infusion pump. The amino acid content of the PN solution was similar to the one used by Wykes, et al. [91], and was based on human milk protein (Vaminolact; Fresenius Kabi, Bad Homburg, Germany). The targeted energy intake was 1100 kJ·kg⁻¹·d⁻¹, with amino acids providing 27% of energy, carbohydrate 37%, and fat 36%. Target nutrient intakes included 18.0 g amino acids·kg⁻¹·d⁻¹, 29.0 g glucose·kg⁻¹·d⁻¹, and 10.4 g fat·kg⁻¹·d⁻¹. These targets were estimated from daily nutritional requirements for sow-fed piglets.
requiring 1050 kJ·kg⁻¹·d⁻¹, and receiving 14.6 g·kg⁻¹·d⁻¹ of amino acids (25% of the total energy intake), 27.4 g·kg⁻¹·d⁻¹ of glucose (37.5% of the total energy intake), and 9.4 g·kg⁻¹·d⁻¹ of lipid (37.5% of the total energy intake)[91].

PN nutrition was prepared by dissolving crystalline amino acids (Table 3.1; Ajinomoto AminoScience, Raleigh, NC, USA) in sterile water (Baxter Corp, Toronto, ON, Canada) and under a nitrogen blanket. Glucose (Dextrose; 90 g/L Sigma-Aldrich, St. Louis, MO, USA) and minerals (Table 3.2; Sigma-Aldrich, St. Louis, MO, USA) including calcium, phosphate, potassium, manganese, magnesium, sodium, and zinc were added to the amino acids to prepare an initial mixture. This mixture was then filtered through a 0.22 µm filter (Millipore Corp., Billerica, MA, USA) into 1L sterile bags (Baxter Corp., Toronto, ON, Canada) using a pressurized pump (Easy-load Masterflex; Millipore Corp., Billerica, MA, USA). The final PN solution was prepared by adding iron dextran (Ferroforte; 1 mL/L; Vetoquinol, Cambridge, ON, Canada), cyanocobalamin (Vitamin B12; 0.2 mL/L; Sandoz Canada Inc., Quebec, Canada), multivitamins (Multi-12/K1 Pediatric; 3 mL/L; Sandoz Canada Inc., Quebec, Canada), more trace elements (Table 3.3; 3 mL/L; Sigma-Aldrich, St. Louis, MO, USA), lipid (Intralipid 20%; 145 mL/L; Baxter, Fresenius Kabi AB, Uppsala, Sweden), and ranitidine (Zantac; 25 mg/L; Sandoz Canada Inc., Quebec, Canada) or famotidine (Famotidine; 5 mg/L; Omega, Montreal, Quebec, Canada) prior to administration.
**Table 3.1** *Amino acid content for 1L PN Mixture*

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Amount (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>5.88</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>4.41</td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>3.35</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>5.81</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.44</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>1.73</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>2.54</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>5.77</td>
</tr>
<tr>
<td>L-Lysine-HCl</td>
<td>5.74</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>1.07</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>2.24</td>
</tr>
<tr>
<td>L-Proline</td>
<td>4.60</td>
</tr>
<tr>
<td>L-Serine</td>
<td>1.78</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.26</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>2.94</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>1.18</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>0.43</td>
</tr>
<tr>
<td>L-Valine</td>
<td>2.94</td>
</tr>
<tr>
<td>Glycyl-Tyrosine dihydrate</td>
<td>1.53</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**Table 3.2** *Mineral content for 1L PN Mixture*

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium D-gluconate monohydrate</td>
<td>6.36</td>
</tr>
<tr>
<td>Potassium acetate</td>
<td>1.46</td>
</tr>
<tr>
<td>Possassium phosphate dibasic trihydrate</td>
<td>1.57</td>
</tr>
<tr>
<td>Potassium phosphate monobasic</td>
<td>1.08</td>
</tr>
<tr>
<td>Magnesium sulfate heptahydrate</td>
<td>0.78</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.15</td>
</tr>
<tr>
<td>Zinc sulfate heptahydrate</td>
<td>0.09</td>
</tr>
</tbody>
</table>

**Table 3.3** *Trace mineral solution*

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium(III) chloride hexahydrate</td>
<td>0.05</td>
</tr>
<tr>
<td>Cupric sulfate pentahydrate</td>
<td>3.12</td>
</tr>
<tr>
<td>Manganese sulfate monohydrate</td>
<td>1.86</td>
</tr>
<tr>
<td>Selenium dioxide</td>
<td>0.12</td>
</tr>
<tr>
<td>Sodium iodide</td>
<td>0.02</td>
</tr>
<tr>
<td>Zinc sulfate heptahydrate</td>
<td>40.68</td>
</tr>
</tbody>
</table>
PN was infused through the jugular venous central catheter immediately after surgery, and was delivered at 50% of the targeted rate. The rate was increased to 75% of the total targeted rate 8 hours after surgery, and to the full rate (13.5 mL·kg⁻¹·hr⁻¹) 16 hours after surgery. On post-operative day 2, enteral nutrition (EN) was introduced via the gastric catheter. The EN solution had a similar amino acid, lipid, and mineral content to the PN solution; however, glucose was substituted with glucose polymer module (Polycose; Abbot Nutrition Canada, Saint-Laurent, Québec, Canada) in order to reduce the osmolarity of the solution. The EN solution was isocaloric and isonitrogenous to the PN solution. Enteral nutrition was started at 10% of the total nutritional fluid rate (1.3 mL·kg⁻¹·hr⁻¹), and was increased by 10% every 24 hours if the piglet was not vomiting, did not appear dehydrated, did not have profound diarrhea, and gained 50 g/day of weight in the first week and 100 g/day in the second week. These parameters for the expected weight gain of surgical short bowel piglets have been previously validated in this laboratory [40]. If stool output was formed or absent, enteral feeds were increased by 10% every 12 hours. With each increase in the rate of EN by 10%, the rate of PN was proportionally decreased by 10% in order to maintain a total nutritional intake of 324 mL·kg⁻¹·day⁻¹ (13.5 mL·kg⁻¹·hr⁻¹). Regardless of an increase in weight, the rate of EN was not advanced beyond 70 mL/hr in order to prevent regurgitation of enteral feeds.

3.6 Glucagon-like peptide-2 or saline infusion

A stock solution of GLP-2 [500 µg/mL] was prepared by dissolving solid GLP-2 (Human GLP-2 (1-33); CS9065; Lot I074 with 96.83% purity confirmed by HPLC; CS Bio Co., Menlo Park, Cam USA) in normal saline (0.9% sodium chlorine; Baxter Corp, Mississauga, ON, Canada). 0.1 M sodium hydroxide (Sodium Hydroxide; 0.1 M; PCCA,
London, ON, Canada) was added to the stock solution to achieve a final pH of 8.2-8.4 [169]. The pH of the solution was determined using a pH meter (ISO 9001 - Waterproof pHTester 10; Eutech Instruments, Oakton Instruments, Vernon Hills, IL, USA). An infusion solution of GLP-2 [4.14 µg/mL] was prepared by diluting the stock solution in normal saline. The final pH of the infusion solution was targeted between 8.2-8.4 with the addition of 0.1 M sodium hydroxide. In a similar manner to the PN and EN solutions, the GLP-2 infusion solution was filtered through a 0.22 µm filter into 500 mL sterile bags using a pressurized pump. Both stock and infusion solutions of GLP-2 were kept at -20°C for a maximum of 2 months after preparation. The volume of infusion GLP-2 required for daily administration was thawed the night before at ambient temperature.

On post-operative day 2, IV GLP-2 at a dose of 11 nmol·kg⁻¹·day⁻¹ was administered to all piglets randomized to the GLP-2 treatment group through the jugular venous catheter. This was achieved by delivering the infusion GLP-2 solution [4.14 µg/mL] continuously over 24 hours at a rate of 0.42 mL·kg⁻¹·hr⁻¹. Piglets randomized to the vehicle group received an equivalent volume of normal saline (0.9% sodium chlorine; Baxter Corp, Mississauga, ON, Canada) delivered at 0.42 mL·kg⁻¹·hr⁻¹ via jugular venous catheters. Both IV GLP-2 and vehicle solutions were delivered by syringe pumps (NE-300 Just Infusion Syringe Pump; New Era Pump Systems, Inc., Farmingdale, NY, USA). The dose and route of GLP-2 administered in this study was selected in accordance with the findings of Burrin et al. who demonstrated that IV GLP-2 administered at 10 nmol·kg⁻¹·day⁻¹ was associated with an increase in villus height, crypt depth, and intestinal epithelial cell proliferation, as well as a decrease in intestinal epithelial cell apoptosis. Lower doses of GLP-2 were found to have a positive effect on villus height, crypt depth,
and cellular proliferation, but no effect on epithelial cell apoptosis [127]. The dose of GLP-2 used in this study was increased by 10% (11nmol·kg\(^{-1}\)·day\(^{-1}\) vs. 10nmol·kg\(^{-1}\)·day\(^{-1}\)) to account for a potential 10% loss in the delivery of infusate from pump failures – a finding that has been previously observed under similar experimental conditions in this laboratory.

3.7 Daily piglet assessment

A physical examination was performed on the piglets daily and included a general assessment (whether the piglets were active or lethargic; loud or quiet; the extent of pain based on a standardized scale), an abdominal examination, and an evaluation of all incisions. Piglets were weighed daily, and both urine and feces were qualitatively and quantitatively assessed. Urine was examined for the presence or absence of blood, and feces were examined for consistency as well as the presence or absence of blood. Urine and fecal output was measured daily, and the combined value constituted the total daily output. Daily fluid balance was calculated by subtracting the total daily output from the total daily input, which was the sum of the PN, EN, and GLP-2 or normal saline infusions. Piglets with excessive gastrointestinal losses, dehydration, suspected sepsis, emesis, hematochezia or melena were managed according to pre-established protocols.

3.8 Clinical chemistry

Whole blood was obtained at baseline, when enteral nutrition constituted 50% of total nutrition, and prior to termination. Whole blood was collected in EDTA-coated tubes (K2 EDTA tube; BD Vacutainer; BD Canada, Mississauga, ON, Canada) to measure total hemoglobin, white blood cells, and platelets. Albumin, total bilirubin, bile acids, aspartate transaminase, alanine aminotransferase, alkaline phosphatase, and
gamma-glutamyl transpeptidase levels were measured from serum samples. Serum was obtained by collecting whole blood in clot-activator tubes (SST Silica Clot Activator tube, BD Vacutainer; BD Canada, Mississauga, ON, Canada), allowing it to sit for 30 minutes, centrifuging the sample at 2500 rounds per minute for ten minutes (RPM) (GP Centriuge, Beckman Coulter Canada, Inc., Mississauga, ON, Canada), and then transferring the serum supernatant to no-additive tubes (No additive, silicone-coated blood collection tube; Monoject; Krackeler Scientific, Inc., Albany, NY, USA). Measurements were obtained by automated procedures at a veterinary laboratory (IDEXX, Markham, ON, Canada).

3.9 Plasma glucagon-like peptide-2 collection and radioimmunoassay

Whole blood was obtained at baseline, when enteral nutrition constituted 50% of total nutrition, and prior to termination. Whole blood was collected in EDTA-coated tubes (K2 EDTA tube; BD Vacutainer; BD Canada, Mississauga, ON, Canada) containing 0.01 mmol aprotinin/L (Calbiochem, La Jolla, CA) and 0.1 mmol diprotin A/L (MP Biomedicals, Aurora, OH). Plasma was obtained by centrifuging the blood sample at 2500 RPM for ten minutes. The supernatant was removed, and several aliquots of plasma were stored at -80°C. Samples were sent to the Holst laboratory in Copenhagen, Denmark, where plasma bioactive GLP-2 was measured. In brief, plasma bioactive GLP-2 was determined by radioimmunoassay with the use of an antibody specific to the N-terminus of GLP-2 [131, 170].
3.10 Serum peptide YY collection and immunoabsorbance assay

Whole blood was obtained at baseline, when enteral nutrition constituted 50% of total nutrition, and prior to termination. Whole blood was collected in clot-activator tubes (SST Silica Clot Activator tube, BD Vacutainer; BD Canada, Mississauga, ON, Canada). Serum was obtained by allowing the blood sample to sit for 30 minutes, and then by centrifuging the sample at 2500 RPM for ten minutes. The supernatant was removed, and several aliquots of serum were stored at -80°C. Total serum peptide YY (PYY) was measured using a commercially available enzyme immuno-absorbance assay kit for PYY (S-1274; Peninsula Laboratories, LLC, San Carlos, CA, USA). The assay was performed according to the protocol supplied by the manufacturer in duplicate for each serum sample and standard. Standards were prepared from synthetic PYY (H-4505; Peninsula Laboratories, LLC, San Carlos, CA, USA) in concentrations of 0-10 ng/mL. Standards and serum samples were added to goat anti-rabbit PYY antibody-coated 96-well plates and incubated with rabbit anti-PYY antiserum for 1 hour at room temperature. A biotinylated tracer was added to each well and incubated overnight at 4°C. Following incubation and plate washing, streptavidin-conjugated horseradish peroxidase (SA-HRP) was added to form a SA-HRP-biotinylated antigen-antibody complex. Finally, 3,3’,5,5’-tetramethylbenzidine (TMB) was added to determine the enzyme activity of each sample or standard. The reactions were terminated by the addition of 2M hydrochloric acid. All plates were analyzed for the absorbance of light at 450 nm by a spectrophotometer (Multiskan Ascent Plate Reader; VWR International, Ltd., Mississauga, ON, Canada).
3.11 Fecal sampling and enteral fat absorption

To determine the amount of enteral fat absorbed by each piglet, fecal matter was collected for a 24- to 48-hour period starting on day 5 and day 12 of the trial. Feces were collected in drainable ostomy pouches secured by skin-barrier appliances (Two-Piece Pouch System; Hollister, Aurora, ON, Canada) that were placed over the perianal region. After 6-8 hours during each fecal collection period, feces were collected from the ostomy pouches and weighed. In order to determine the amount of enteral fat delivered to piglets, enteral nutrition bags were weighed at the start and end of each 6- to 8-hour period. If leakage of feces was suspected during the 6- to 8-hour collection period, these samples were discarded from the analysis. All fecal matter was freeze-dried for six days prior to performing the fat-content analysis. Each fecal collection was analyzed in duplicate with samples weighing 1-2g each. Over a 6-hour period, fat was extracted from each sample by petroleum ether distillation using the Goldfisch apparatus (method Aa 438, AOAC 2000), and the fat content of each sample was determined. Finally, fat absorption (g·kg⁻¹·day⁻¹) was calculated by subtracting the fat content of all the feces obtained over a collection period from the total amount of lipid delivered during the same collection period.

3.12 Terminal laparotomy and tissue specimens

At the end of the trial (day 14), piglets underwent a general anaesthetic and received a laparotomy. Intestinal adhesions were lysed and the intestine was measured in length from the ligament of Treitz to the terminal ileum in sham and JI surgical groups, and from the ligament of Treitz to the jejuno cola anastomosis in the JC group. Gross examination of the small intestine and colon was performed to identify any areas of
diseased bowel. In all piglets, a 2 cm specimen of intestinal tissue was obtained at 60 cm
distal to the ligament of Treitz (jejunum), 20 cm proximal to the ileocecal valve when
applicable (terminal ileum), and 5 cm distal to the ileocecal valve or jejunocolic
anastomosis (colon). A 2x1x2 cm segment of liver tissue also was obtained. In cases
where regions of bowel appeared diseased, additional specimens were obtained. All
tissue specimens were preserved in a 10% buffered formaldehyde solution (Histoprep;
Fisher Scientific, Ottawa ON) for histological analysis. The entire small intestine was
subsequently detached from the mesentery, cleared of intestinal contents, and weighed.
The liver and spiral colon were also removed and weighed.

3.13 Histology

Histological specimens were prepared and analyzed by a certified veterinary
pathologist at the University of Alberta, Dr. Patrick Nicholas Nation, who was blinded to
the treatment-arms of the study. In brief, 2-3 mm cross-sections of intestinal tissue were
obtained from the specimens preserved in formaldehyde. These were embedded in
paraffin and placed in cassettes according to standard techniques. Five-micrometer
sections were obtained from the fresh-frozen paraffin-embedded intestinal specimens,
and stained with hematoxylin and eosin (H&E) according to standard procedures. Ten
well-oriented villi and crypts were measured on 2-3 H&E stained-sections at 100X
magnification with the Nikon Eclipse 80i microscope. Villus height was determined by
measuring the length of the villus from the villar-crypt junction to the tip of the villus,
and crypt depth was measured from the base of the crypt to the villar-crypt junction.
3.14 Immunohistochemistry

Intestinal specimens were stained for Ki-67 (a marker of cellular proliferation [171]) and cleaved caspase-3 (a marker of cellular apoptosis [172]). Immunohistochemical staining was conducted at Prairie Diagnostic Services, Saskatoon, SK, Canada using a commercial staining platform (Benchmark Staining Platform, Ventana Medical Systems Inc., Tucson, AZ, USA) and a streptavidin–biotin detection system (BMK iVIEW DAB Paraffin detection kit, Ventana Medical Systems, Inc., Tucson, AZ, USA). From each fresh-frozen paraffin-embedded intestinal sample three 4µm serial sections were cut – one for each primary antibody and one primary antibody omission control. According to the protocol supplied by the manufacturer, heat-induced epitope retrieval consisted of applying a cell conditioner to sections and then heating to 95°C for 8 minutes, followed by 20 minutes at 100°C. The Ki-67 (Mouse monoclonal to Ki-67 Clone MIB-1 (M7240), Dako Canada Inc., Mississauga, ON, Canada) and Cleaved Caspase-3 antibodies (Rabbit polyclonal to Cleaved Caspase-3, Asp175 (9661), Cell Signaling Technology Inc., Danvers, MA, USA) were used at a dilution of 1:100. For each staining run, sections of normal intestine were stained to ensure consistency of positive staining. Slides were prepared according to standard techniques. All slides were scored by a certified veterinary pathologist at the University of Alberta, Dr. Patrick Nicholas Nation, who was blinded to the treatment-arms of the study.

3.15 Ki-67

Cellular proliferation was assessed using Ki-67 immuohistochemistry staining. For each intestinal specimen, 20 well-oriented crypts were identified from an average of 2-3 slides using the Nikon Eclipse 80i microscope. Along the right half of each crypt,
cell positions were identified according to the methods reported by Potten and Veradi et al. [173-175]. The cell located at the base of the crypt, the Paneth cell, was designated as cell-position 1. Cells on the right-column of each half-crypt were divided into eight zones (3 cells/zone) up to the twenty-fourth position. Ki-67 expression was quantified by determining the percentage of positively stained cells per half-crypt zone.

3.16 Cleaved Caspase-3

Cellular apoptosis was assessed using cleaved caspase-3 staining. For each intestinal specimen, well-oriented villi were identified from an average of 2-3 slides using the Nikon Eclipse 80i microscope. According to the technique reported by Shin et al., positively stained cells were identified and expressed as a percentage of the first 60 cells from the villus tip (30 on each side) for 20 well-oriented half-villi [176].

3.17 Quantitative real-time polymerase chain reaction (qRT-PCR): GLP-2 receptor mRNA quantification

As previously described [177], total mRNA was extracted from tissue samples of jejunum, ileum, and colon at baseline and termination using the TRIzol reagent (Invitrogen Life Technologies Inc., Burlington, ON, Canada) according to the manufacturer’s instructions. For each respective tissue sample, 1µg of mRNA was reverse transcribed with Superscript II enzyme (Invitrogen Life Technologies Inc., Burlington, ON, Canada) and random hexamer primers (Amersham, Arlington Heights, IL, USA) to synthesize cDNA. Two-step qRT-PCR analysis was performed using Express SYBR GreenER (Invitrogen Life Technologies Inc., Burlington, ON, Canada) and porcine-specific primers.
For each sample, DNA amplification was performed in duplicate with the 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) under the following conditions:

Step 1: 95°C for 20 seconds.

Step 2: 40 cycles of 95°C for 1 second and 60°C for 20 seconds.

Step 3 (dissociation): 95°C for 15 seconds, 60°C for 15 seconds, and 95°C for 15 seconds.

Data analysis was performed using the 7900HT Fast Real-Time PCR System software (SDS 2.3; Applied Biosystems, Foster City, CA, USA). Relative GLP-2R mRNA quantification was determined using the ΔΔC(t) method with ribosomal 18S as the internal control. The vehicle-treated group within each surgical group (Sham, JI, and JC) was used as a respective reference control, and GLP-2R mRNA expression in GLP-2 treated animals is expressed as a fold increase over vehicle-treated animals within the same surgical group. Primer sequences are as follows (University Core DNA Services, University of Calgary):

GLP-2R (133-bp amplicon):  forward 5’-GCC TTC ATA CTT ACC TTG TGT GAG- 3’ and reverse 5’-TTC TCG GAG CAT CGG AGT CAT C- 3’

18S (196-bp amplicon):  forward 5’-GGT GGT GCC CTT CCG TCA- 3’ and reverse 5’- CGA TGC GGC GGC GTT ATT- 3’
3.18 Statistical analysis

Experimental data are expressed as mean ± SE. Some data are expressed as counts. Data expressed as means were compared by 2-way ANOVA for a 2x2 factorial design of surgery and treatment factors. If the interaction term between the surgery and treatment factors was significant, a subgroup analysis was performed using the Student t test to compare differences between treatment groups within the same surgical group, or 1-way ANOVA with Tukey’s post hoc analysis to compare differences among the three surgical groups within the same treatment group. Proportions were compared with the Chi-square (and Fischer’s exact) test. A linear regression analysis was performed to assess the degree of correlation between two variables, and the coefficient of determination ($r^2$) for each analysis is reported. P values < 0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism 5.03 for Windows (GraphPad Software Inc., San Diego, CA, USA).
CHAPTER 4

RESULTS
4 Results

4.1 Animals

4.1.i Flow of Animals

Fifty-one male Large/Landrace White Cross piglets were randomized to three surgical groups (sham, JI, or JC) and two treatment groups (saline or GLP-2). All randomization was carried out prior to surgery. The flow of animals during the study is summarized in Figure 4.1. Twelve piglets were randomized to the sham group, and seven of these animals were further assigned to the saline arm and five animals to the GLP-2 arm. After initiation of therapy, two sham animals were excluded from the saline arm. One piglet was excluded due to a case of congenital urethral aplasia (euthanized on day 3), and the other piglet was excluded on account of severe internal hemorrhage secondary to crush-injuries from the sow (euthanized on day 5). No sham piglets were excluded from the GLP-2 treatment group after commencing therapy. Eighteen piglets were randomized to the JI group; 11 JI piglets were further assigned to the saline arm, and 7 piglets to the GLP-2 arm. Of the JI saline treated animals, 4 animals were excluded after commencing therapy. Two piglets developed an early post-operative bowel obstruction and were euthanized on day 7 and 9 of the trial. The gastric feeding tube in one piglet was displaced, and could not be reinstated (piglet euthanized on day 8), and central venous access was lost in one piglet that subsequently developed significant interstitial edema (piglet euthanized on day 13). One JI piglet in the GLP-2 treatment arm developed sepsis and was excluded from the final results of study, even though it was maintained to the end of the trial period. Twenty-one piglets were randomized to the JC surgical group, and three piglets were excluded prior to the initiation of treatment.
Two of these piglets incurred anaesthetic complications immediately after surgery and failed extubation (piglets euthanized on day 0), and one piglet had complete gastric necrosis and was euthanized on day 1. All of these piglets were euthanized prior to the delivery of vehicle or GLP-2 therapy. Eighteen JC piglets were then randomized to the treatment groups: 8 animals to the saline arm, and 10 animals to the GLP-2 arm. After the initiation of treatment, one JC piglet in the saline arm developed an anastomotic leak and was euthanized in day 3. Two piglets in the GLP-2 arm died unexpectedly prior to the trial end date. The cause of death in one animal was severe dehydration (piglet found dead on day 13), and the cause of death in the second animal was undetermined (piglet found dead on day 9), however was likely related to unexplained neurological abnormalities. One piglet in the saline arm and two piglets in the GLP-2 arm developed sepsis and were excluded from the final results of the study.

Figure 4.1. Flow of animals
4.1.ii Morbidity and Mortality

The number of cases of morbidity or mortality for each surgical and treatment group, which resulted from causes discussed in the previous section, are summarized in

Figure 4.2. The proportion of cases of morbidity and mortality for saline and GLP-2 treated animals within the same surgical groups were compared using the Chi-square (and Fischer’s exact) test, and no significant differences in proportions were found.

Figure 4.2. Number of cases with no complications and morbidity/mortality.
4.2 Baseline Piglet Characteristics

Piglet characteristics obtained at the start of the trial (surgery) are summarized in Figure 4.3; these include piglet age, weight, pre- and post-resection small intestinal length. The mean age of all piglets included in the trial was 3.7 ± 0.1 days, and the differences in age among surgical and treatment groups were not statistically different (Figure 4.3.a). The mean weight of all piglets prior to surgery was 2.24 ± 0.3 kg, and the differences in piglet weight among surgical and treatment groups were not statistically significant (Figure 4.3.b). Small intestinal length was measured prior to resection using a pre-measured silk suture. The mean small intestinal length for all animals was 662.9 ± 9.4 cm, and the differences in intestinal length among all the groups were not statistically different (Figure 4.3.c). Sham piglets did not undergo surgical resection; hence the post-resection lengths were only measured in JI and JC animals. The mean post-resection lengths after a 75% proximal or distal-intestinal resection in JI saline, JI GLP-2, JC saline, JC GLP-2 groups were 162.2 ± 4.4, 163.9 ± 4.0, 162.8 ± 6.0, and 170.2 ± 4.4 cm. The differences in post-resection lengths among these groups were not statistically different (Figure 4.3.d).
Figure 4.3  *Baseline piglet characteristics at surgery (day 0)*. Piglet age at surgery (a), Piglet weight at surgery (b), Small intestinal length prior to surgical resection (c), Small intestinal length after surgical resection (d). Comparison of means made by 2-way ANOVA shown in table.

4.3  **Trial Characteristics**

Trial characteristics, including percentage of PN, EN, and GLP-2 administered to piglets (calculated as percent of volume delivered relative to expected volume for a 8-24 hour period), trial end day, and number of PN and EN pump failures are summarized in Figure 4.4. The differences in percentage of PN administered to the various surgical and treatment groups were not statistically significant (*Figure 4.4.a*). Similarly, the differences in percentage of EN administered to the various surgical and treatment groups were not statistically significant (*Figure 4.4.b*). When comparing the percentage of GLP-2 delivered to piglets in the different surgical and treatment groups by 2-way ANOVA, the interaction term was found to be significant (*Figure 4.4.c*); hence, a subgroup analysis was performed between treatment groups within the same surgical
group using the Student t test. The percent volume of GLP-2 delivered to sham GLP-2 animals was less than the percent volume of saline administered to sham saline animals (89 ± 4 vs. 98 ± 1%, p < 0.05). The differences in GLP-2 and saline delivered to JI saline and JI GLP-2, and JC saline and JC GLP-2 animals were not statistically different. The number of days that piglets were kept on the trial did not differ among surgical and treatment groups (Figure 4.4.d).

The number of EN pump failures (from air or solid occlusions in the lines) did not differ among the different surgical and treatment groups (Figure 4.4.f). The number of PN pump failures was significantly higher in all saline treated groups compared to GLP-2 treated groups (Figure 4.4.e). Despite the higher number of pump failures in the saline treated groups, the percentage of PN administered to all groups regardless of treatment or surgical type was not statistically significant (Figure 4.4.a).
Figure 4.4  Trial Characteristics. (a) Percentage of PN delivery over trial, (b) Percentage of EN delivery over trial, (c) Percentage of GLP-2 delivery over trial, (d) Trial end day, (e) Number of PN pump failures, (f) Number of EN pump failures. Comparison of means made by 2-way ANOVA shown in table. * Indicates p <0.05 for subgroup comparison of means by the Student’s t test.

4.4  Bioactive plasma glucagon-like peptide-2 levels

Bioactive plasma GLP-2 levels at the time of surgery (day 0) did not differ among all the groups (Figure 4.5.a). When all animals achieved 50% nutrition by enteral feeds (50% EN), GLP-2 levels were higher in all groups receiving exogenous GLP-2 relative to saline treated animals for the same surgical group (Figure 4.5.b). At 50% EN, sham saline, sham GLP-2, JI saline, JI GLP-2, JC saline, JC GLP-2 piglets had plasma GLP-2
levels of 33.5 ± 9.1, 263.0 ± 85.7, 103.3 ± 9.9, 296.7 ± 37.5, 68.7 ± 11.1, and 325.8 ± 55.4 pmol/L, respectively. At termination, there were no significant differences in plasma GLP-2 levels between saline and GLP-2 treated animals for the same surgical group (Figure 4.5.c); however, there was a trend towards higher levels in groups receiving exogenous GLP-2. Bioactive GLP-2 levels at termination for sham saline, sham GLP-2, JI saline, JI GLP-2, JC saline, JC GLP-2 piglets were 52.6 ± 6.9, 299.28 ± 163.3, 161.0 ± 33.6, 355.8 ± 90.7, 108.2 ± 22.3, and 150.2 ± 20.3 pmol/L, respectively.

When a subgroup analysis was performed comparing endogenous bioactive GLP-2 levels at 50% EN among the different surgical groups for saline treated animals, JI saline animals had significantly higher levels compared to sham saline animals (103.3 ± 9.9 vs. 33.5 ± 9.1 pmol/L), while there was no difference in levels between JC saline and sham saline animals (68.7 ± 11.1 vs. 33.5 ± 9.1 pmol/L). Although JC saline animals trended towards lower GLP-2 levels at 50% EN than JI saline animals (68.7 ± 11.1 vs. 103.3 ± 9.9 pmol/L), this difference was not statistically significant (Figure 4.6.a). A similar trend to what was observed at 50% EN was also observed at termination, with higher endogenous bioactive plasma GLP-2 levels in the JI saline group compared to the sham saline group (161.0 1 ± 33.6 vs. 52.6 ± 6.9 pmol/L), and no differences between sham saline and JC saline animals (52.6 ± 6.9 vs. 108.2 ± 22.3 pmol/L). Although there was a trend towards lower GLP-2 levels in JC saline animals compared to JI saline animals (161.0 1 ± 33.6 vs. 108.2 ± 22.3 pmol/L), this was not statistically significant (Figure 4.6.c).

For sham, JI, and JC piglets that received exogenous GLP-2, there were no statistically significant differences in bioactive plasma GLP-2 levels at 50% EN (Figure
At termination, JC GLP-2 treated animals had significantly lower bioactive GLP-2 levels than JI GLP-2 animals (150.2 ± 20.3 vs. 355.8 ± 90.7 pmol/L), while there was no difference between JI GLP-2 and sham GLP-2 treated animals (355.8 ± 90.7 vs. 299.28 ± 163.3 pmol/L). Although JC saline animals trended towards lower GLP-2 levels at termination than sham GLP-2 animals (150.2 ± 20.3 vs. 299.28 ± 163.3 pmol/L), this difference was not statistically significant (Figure 4.6.d).

Plasma GLP-2 levels for sham saline treated animals at surgery (day 0), 50% EN, and termination (day 14) were 23.4 ± 4.0, 33.5 ± 9.1, and 52.6 ± 6.9 pmol/L, respectively. For the sham animals, GLP-2 levels at termination were significantly higher than at surgery. There was a trend towards increasing levels of plasma GLP-2 over the course of the trial; however, this was not statistically significant (Figure 4.7.a). For JI saline animals, plasma GLP-2 levels were higher at 50% EN (103.3 ± 9.9 pmol/L) and termination (161.0 ± 33.6 pmol/L) as compared to at the time of surgery (20.8 ± 2.3 pmol/L). There was no difference in plasma GLP-2 levels for JI saline animals at 50% EN and termination; however, there was a trend towards higher levels for the latter period (Figure 4.7.c). Plasma GLP-2 levels for JC saline treated animals at surgery, 50% EN, and termination were 30.7 ± 6.0, 68.7 ± 11.1, and 108.2 ± 22.3 pmol/L, respectively. For JC saline animals, GLP-2 levels at termination were significantly higher than at surgery. There was a trend towards increasing levels of plasma GLP-2 over the course of the trial; however, this was not statistically significant (Figure 4.7.e).

For sham GLP-2 treated animals, plasma GLP-2 levels at 50% EN (263.0 ± 85.7 pmol/L) and termination (299.3 ± 163.3 pmol/L) were higher than at the time of surgery (28.0 ± 4.2 pmol/L), while there was no significant difference between GLP-2 levels at
50% EN and termination (263.0 ± 85.7 pmol/L vs. 299.3 ± 163.3 pmol/L) (**Figure 4.7.b**). Plasma GLP-2 levels were higher at 50% EN (296.7 ± 37.5 pmol/L) and termination (355.8 ± 90.7 pmol/L) compared to at the time of surgery (18.6 ± 4.3 pmol/L) for JI GLP-2 treated animals, and there was no significant difference in GLP-2 levels at 50% EN and termination for these animals (**Figure 4.7.d**). For JC GLP-2 treated piglets, plasma GLP-2 levels at 50% EN (325.8 ± 55.4 pmol/L) and termination (150.2 ± 20.3 pmol/L) were significantly higher than at the time of surgery (25.2 ± 3.7 pmol/L); however, GLP-2 levels for JC GLP-2 animals were significantly lower at termination than at 50% EN (**Figure 4.7.f**).

![Plasma GLP-2 Concentration](image)

**Figure 4.5** Plasma GLP-2 concentration over the course of the trial. (a) Surgery (day 0), (b) When piglets achieved 50% calories as EN, (c) Termination (day 14). Comparison of means made by 2-way ANOVA shown in table. * Indicates p <0.05 for subgroup comparison of means by the Student’s t test.
Figure 4.6  Plasma GLP-2 concentrations for sham, JI, and JC surgical groups at selected points in time during the trial. (a) GLP-2 concentration for saline treated piglets at 50% EN, (b) GLP-2 concentration for GLP-2 treated piglets at 50% EN, (c) GLP-2 concentration for saline treated piglets at termination, (d) GLP-2 concentration for GLP-2 treated piglets at termination. * Indicates p <0.05 for comparison of means made by 1-way ANOVA.
Figure 4.7 Plasma GLP-2 concentrations at selected points in time during the trial (a) Sham saline treated piglets, (b) Sham GLP-2 treated piglets, (c) JI saline treated piglets, (d) JI GLP-2 treated piglets, (e) JC saline treated piglets, (f) JC GLP-2 treated piglets. * Indicates p <0.05 for comparison of means made by 1-way ANOVA.

4.5 Clinical and Functional Outcomes

4.5.i Exogenous glucagon-like peptide-2 improves outcomes relating to parenteral and enteral nutrition in piglets with a distal-intestinal resection

Clinical outcomes relating to PN and EN, including the number of days on PN, the number of days during which EN was the sole source of nutrition, the highest percentage of EN tolerated over the course of the trial, and the percentage of EN at
termination for the different surgical and treatment groups are summarized in Figure 4.8. Following comparisons by 2-way ANOVA, the interaction term between the surgery and treatment factors was significant for all the aforementioned outcomes; hence, a subgroup analysis was performed using the Student t test to compare outcomes between the saline and GLP-2 treated animals within same surgical group. For sham saline and sham GLP-2 animals, as well as JI saline and JI GLP-2 animals, there were no differences in the number days on PN, the number days on EN as the sole nutrition source, the highest percentage of EN tolerated over the course of the study period, and the percentage of EN at termination. Clinical outcomes were found to be more favourable in JC GLP-2 treated animals, as compared to JC saline piglets. JC GLP-2 treated piglets had fewer days on PN (10.0 ± 0.6 days vs. 13.8 ± 0.2), more days on EN alone (4.0 ± 0.6 vs. 0.2 ± 0.2 days), a higher percentage of EN achieved over the study period (100 ± 0 vs. 73 ± 7%), and a higher percentage of EN at termination (92 ± 5 vs. 52 ± 10%), as compared to saline controls.
Figure 4.8 Clinical outcomes relating to PN and EN. (a) Number of days on PN, (b) Number of days on EN as sole nutrition source, (c) Highest percentage of EN tolerated over course of trial period, (d) Percentage of EN at termination. Comparison of means made by 2-way ANOVA shown in table. * Indicates p <0.05 for subgroup comparison of means by the Student’s t test.

4.5.ii Exogenous glucagon-like peptide-2 improves fecal output but does not affect enteral fat absorption in piglets with a distal-intestinal resection

Clinical outcomes relating to fecal output, including the day on which the first bowel movement occurred and the number of days of diarrhea for each surgical and treatment group are summarized in Figure 4.9. Surgical group was a significant factor affecting the first bowel movement day, with sham piglets having a bowel movement on significantly later days during the trial period compared to JI and JC piglets (Figure 4.9.a). There was no effect of treatment on the first bowel movement day. The interaction between surgical and treatment factors was significant when comparing the number of days of diarrhea during the trial, hence a subgroup analysis was performed (Figure 4.9.b). The difference in the number of days of diarrhea between sham saline
and sham GLP-2 animals, and between JI saline and JI GLP-2 animals, was not significant. JC GLP-2 treated animals had fewer days of diarrhea than JC saline treated animals (8.0 ± 0.7 vs. 12.3 ± 0.4 days).

Figure 4.9  Clinical outcomes relating to fecal output. (a) Day of first bowel movement, (b) Number of days of diarrhea during the trial period. Comparison of means made by 2-way ANOVA shown in table. * Indicates p <0.05 for subgroup comparison of means by the Student’s t test.

Data on enteral fat absorption from days 12-14 for JI and JC piglets are summarized in Figure 4.10, and the change in enteral fat absorption from study days 5-7 to days 12-14 for JI and JC animals are summarized in Figure 4.11. No significant differences were observed in the absorption of enteral fat among the surgical and treatment groups. Enteral fat absorption for sham animals is not reported because nearly all of these animals did not produce feces during the fecal collection periods of the trial.
Figure 4.10  Enteral Fat Absorption for Days 12-14. (a) Expressed in g/kg/day, (b) Expressed in g/kg/cm/day, (c) Expressed in g/day. Comparison of means made by 2-way ANOVA shown in table.
Figure 4.11  Change in enteral fat absorption from days 5-7 and days-12-14.  (a) Expressed in g/kg/day, (b) Expressed in g/kg/cm/day, (c) Expressed in g/day. Comparison of means made by 2-way ANOVA shown in table.
4.5.iii  Exogenous glucagon-like peptide-2 does not affect number of septic episodes

The number of septic episodes, both presumed and culture positive, were measured during the trial. A septic episode was recorded as a presumed episode if piglets demonstrated clinical signs of systemic infection, including lethargy, dehydration, increased stool output, or decreased urine output. A sepsis protocol was followed, and all presumed septic episodes were treated with antibiotics. A presumed septic episode was documented as a positive case of sepsis if a specific pathogen was identified from aerobic or anaerobic blood cultures. Both sham saline and sham GLP-2 piglets did not incur any septic episodes. Presumed sepsis occurred in two JI saline animals (n=7), however no positive cases were identified. Two cases of presumed sepsis occurred in the JI GLP-2 animals (n=7), and one case was found to be culture positive with growth of *Enterococcus faecium*. Presumed sepsis occurred in four JC saline animals (n=7), and one of these cases was found to be culture positive with growth of *Enterococcus gallinarum*. Three cases of presumed sepsis occurred in the JC GLP-2 animals (n=8), and one was found to be culture positive with growth of *Enterococcus faecium*. The proportion of presumed, negative, and positive septic episodes for saline and GLP-2 treated animals within the same surgical groups were compared using the Chi-square (and Fischer’s exact) test. No significant differences in proportions were found (Figure 4.12).
Figure 4.12  *Number of positive (+), negative (-), and presumed (?) septic episodes.*

**4.5.iv  Clinical Chemistry**

Clinical chemistry, including plasma hemoglobin (Hb), white cell count (WCC), platelets (Plt), albumin, total bilirubin, bile acids, aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT) were obtained prior to surgery (day 0) and termination (day 14) for each piglet. These results are summarized in *Table 4.1*. Within the same surgical and treatment group, baseline values were compared to termination values using the Student t test. Similarly, during the same trial period (baseline or termination), saline treated animals were compared to GLP-2 treated animals within the same surgical group using the Student t test. At termination, sham saline animals had a significantly lower WCC relative to baseline; similarly, JI saline animals had a significantly lower WCC relative to baseline. All piglets had significantly higher albumin at termination relative to baseline. Total bilirubin was lower at termination than
at baseline for sham saline animals and JI GLP-2 piglets. At termination, ALP levels were lower for all animals compared to baseline. Sham saline, JI saline, and JC saline piglets had lower AST levels at termination relative to baseline. However, GGT levels were elevated in JI saline and JC GLP-2 animals at termination compared to baseline.

When comparing GLP-2 treated to saline treated animals within the same surgical group, JI GLP-2 piglets had lower total bilirubin at termination compared to JI saline piglets. Even though total bilirubin, AST, and GGT levels were higher at baseline in JC GLP-2 animals compared to JC saline piglets, there were no differences between treatment groups at termination.
Table 4.1 Clinical Chemistry prior to surgery and termination

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>GLP-2</th>
<th>JI</th>
<th>GLP-2</th>
<th>JC</th>
<th>GLP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline (n=5)</td>
<td>GLP (n=5)</td>
<td>Saline (n=7)</td>
<td>GLP-2 (n=6)</td>
<td>Saline (n=6)</td>
<td>GLP-2 (n=6)</td>
</tr>
<tr>
<td>Hb (g/L) [90-150 g/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>72.3±3.7</td>
<td>77.5±4.2</td>
<td>82.3±3.1</td>
<td>82.4±3.1</td>
<td>77.5±4.2</td>
<td>80.6±3.4</td>
</tr>
<tr>
<td>Termination</td>
<td>70.8±4.5</td>
<td>80.3±8.6</td>
<td>82.4±3.1</td>
<td>85.6±5.9</td>
<td>80.4±4.9</td>
<td></td>
</tr>
<tr>
<td>WCC (10^9/L) [11-21 x 10^9/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.1±1.4</td>
<td>11.0±1.2</td>
<td>11.4±2.3</td>
<td>11.7±1.3</td>
<td>12.3±1.5</td>
<td></td>
</tr>
<tr>
<td>Termination</td>
<td>6.0±1.4†</td>
<td>6.9±0.7†</td>
<td>9.0±1.0</td>
<td>12.0±2.6</td>
<td>9.2±4.2</td>
<td></td>
</tr>
<tr>
<td>Plt (10^9/L) [100-900 x 10^9/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>307.5±32.7</td>
<td>305.2±33.9</td>
<td>325.3±40.1</td>
<td>301.2±56.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Termination</td>
<td>358.8±138.7</td>
<td>385.4±111.5</td>
<td>449.4±69.8</td>
<td>273.4±91.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L) [9-32 g/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>11.8±0.6</td>
<td>12.8±1.3</td>
<td>12.3±0.9</td>
<td>13.8±0.6</td>
<td>13.8±0.6</td>
<td></td>
</tr>
<tr>
<td>Termination</td>
<td>20.0±0.9†</td>
<td>21.9±1.3†</td>
<td>22.7±1.4†</td>
<td>21.6±1.0†</td>
<td>21.4±0.8†</td>
<td></td>
</tr>
<tr>
<td>Total Billirubin (µmol/L) [0-6 µmol/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.8±0.3</td>
<td>3.2±0.4</td>
<td>3.0±0.3</td>
<td>3.0±0.0</td>
<td>3.8±0.2*</td>
<td></td>
</tr>
<tr>
<td>Termination</td>
<td>2.5±0.3†</td>
<td>3.4±0.4</td>
<td>2.1±0.1*</td>
<td>2.4±0.4</td>
<td>3.2±0.9</td>
<td></td>
</tr>
<tr>
<td>Bile Acids (µmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.0±0.9</td>
<td>9.1±1.0</td>
<td>7.6±0.9</td>
<td>7.8±0.3</td>
<td>7.4±0.9</td>
<td></td>
</tr>
<tr>
<td>Termination</td>
<td>6.3±1.2</td>
<td>8.5±1.4</td>
<td>12.6±3.4</td>
<td>6.4±2.1</td>
<td>7.4±1.8</td>
<td></td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>17.7±2.3</td>
<td>18.2±1.4</td>
<td>17.8±0.4</td>
<td>20.4±0.5*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Termination</td>
<td>9.8±0.6†</td>
<td>14.3±2.2†</td>
<td>11.7±1.7†</td>
<td>12.8±3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP (IU/L) [180-460 IU/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1273±149</td>
<td>1448±254</td>
<td>1705±230</td>
<td>1640±142</td>
<td>1754±224</td>
<td></td>
</tr>
<tr>
<td>Termination</td>
<td>618±85†</td>
<td>684±76†</td>
<td>709±45†</td>
<td>685.6±55†</td>
<td>607±88†</td>
<td></td>
</tr>
<tr>
<td>GGT (IU/L) [8-40 IU/L]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>30.0±6.1</td>
<td>26.3±4.2</td>
<td>30.0±3.4</td>
<td>23.4±3.3</td>
<td>32.2±1.9*</td>
<td></td>
</tr>
<tr>
<td>Termination</td>
<td>37.3±8.0</td>
<td>46.0±6.0†</td>
<td>37.0±5.5</td>
<td>42.9±8.2</td>
<td>63.6±7.3†</td>
<td></td>
</tr>
</tbody>
</table>

† significantly different result compared to baseline (p<0.05)
* significantly different result compared to saline treated animals within the same surgical group (p<0.05)
[ ] indicate the range of normal values
4.6 Morphological outcomes

4.6.i Exogenous glucagon-like peptide-2 does not affect piglet weight

The weight of each animal was measured daily, and the mean weights of animals for each surgical and treatment group from day 0 to day 14 of the trial are presented in Figure 4.13. There were no significant differences in daily weight between saline and GLP-2 treated animals within the same surgical group. Daily weight gain was the highest in sham animals, followed by JI, and JC animals (Figure 4.14.a). Furthermore, there were no significant differences in the weight gained from surgery (day 0) to termination (day 14) between saline and GLP-2 treated animals within the same surgical group. (Figure 4.14.b).
Figure 4.13 *Daily weight gain during trial.*

Figure 4.14 *Piglet weight at termination (day 14).*  (a) Absolute weight, (b) Change in piglet weight relative to initial weight at surgery (day 0). Comparison of means made by 2-way ANOVA shown in table.
4.6.ii Exogenous glucagon-like peptide-2 increases small intestinal length in piglets with a proximal and distal-intestinal resection

Small intestinal length was measured at surgery (day 0) and termination (day 14) for all animals. Absolute intestinal length at termination was significantly higher in JI GLP-2 piglets (280 ± 11 vs. 214 ± 6 cm) and JC GLP-2 piglets (204 ± 12 vs. 155 ± 8 cm) relative to the respective saline treated piglets. The change in small intestinal length from surgery to termination (expressed as a percent increase) was also higher for JI GLP-2 piglets (71 ± 8 vs. 32 ± 2%) and JC GLP-2 piglets (19 ± 4 vs. -5 ± 3 %) relative to the saline treated animals. These results are summarized in Figure 4.15.

Figure 4.15 Small intestinal length at termination (day 14). (a) Absolute length, (b) Percent change in length relative to length measured at surgery (day 0). Comparison of means made by 2-way ANOVA shown in table. * Indicates p <0.05 for subgroup comparison of means by the Student’s t test.

4.6.iii Exogenous glucagon-like peptide-2 increases colonic weight in piglets with distal-intestinal resection, but does not affect small intestinal or liver weight

Mean small intestinal, colonic, and liver weight relative to piglet body weight at termination are summarized in Figure 4.16. Surgery was a significant factor affecting small intestinal weight per piglet body weight, with sham and JI animals having higher values than JC animals. GLP-2 therapy had no effect on small intestinal weight per piglet body weight for all surgical groups (Figure 4.16.a). There were no significant
differences in liver weight per piglet body weight among all surgical and treatment groups (Figure 4.16.b). The interaction term between surgical and treatment factors was significant by a 2-way ANOVA comparison of colonic weight per piglet body weight; hence, a subgroup analysis comparing colonic weight per piglet body weight for saline treated and GLP-2 treated animals within the same surgical group was performed.

Colonic weight per piglet body weight was significantly higher in saline treated sham animals compared to GLP-2 treated sham piglets (12.0 ± 0.7 vs. 9.2 ± 0.7 g/kg), while GLP-2 treated JC animals had higher values relative to saline treated JC piglets (30.1 ± 1.8 vs. 20.3 ± 3.2 g/kg) (Figure 4.16.c).

Figure 4.16 Tissue weight per piglet body weight. (a) Small Intestinal weight per piglet body weight. (b) Liver weight per piglet body weight. (c) Colonic weight per piglet body weight. Comparison of means made by 2-way ANOVA shown in table. * Indicates p <0.05 for subgroup comparison of means by the Student's t test.
4.7  **Endogenous bioactive plasma glucagon-like peptide-2 levels do not correlate with small intestinal length and enteral nutrition feed levels at termination**

Linear regression analyses were performed to determine whether endogenous bioactive plasma GLP-2 for the different saline treated surgical groups correlated with small intestinal length and enteral feed levels at termination. The goodness of fit between endogenous bioactive plasma GLP-2 levels at termination and small intestinal length was weak for all saline treated animals, and the slope for the regression analysis was not significantly different from zero (*Figure 4.17*). Furthermore, when the data was analyzed for each saline treated surgical group, the goodness of fit between endogenous bioactive plasma GLP-2 levels at termination and small intestinal length was weak for sham saline, JI saline, and JC saline animals, and the slope for each regression analysis was not significantly different from zero (*Figure 4.18*).

Similarly, a linear relationship between plasma GLP-2 levels and enteral feed levels at termination was neither observed for all saline treated piglets (*Figure 4.19*), nor each specific saline treated surgical group (*Figure 4.20*).
Figure 4.17  Small intestinal length vs. endogenous GLP-2 levels for all saline treated piglets.
Figure 4.18  Small intestinal length vs. endogenous GLP-2 levels. (a) Sham, saline piglets, (b) JI, saline piglets, (c) JC, saline piglets.
Figure 4.19  Enteral feed levels vs. endogenous GLP-2 levels at termination for all saline treated piglets.
Figure 4.20  Enteral feed levels vs. endogenous GLP-2 levels at termination. (a) Sham saline treated piglets, (b) JI saline treated piglets, (c) JC GLP-2 treated piglets.
4.8 Histological outcomes

4.8.i Exogenous glucagon-like peptide-2 increases jejunal villus height and crypt depth in piglets with a proximal-intestinal resection and increases jejunal crypt depth in piglets with a distal-intestinal resection

Data on small intestinal mucosal morphometry are summarized in Figure 4.21. Figures 4.22 and 4.23 contain representative H&E-stained intestinal sections (100x magnification) from jejunal and ileal tissue for each surgical and treatment group.

Jejunal villus height was significantly higher in JI GLP-2 treated animals compared to saline treated JI piglets (1006 ± 92 vs. 741 ± 37 µm). There were no differences in jejunal villus height between sham saline and GLP-2 treated animals, and JC saline and JC GLP-2 treated piglets. Relative to saline treated piglets, jejunal crypts were deeper in GLP-2 treated JI animals (234 ± 9 vs. 177 ± 14 µm) and GLP-2 treated JC animals (248 ± 21 vs. 172 ± 12 µm). There were no differences in jejunal crypt depth between sham saline and sham GLP-2 treated animals (182 ± 9 vs. 197 ± 22 µm) (Figure 4.21.b).

In the ileum of sham and JI animals, there were no differences in villus height and crypt depth between saline and GLP-2 treated groups (Figures 4.21.c and 4.21.d).
Figure 4.21 *Small intestinal mucosal morphometry.* (a) Jejunal villus height, (b) Jejunal crypt depth, (c) Ileal villus height, (d) Ileal crypt depth. Comparison of means made by 2-way ANOVA shown in table. * Indicates p <0.05 for subgroup comparison of means by the Student’s t test.
Figure 4.22  H&E-stained intestinal sections (100x magnification) from jejunal tissue. (a) Sham saline piglets, (b) Sham GLP-2 piglets, (c) JI saline piglets, (d) JI GLP-2 piglets, (e) JC saline piglets, (f) JC GLP-2 piglets.
Figure 4.23  *H&E*-stained intestinal sections (100x magnification) from ileal tissue.  (a) Sham saline piglets, (b) Sham GLP-2 piglets, (c) JI saline piglets, (d) JI GLP-2 piglets.
4.8.ii Exogenous glucagon-like peptide-2 does not affect jejunal crypt cellular proliferation and villus apoptosis but decreases the relative fold increase in apoptosis for JI and JC groups

A positional analysis of half-crypt cellular proliferation was performed for jejunal mucosa with Ki67 immunohistochemistry; the results are summarized in Figure 4.24. There were no significant differences in jejunal Ki67 immunoreactivity in a given crypt zone for all surgical and treatment groups. Jejunal villus apoptosis was evaluated with cleaved caspase-3 immunohistochemistry, and quantified by counting the number of positively stained cells per 60 cells from the villus tip. There were no statistically significant differences in jejunal villus cleaved caspase-3 immunoreactivity for the different surgical and treatment groups, and these results are summarized in Figure 4.25. However, when jejunal villus cleaved caspase-3 immunoreactivity for GLP-2 treated piglets relative to saline treated control piglets in the same surgical group (fold of control) was evaluated, JI and JC animals had significantly lower fold of control cleaved caspase-3 immunoreactivity (1.2 ± 0.2 and 1.1 ± 0.1 fold of control, respectively) than the sham group (2.3 ± 0.6 fold of control) (Figure 4.26).
Figure 4.24 Ki67 immunoreactivity for jejunal half-crypt zones. (a) Crypt zone 1, (b) Crypt zone 2, (c) Crypt zone 3, (d) Crypt zone 4, (e) Crypt zone 5, (f) Crypt zone 6, (g) Crypt zone 7, (h) Crypt zone 8. Comparison of means made by 2-way ANOVA shown in table.
Figure 4.25  Jejunal villus cleaved-caspase 3 immunoreactivity. Comparison of means made by 2-way ANOVA shown in table.

Figure 4.26  Jejunal villus cleaved caspase-3 immunoreactivity for GLP-2 treated piglets relative to saline treated control piglets in the same surgical group. * Indicates p <0.05 for comparison of means made by 1-way ANOVA.
4.9 **Exogenous glucagon-like peptide-2 (GLP-2) increases GLP-2 receptor mRNA expression in jejunal, ileal, and colonic tissue**

Glucagon-like peptide-2 receptor mRNA expression was quantified in intestinal tissue using qRT-PCR with ribosomal 18S mRNA as an internal control. At surgery (day 0), no differences in GLP-2 receptor mRNA expression in jejunal, ileal, and colonic tissue were observed among all groups (*Figure 4.27*). At the end of the trial period (day 14), jejunal GLP-2R mRNA expression was significantly higher in JI GLP-2 treated animals compared to JI saline treated animals (2.5 ± 1.1 vs. 0.2 ± 0.1; *Figure 4.28.a*). There were no statistically significant differences in jejunal GLP-2R mRNA expression between GLP-2 and saline treated animals for the sham and JC groups. In the ileum, GLP-2R mRNA expression was significantly higher in sham GLP-2 (11.1 ± 5.7 vs 0.8 ± 0.5) and JI GLP-2 (2.4 ± 0.4 and 0.2 ± 0.1) treated animals compared to the respective saline treated animals (*Figure 4.28.c*). In the colon, JI GLP-2 treated piglets (7.6 ± 3.1 vs. 0.6 ± 0.1) and JC GLP-2 treated piglets (18.5 ± 7.9 and 1.2 ± 0.5) had significantly higher GLP-2R mRNA expression compared to the respective saline treated animals (*Figure 4.28.d*).
Figure 4.27  Relative GLP-2 Receptor mRNA expression in intestinal tissue at surgery (day 0). (a) Jejunum, (b) Ileum, (c) Colon. Comparison of means made by 2-way ANOVA shown in table or by the Student’s t test as indicated.

Figure 4.28 Relative GLP-2 Receptor mRNA expression in intestinal tissue at termination (day 14). (a) Jejunum, (b) Ileum, (c) Colon. Comparison of means made by 2-way ANOVA shown in table. * Indicates p < 0.05 for subgroup comparison of means by the Student’s t test.
4.10 Total endogenous serum peptide YY levels

Total serum PYY levels at the time of surgery (day 0) did not differ among all the surgical and treatment groups (Figure 4.29.a). Total serum PYY levels when animals achieved 50% nutrition by enteral feeds for sham saline, sham GLP-2, JI saline, JI GLP-2, JC saline, and JC GLP-2 groups were 2.3 ± 0.1, 2.8 ± 0.3, 2.0 ± 0.1, 2.3 ± 0.3, 2.4 ± 0.2, and 2.5 ± 0.2, respectively, and did not differ among all groups (Figure 4.29.b). At termination, total serum PYY levels were 2.6 ± 0.1, 3.1 ± 0.2, 2.2 ± 0.1, 2.5 ± 0.1, 2.3 ± 0.2, and 2.0 ± 0.4, respectively, and there were no statistically significant differences between saline and GLP-2 treated animals for the same surgical group (Figure 4.29.c).

When a subgroup analysis was performed comparing total serum PYY levels among the different surgical groups for saline treated animals, no differences were observed among sham saline, JI saline, and JC saline animals at 50% EN and at termination (Figure 4.30.a and 4.30.c). For piglets that received exogenous GLP-2, there were no significant differences in serum PYY levels for sham, JI, and JC piglets at 50% EN (Figure 4.30.b). At termination, sham GLP-2 animals (3.1 ± 0.2 ng/mL) had significantly higher serum PYY levels than JI GLP-2 (2.5 ± 0.1 ng/mL) and JC GLP-2 animals (2.0 ± 0.4 ng/mL), while there were no differences between JI GLP-2 and JC GLP-2 animals (Figure 4.30.d).

Total serum PYY levels at surgery, 50% EN, and termination for each surgical and treatment group are summarized in Figure 4.31. Total serum PYY levels for sham saline (Figure 4.31.a), sham GLP-2 (Figure 4.31.b), JI saline (Figure 4.31.c), JI GLP-2 (Figure 4.31.d), and JC saline (Figure 4.31.e) animals did not differ at surgery, 50% EN, and termination for each respective group. At surgery, 50% EN, and termination, PYY
levels for JC GLP-2 treated animals were 3.0 ± 0.2, 2.5 ± 0.2, and 2.0 ± 0.4 ng/mL, respectively. For JC GLP-2 treated animals, PYY levels at termination were significantly lower than at surgery (2.0 ± 0.4 vs. 3.0 ± 0.2 ng/mL), and there were no differences in PYY levels at surgery and 50% EN (3.0 ± 0.2 vs. 2.5 ± 0.2 ng/mL), or at 50% EN and termination (2.5 ± 0.2 vs. 2.0 ± 0.4 ng/mL) (Figure 4.31.f). Hence, there was a trend towards decreasing PYY levels over the trial period for JC GLP-2 treated animals; however, this trend was not statistically significant.

Figure 4.29 Serum PYY concentration over the course of the trial. (a) Surgery (day 0), (b) When piglets achieved 50% calories as EN, (c) Termination (day 14). Comparison of means made by 2-way ANOVA shown in table. * Indicates p <0.05 for subgroup comparison of means by the Student’s t test.
Figure 4.30  Serum PYY concentrations for sham, JI, and JC surgical groups at selected points in time during the trial  (a) PYY concentration for saline treated piglets at 50% EN, (b) PYY concentration for GLP-2 treated piglets at 50% EN, (c) PYY concentration for saline treated piglets at termination, (d) PYY concentration for GLP-2 treated piglets at termination. * Indicates p <0.05 for comparison of means made by 1-way ANOVA.
Figure 4.31 Serum PYY concentrations at selected points in time during the trial. (a) Sham saline treated piglets, (b) Sham GLP-2 treated piglets, (c) JI saline treated piglets, (d) JI GLP-2 treated piglets, (e) JC saline treated piglets, (f) JC GLP-2 treated piglets. * Indicates p <0.05 for comparison of means made by 1-way ANOVA.
4.11 Total endogenous serum PYY levels do not correlate with small intestinal length and enteral feed levels at termination

Linear regression analyses were performed to determine whether total endogenous serum PYY levels for the different surgical and treatment groups correlated with small intestinal length and enteral feed levels at termination. The goodness of fit between total endogenous PYY levels at termination and small intestinal length was weak for saline treated piglets and GLP-2 treated piglets, and the slope for each regression analysis was not significantly different from zero (Figure 4.32). Similarly, when the data was analyzed for specific surgical and treatment conditions, no linear relationship was identified between total endogenous PYY levels at termination and small intestinal length for each respective group (Figure 4.33).

The goodness of fit between total endogenous serum PYY levels and enteral feed levels at termination was weak for saline treated piglets and GLP-2 treated animals, and the slope for each regression analysis was not significantly different from zero (Figure 4.34). Similarly, when the data was analyzed for specific surgical and treatment conditions, no linear relationship was identified between total endogenous PYY levels and enteral feed levels at termination for each respective group (Figure 4.35).
Figure 4.32  Small intestinal length vs. endogenous PYY levels.  (a) Saline treated piglets, (b) GLP-2 treated piglets.
Figure 4.33 Small intestinal length vs. endogenous PYY levels. (a) Sham saline treated piglets, (b) Sham GLP-2 treated piglets, (c) JI saline treated piglets, (d) JI GLP-2 treated piglets, (e) JC saline treated piglets, (f) JC GLP-2 treated piglets.
Figure 4.34  *Enteral feed levels vs. endogenous PYY levels at termination.* (a) Saline treated piglets, (b) GLP-2 treated piglets.
Figure 4.35 Enteral feed levels vs. endogenous PYY levels at termination. (a) Sham, saline piglets, (b) Sham, GLP-2 piglets, (c) JI, saline piglets, (d) JI, GLP-2 piglets, (e) JC, saline piglets, (f) JC, GLP-2 piglets.
CHAPTER 5

DISCUSSION
5 Discussion

5.1 Quality of Animal Study

Prior to engaging in a general discussion of the results of this thesis, it is important to begin with an assessment of the quality of this study. In order to evaluate the quality of animal research and improve the successful translation of such research to humans, van der Worp et al. have suggested that eight particular aspects of a study should be reported in the manuscript: (1) sample size calculation; (2) inclusion and exclusion criteria; (3) treatment allocation; (4) blinding to treatment allocation; (5) flow of animals during the study, particularly excluded animals; (6) control of physiological variables; (7) control of study conduct; and (8) statistical methods [103]. To assess the quality of the neonatal piglet model of SBS used in this study for testing the therapeutic potential of GLP-2, each of these eight aspects will be discussed in detail.

5.1.i Sample size calculation

As reported in the methods section of this thesis, a 20% increase in small intestinal length (effect size, $\delta$) between GLP-2 treated and control animals was used as the primary outcome for determining sample size. Small intestinal length was selected as the primary outcome for the sample size calculation because small intestinal length strongly correlates with favourable outcomes in adult and pediatric patients with SBS [11-16]. With a two-tailed statistical significance of 0.05 ($\alpha$), a power of 80% (1-$\beta$), and a standard deviation of 15 cm (as reported by Sangild et al. [128]), a sample size of 8 animals in each experimental group was required. The original sample size in each group was calculated with the expectation of observing a 20% difference in small intestinal length between JC GLP-2 treated and JC saline animals; however, some animals were
excluded from the results of the study (discussed in detail below) and the final number of animals was less than originally planned. With a final sample size of 6 animals in each of the JC GLP-2 and saline groups, a 24% difference in mean intestinal length between the two groups was observed. Hence, a sample size of 6 was adequate to observe the desired effect on small intestinal length.

With regard to other secondary outcomes, there were many trends observed in this thesis that did not reach statistical significance; hence, in future work, it would be worthwhile to determine sample size based on more than one primary outcome, so that a larger number of animals in each group may produce results with statistically significant differences.

5.1.ii Inclusion and exclusion criteria

Male Large/Landrace White Cross piglets between 2-5 days of age, and weighing 1.8-2.6 kg were used in this study. Piglets of this age and size have similar neonatal birth parameters to human infants, including birth weight, small intestinal length, and large intestinal length [94], and therefore piglets outside of these age and weight ranges were excluded from the study. There were no statistically significant differences in piglet age, weight, or pre-resection small intestinal length among piglets assigned to the different surgical and treatment groups.

5.1.iii Treatment allocation

All animals were randomized to surgical and treatment groups using a completely randomized design. Surgical and treatment levels included sham saline, sham GLP-2, JI saline, JI GLP-2, JC saline, and JC GLP-2. Prior to the start of the study, complete
randomization of animals to each level was determined using the card-shuffling technique.

5.1.iv Blinding to treatment allocation

Blinding to treatment allocation was achieved for all histological analyses, as the certified veterinary pathologist who performed the analyses was blinded to the treatment-arms of the study. All individuals who were involved in surgical procedures, daily animal care, delivery of nutrition and treatment or vehicle, whole blood and tissue collection, and statistical analyses were not blinded to treatment allocation. A lack of blinding to treatment could have biased some of the results of this study, particularly with regard to clinical outcomes. The decision to advance EN and wean PN was based on several objective variables, including expected daily weight gain, stool and urine output, and the absence of emesis; however, enteral nutrition was only advanced if these criteria were met and if the animal also appeared well. The subjective assessment of animal wellness was based on an assessment of piglet activity level and hydration status. Hence, it is possible that when a decision to advance enteral feeds was contingent on a subjective assessment of the animal’s activity level and hydration status, the decision to advance enteral feeds in GLP-2 treated animals could have been favoured, while the decision to hold enteral feeds in saline treated animals could have been favoured.

5.1.v Flow of animals

Fifty-one piglets were used in this study. Of these animals, 16 were excluded from the study. Three animals were excluded prior to treatment allocation on the basis of anaesthetic complications (n=2), and unexplained complete gastric necrosis (n=1). After treatment allocation, 13 animals were excluded for reasons including sepsis (n=4), small
bowel obstruction (n=2), anastomotic leak (n=1), severe dehydration (n=1), loss of
gastric feeding tube (n=1), loss of central venous access (n=1), congenital urethral aplasia
(n=1), traumatic injuries from sow-crushing (n=1), and unexplained neurological
abnormalities (n=1). Even though a small number of animals died from the
aforementioned causes, there were no differences in the number of cases of morbidity or
mortality among all surgical and treatment groups. Nevertheless, several measures could
be undertaken in the future to prevent mortality from these causes.

Complications related to surgery were the most common cause for the exclusion
of animals from this study. Bowel obstructions, anastomotic leaks, displaced feeding
tubes, and the loss of central venous access are undesirable complications of surgery.
Optimal surgical techniques and animal care may minimize the occurrence of these
complications; however, even in the most skilled surgical hands, these complications may
occur.

Sepsis was the second most common cause for the exclusion of animals from this
study. Bacterial overgrowth and translocation in the setting of intestinal resection, as
well as the presence of a central line are risk factors for sepsis [7]. Even though there
were no differences in the number of presumed, positive, and negative septic episodes
between all surgical and treatment groups, no septic episodes occurred in sham animals.
Both proximal and distal-intestinal resection groups incurred presumed and positive
septic episodes. This may suggest that sepsis in the neonatal piglet models of SBS more
likely results from causes related to intestinal surgery and the disease model, rather than
central venous catheter related infections. Diligent animal care, as well as maintaining
patency and cleanliness of central venous catheters may nevertheless prevent the
occurrence of septic episodes. In cases of positive septic episodes, early identification, appropriate administration of antibiotics, and maintenance of adequate fluid balance may reduce mortality.

Two animals died from anaesthetic complications; hence, it would be worthwhile to re-evaluate current anaesthetic devices and materials in order to ensure that optimal conditions are being used during surgery. One animal died from sow-crush injuries and one animal had congenital urethral aplasia. An examination of all internal organs (including retroperitoneal organs, such as the kidneys) to evaluate for potential sow-crush injuries, as well as an examination of the urethra to ensure the presence of its patency, may reduce mortality. These measures are easy to perform at the time of surgery, and hence should become a routine part of future studies.

Lastly, one animal in the JC group died from severe dehydration. Although diarrhea is observed in both JI and JC animals, the latter have longer periods of diarrhea and are more susceptible to dehydration from severe gastrointestinal losses. For future studies, in order to prevent severe fluid imbalances, advancement of enteral feeds in JC animals should be done more judiciously, and capping of enteral feeds at a certain percentage of the total daily caloric intake should be considered.

5.1.vi Control of physiological variable

Total daily nutrient intake was controlled during this study. The targeted energy intake was 1100 kJ·kg⁻¹·d⁻¹, with amino acids providing 27% of energy, carbohydrate 37%, and fat 36%. As PN was weaned, EN was proportionally advanced to maintain the same daily nutrient intake for all animals. PN, EN, and GLP-2 or saline therapy was delivered through infusion pumps. The expected volume of PN, EN, or assigned therapy
was calculated for each animal on a daily basis according to individual piglet weight and parameters defined for advancement of enteral nutrition. On occasion, infusion pump failures would occur as a result of air within the lines, kinking of the lines, or clogging of the lines with infusate, and the delivery of infusate would cease until the cause identified and corrected. As a consequence, the amount of PN, EN, GLP-2 or saline actually delivered to each animal was determined as percentage of the expected amount. There were no statistically significant differences in the percentage of PN and EN delivered to piglets assigned to the different surgical and treatment groups. The actual, relative to expected, amount of saline delivered to sham saline piglets was higher than the amount of GLP-2 delivered to sham GLP-2 animals, and there were no differences between the amount of GLP-2 and saline delivered to respective JI and JC animals.

5.1.vii Control of study conduct

No third party member controlled any part of this study.

5.1.viii Statistical methods

The statistical methods used in this study are discussed in detail in the Methods section.

5.2 Endogenous bioactive glucagon-like peptide-2 levels in proximal-intestinal and distal-intestinal resection neonatal piglet models of SBS

Previously, it has been shown that endogenous bioactive GLP-2 levels are reduced in a distal-intestinal resection neonatal piglet model of SBS that lacks remnant ileum, compared with a proximal-intestinal resection neonatal piglet model of SBS in which the ileum is present [151]. Hence, in this thesis, similar results with regard to endogenous GLP-2 levels were expected, and it was hypothesized that endogenous bioactive GLP-2 levels would be lower in a distal-intestinal resection neonatal piglet model of SBS that
lacks remnant ileum (type 2 remnant anatomy) compared with a proximal-intestinal resection neonatal piglet model of SBS in which the ileum is present (type 1 remnant anatomy) (Hypothesis 1).

Using the same neonatal piglet models of SBS used in this thesis, Hua found endogenous bioactive GLP-2 levels in JI piglets to be significantly higher at the end of a 14-day trial period than in JC piglets and sham piglets, while no differences in plasma levels were observed between the latter two groups [151]. Although no significant differences in bioactive plasma GLP-2 levels between JC and JI saline treated animals at 50% enteral feeds or at termination were observed, there was a trend towards lower GLP-2 levels in JC saline piglets at both time points during the trial. Moreover, there were no differences in bioactive plasma GLP-2 levels between sham and JC saline treated animals at 50% enteral feeds and at termination, while JI saline treated animals had significantly higher bioactive GLP-2 levels than sham saline treated animals at both time points.

These findings may in part be explained by the fact that JI animals have both an ileal and colonic intestinal remnant; hence, both ileal and colonic L cells are contributing to endogenous GLP-2 secretion in JI animals. JC animals, on the other hand, only have a colonic remnant and colonic L cells. Therefore, it may be the case that either there are fewer total L cells in JC animals compared to JI animals, or that colonic L cells alone do not have the capacity to secrete GLP-2 in the same manner as the combination of ileal and colonic L cells. For future work, it would be worthwhile to characterize ileal and colonic L cell populations by immunohistochemistry in the different surgical groups.

Previously, several studies have demonstrated higher plasma GLP-2 levels in proximal-intestinal resection rodent models of SBS as compared to surgical controls
The literature on endogenous GLP-2 levels after distal-intestinal resection is more conflicting, with some studies demonstrating no differences in plasma GLP-2 levels between distal-intestinal resection rodent models of SBS and surgical controls [153, 154] (similar to the results in this thesis), and others demonstrating an increase [155].

Because intestinal resection and enteral nutrients have an effect on endogenous GLP-2 production, it is worth mentioning that at 50% enteral feeds the percentage of calories from enteral nutrients is the same for all animals. Therefore, when comparing endogenous GLP-2 levels among the different surgical groups at 50% enteral feeds, the confounding effect of enteral nutrients on endogenous GLP-2 production is eliminated. At termination, however, since enteral nutrient load is variable for each individual animal, enteral nutrients confound the effect of remnant intestinal anatomy on endogenous GLP-2 levels.

Furthermore, when endogenous bioactive plasma GLP-2 levels at different time points during the trial (surgery, 50% enteral feeds, and termination) were compared for each saline treated surgical group, endogenous GLP-2 levels were found to be higher at termination compared to at the time of surgery for all surgical groups. Since sham saline animals did not undergo intestinal surgery, and were receiving 100% nutrition by the enteral route at termination, the observed increase in endogenous GLP-2 levels in these animals was likely due to an increase in enteral nutrition. However, in JI and JC saline treated animals, enteral nutrients confound the effect of remnant intestinal anatomy on endogenous GLP-2 levels.
5.3 **Exogenous glucagon-like peptide-2 and intestinal adaptation in proximal-intestinal and distal-intestinal resection neonatal piglet models of SBS**

In addition to the finding that endogenous bioactive GLP-2 levels are reduced in a distal-intestinal resection neonatal piglet model of SBS compared with a proximal-intestinal resection neonatal piglet model of SBS, Hua also demonstrated that animals with a distal-intestinal resection have blunted clinical and morphological outcomes of intestinal adaptation than animals that have undergone a proximal-intestinal resection [151]. Hence, in this thesis, it was also hypothesized that exogenous administration of human GLP-2 would improve clinical, functional, morphological, and histological outcomes of intestinal adaptation in a distal-intestinal resection neonatal piglet model of SBS that lacks remnant ileum and represents the type 2 anatomical subtype of SBS (*Hypothesis 2*).

5.3.i **Clinical and functional outcomes of intestinal adaptation**

Clinical trials testing the efficacy of native human GLP-2 and (Gly³)GLP-2 therapy in adult patients with SBS include outcomes that are typically used by physicians to monitor the progression and treatment of patients with SBS. These outcomes include parenteral nutrient requirements (amount and volume), enteral nutrient intake, body weight changes, urine volume, oral fluid intake, fecal output, and overall fluid and electrolyte balances [44, 167, 168]. Although several studies investigating the effect of GLP-2 therapy in animal models of SBS are reported in the literature, with the exception of body weight changes, other clinically relevant outcomes of intestinal adaptation are lacking in these studies. This study is novel in that it is the first to report the effects of human GLP-2 therapy on clinically relevant outcomes of intestinal adaptation, including
parenteral nutrient requirements, enteral nutrient intake, body weight changes, and the number of days of diarrhea, in animal models of SBS.

The results of this study support the second hypothesis, as exogenous human GLP-2 administration was found to augment clinical outcomes of intestinal adaptation in a distal-intestinal resection neonatal piglet model of SBS. Specifically, JC GLP-2 treated piglets had fewer days on PN, more days on EN alone, a higher percentage EN tolerated, a higher percentage of enteral nutrition at the end of the trial period, and fewer days of diarrhea, as compared to JC saline treated piglets. JI GLP-2 treated animals tolerated a higher percentage of enteral feeds than JI saline treated piglets, however no differences in all other clinical outcomes were observed between these two groups. Furthermore, no differences in daily body weight or final body weight between saline and GLP-2 treated piglets for each respective surgical group were observed, suggesting an appropriate delivery of nutrients (either as PN or EN) to all animals.

Since JC GLP-2 treated piglets had fewer days of diarrhea and better enteral tolerance relative to JC saline treated animals, it was also expected that nutrient absorption and measures of functional adaptation would be improved in these animals. As an indicator of functional adaptation, enteral fat absorption studies were performed for a 24- to 48- hour period starting on day 5 and 12 of the trial. In each respective surgical group, no differences in enteral fat absorption between GLP-2 treated and saline treated animals were observed. Previous animal studies that have evaluated the effect of GLP-2 therapy on functional intestinal adaptation in the normal gastrointestinal tract have demonstrated a positive relationship between GLP-2 therapy and various measures of functional adaptation. In rodents with a normal gastrointestinal tract, native rat or human
GLP-2 has been shown to increase carbohydrate, protein, and lipid absorption [120-122], and up-regulate the expression of epithelial nutrient transporters and digestive enzymes [120, 123, 124]. Similarly, human GLP-2 has also been shown to increase carbohydrate and protein absorption, and up regulate the expression of epithelial nutrient transporters and digestive enzymes in piglets [128-130].

In rodents and piglet models of SBS, the effects of GLP-2 therapy on parameters of functional adaptation are not as clear. In a proximal-intestinal resection rodent model of SBS, Kaji et al. demonstrated an increase in ileal SGLT1 and GLUT5 expression in animals administered human GLP-2 as compared to non-treated controls [159], while in a proximal-intestinal resection juvenile piglet model of SBS, Pereria-Frantini et al. found a decrease in jejunal and ileal maltase activity with human GLP-2 administration [164]. In the latter study, no differences in jejunal and ileal sucrase and lactase activity were observed between GLP-2 treated and non-treated groups. Similarly, Sigalet et al. observed no differences in jejunal SGLT1 and GLUT5 expression between GLP-2 treated and non-treated animals in a distal-intestinal resection rodent model of SBS [166]. The effect of GLP-2 administration on enteral fat absorption in animal models of SBS has not been previously studied and, based on the current literature, it is difficult to draw conclusions about the effect of exogenous GLP-2 on carbohydrate and protein absorption in the setting of SBS. In future studies, it would be important to evaluate enteral carbohydrate and protein absorption, in addition to fat absorption. Particularly, it would be worthwhile to examine the effect of remnant intestinal anatomy, with or without GLP-2 therapy, on outcomes of functional intestinal adaptation that have been studied previously in other animal models of SBS, including SGLT1 expression; maltase,
sucrase, and lactase activity; and *in vivo* intestinal absorption studies with methylglucose or galactose.

The large variability in the enteral fat absorption data may also partly explain the absence of observed differences between GLP-2 and saline treated groups. This variability may have resulted from the small sample size of eligible animals for the enteral fat analysis, as only those animals for which adequate feces were collected at both collection periods during the trial were included in the final enteral fat analysis. For future studies, the methods of fecal collection must be improved to reduce this variability and increase sample size.

Sepsis from central line infections or bacterial overgrowth and translocation is a common complication in patients with SBS. As a clinical outcome, the number of culture positive, culture negative, and presumed septic episodes that occurred within each surgical and treatment arm was measured. No differences in the proportion of culture positive, culture negative, or presumed septic episodes were observed between GLP-2 and saline treated animals within each respective surgical group.

Clinical chemistry was also measured at the start and end of the trial period, and the following trends were observed: (1) An increase in serum albumin levels for all groups at the end of the trial period, compared to the start of the trial. This is indicative of an improvement in the overall nutritional status of all animals over the course of the study period. (2) A decrease in serum ALP levels for all groups at the end of the trial period, compared to the start of the trial. Neonatal ALP levels are typically higher at birth, and gradually decline with increasing age. The trend towards lower ALP levels in
all groups at the end of the trial period may have been due to the advancing age and maturation of the piglets over the course of the trial.

5.3.ii Morphological outcomes

In order to explain the observed differences in clinical outcomes between JC saline and JC GLP-2 treated animals, it was also expected that similar differences in morphological outcomes between the two groups would be observed. In patients with SBS, a strong predictor of clinical success and weaning from PN is remnant intestinal length [3, 11-16, 20]. Hence, it was expected that the groups of animals that had better clinical outcomes would also have longer small intestinal lengths.

JC GLP-2 treated animals had better clinical outcomes than JC saline treated piglets, and both absolute small intestinal length and the percent change in intestinal length were higher in the JC GLP-2 treated group, compared to the JC saline group. Interestingly, even though both absolute small intestinal length and the percent change in small intestinal length were higher for JI GLP-2 treated animals relative to JI saline treated piglets, no differences in clinical outcomes between these two groups were observed. The absence of better clinical outcomes in JI GLP-2 treated animals as compared to saline treated JI piglets, despite an increase in absolute and relative small intestinal length, may suggest that other methods of intestinal adaption (microscopic structural adaptation and functional adaptation) rather than macroscopic structural adaptation are occurring in piglets with remnant ileum – regardless of exogenous GLP-2 administration. The presence of remnant ileum may induce mechanisms of intestinal adaptation other than a macroscopic increase in intestinal length, such that both saline treated and GLP-2 treated JI animals fair equally well with regard to clinical outcomes.
Again, for future work, it would be worthwhile to examine the effect of remnant intestinal anatomy, with or without GLP-2 therapy, on outcomes of functional intestinal adaptation that have been studied previously in other animal models. Intestinal SGLT1 expression, maltase, sucrase, or lactase activity, and in vivo intestinal absorption studies with methylglucose or galactose could be performed to determine the effect of exogenous GLP-2 on intestinal absorption in neonatal piglet models of SBS; in vivo permeability studies with lactulose or mannitol could be performed to understand the effect on intestinal permeability; and intestinal mucosal eNOS expression could be determined to understand the effect on intestinal blood flow.

Furthermore, endogenous GLP-2 levels were found to be higher at both 50% enteral feeds and at termination in JI saline animals as compared to sham saline animals. This finding supports the idea that endogenous GLP-2 secretion in piglets with remnant ileum may be sufficient to improve clinical outcomes of intestinal adaptation. The increase in intestinal length of JI GLP-2 animals is impressive, but does not correlate with an improvement in clinical outcomes relative to JI saline treated animals. Finally, it must be considered that in contrast to patients with SBS, remnant intestinal length may not be a strong predictor of clinical success in neonatal piglet models of SBS, and that other forms of intestinal adaptation, such as microscopic and functional adaptation, may have a more important role.

One puzzling finding in this study was the absence of an observed difference in small intestinal weight at the end of the trial period between saline and GLP-2 treated JI and JC piglets, despite observed differences in intestinal length. Previous studies in rodent models of SBS that have demonstrated an increase in small intestinal length with
GLP-2 therapy have also demonstrated a corresponding increase in small intestinal weight. In a 90% proximal-intestinal resection rodent model of SBS, Martin et al. found an increase in small intestinal length of GLP-2 treated animals relative to non-treated controls (15.3±2.6 vs. 10±0.2 cm); similarly, GLP-2 treated rodents had heavier small intestines than non-treated controls (2.1±0.09 vs. 0.98 ±0.4 g) [160]. Kaji et al. also demonstrated an increase in small intestinal length in rodents that underwent a 90% proximal-intestinal resection with GLP-2 therapy delivered in the first week of a two-week study, and that these animals also had an increase in small intestinal weight relative to untreated animals. In the latter study, only GLP-2 therapy administered during the first week of the trial was associated with an increase in small intestinal length, while GLP-2 therapy administered in the second week of the study (no treatment in the first week) was not associated with an increase in small intestinal length [159]. Moreover, the animals that received GLP-2 therapy during the second week of the trial did not demonstrate an increase in small intestinal weight relative to non-treated animals. The results of the latter study may suggest that early administration of GLP-2 therapy is necessary to observe a positive effect on intestinal length.

It seems reasonable to observe an increase in intestinal weight with an increase in intestinal length, since an increase in tissue mass is necessary for an increase in length to occur. However, an increase in intestinal weight may occur in the absence of an increase in intestinal length, since the former can result from mucosal surface hyperplasia and/or hypertrophy. One possible explanation for the observation of longer small intestines with GLP-2 therapy in JI and JC animals, without an associated increase in small intestinal weight, might be due to an associated decrease in mucosal enteroocyte density along the
longitudinal intestinal axis. In order to prove this theory, mucosal density studies would need to be performed in future experiments. It is also possible that the increase in intestinal length observed in JI and JC GLP-2 treated animals was associated with atrophic changes in the submucosal and muscular layers of the small intestine. These layers of the small intestine were not examined in this study, but it would be worthwhile to do so in future experiments.

Another possible cause for the observed discrepancy in small intestinal length and weight for GLP-2 treated JI and JC animals may have been due to the methodology used for weighing small intestines at termination. Small intestines were removed from each animal by separating the serosal surface of the intestine from the mesentery with sharp dissection. Prior to determining small intestinal mass, intestinal contents were cleared by milking the intestine. In most other studies in which small intestinal mass was measured, the lumen of the intestinal specimen was cleared, flushed with saline, and then weighed [159-161, 166]. Although saline flushing facilitates removal of intestinal contents, this process may damage mucosal integrity, and diminish the quality of intestinal tissue specimens used for histology. Based on this, a decision was made not to flush intestines with saline in this study, and the possible presence of residual intestinal contents in the removed intestines may have contributed to the discrepancy between intestinal length and weight observed in this study.

5.3.iii Endogenous bioactive glucagon-like peptide-2 levels, small intestinal length, and enteral nutrients

As discussed previously, small intestinal resection and enteral nutrients are known to have an effect on endogenous GLP-2 production. In neonatal patients with SBS, Sigalet et al. have demonstrated a positive correlation between remnant small intestinal
length and endogenous bioactive GLP-2 levels, as well as a positive correlation between percent calories consumed as enteral nutrients and endogenous bioactive GLP-2 levels [158]. Hence, it was expected that similar results would be observed in this thesis. However, neither small intestinal length nor enteral nutrient levels at termination correlated with endogenous bioactive GLP-2 levels in sham, JI, and JC saline treated groups. The absence of a positive correlation between small intestinal length and endogenous GLP-2 levels at termination may have resulted from a confounding effect of enteral nutrient level (which varied for each individual animal) at this point during the trial. Similarly, the absence of a positive correlation between enteral nutrient levels and endogenous GLP-2 levels at termination may have resulted from a confounding effect of small intestinal length (which also varied for each individual animal) at this point during the trial.

5.3.iv Histological outcomes

In order to explain the observed differences in clinical outcomes between JC saline and JC GLP-2 treated animals, it was also expected that similar differences in microscopic intestinal architecture would be observed between the two groups. In the jejunum, crypts were found to be significantly deeper in JC and JI GLP-2 treated animals relative to the respective saline controls, and jejunal villi were found to be significantly longer for JI GLP-2 treated animals relative to JI saline treated piglets. Even though the results were not statistically significant, there was a trend towards longer jejunal villi for both JC and sham GLP-2 treated animals relative to the respective treatment controls. Since enterocyte proliferation originates within intestinal crypts and subsequently continues along the crypt-villus axis, an increase in jejunal villus height in JC GLP-2
treated animals may not have been observed, even though an increase in crypt depth was observed, if the study period concluded prior to the occurrence of villus hyperplasia. Using a distal-intestinal resection rodent models of SBS, Sigalet et al. and Liu et al. have demonstrated increases in both jejunal villus height and crypt depth with administration of GLP-2 therapy [153, 166]. In cases of massive intestinal resection, remnant ileum has the capacity to increase nutrient absorption and take-on the function of the jejunum, while the adaptation of the jejunum is not as pronounced [10, 18, 27]. Hence, evidence for the presence of microscopic intestinal hyperplasia and adaptation in the jejunum of surgically resected GLP-2 treated neonatal piglets relative to saline treated animals is an encouraging finding.

In the remnant ileum of JI animals, no differences in villus height and crypt depth between GLP-2 and saline treated animals were observed. In contrast to these findings, Pereria et al. found a decrease in ileal villus height and crypt depth between GLP-2 treated and non-treated juvenile piglets after proximal-intestinal resection [164]. Piglets used in the latter study were older than the animals used in this study (4 weeks vs. 2-5 days), and GLP-2 was administered twice daily via a subcutaneous route instead of as a continuous infusion. These methodological differences may account for the observed differences in ileal villus height and crypt depth between this study and the one conducted by Pereria et al. In proximal-intestinal resection rodent models of SBS, GLP-2 therapy is typically associated with an increase in jejunal and ileal villus height and crypt depth [150, 159-161, 163]. It is possible that microscopic ileal adaptation is more pronounced with exogenous GLP-2 administration in proximal-intestinal resection rodent models of SBS than in similar piglet models.
In this study, the differences observed in small intestinal length and jejunal villus architecture between JI GLP-2 and JI saline piglets may suggest that the anatomical region of the small intestine that was lengthened in the former group was the jejunum rather than the ileum, since no differences in ileal microscopic architecture were observed between the two JI groups. In this study, relative macroscopic growth for the different anatomical regions of the small intestine in JI animals was not determined; however, this kind of evaluation would be informative for future studies.

5.4 Mechanisms of intestinal adaptation

5.4.i Crypt cellular proliferation and villus apoptosis

In order to understand in detail the differences in intestinal villus length and crypt depth observed between saline and GLP-2 treated groups, intestinal cellular proliferation and apoptosis were evaluated. In groups with longer villi and/or deeper crypts, an associated increase in crypt cellular proliferation and/or a decrease in villus apoptosis was expected. Enterocyte cellular hyperplasia in the setting of exogenous GLP-2 administration has been extensively studied in rodents with intact intestinal anatomy, and both an increase in epithelial cell proliferation and a decrease in apoptosis is observed with GLP-2 administration [116-119]. In a proximal-intestinal resection model of SBS, Martin et al. demonstrated that GLP-2 therapy was associated with an increase in ileal cell proliferation with no change in ileal enterocyte apoptosis [160], and in a distal-intestinal resection model of SBS, Sigalet et al. found both an increase in jejunal cellular proliferation and apoptosis with GLP-2 administration [166]. Both of these studies demonstrated increases in intestinal villus height and crypt depth with GLP-2 therapy.
In TPN-fed neonatal piglets with intact intestinal anatomy, GLP-2 administration has been shown to decrease enterocyte apoptosis, and in one study, a higher dose of GLP-2 was associated with a decrease in intestinal epithelial apoptosis and an increase in cellular proliferation [126, 127]. These studies have also demonstrated an increase in intestinal villus height and crypt depth with exogenous GLP-2 administration.

In contrast to what is previously reported in the literature, in this study, jejunal villus height and/or crypt depth in GLP-2 treated JI and JC piglets relative to saline treated animals in the same surgical groups were increased; however, no differences in villus apoptosis or crypt cellular proliferation were observed. Pereira-Frantini et al. found both an increase in enterocyte proliferation and apoptosis, and a decrease in ileal villus height and crypt depth with GLP-2 administration in a proximal-intestinal resection juvenile piglet model of SBS [164]. In the latter study, the resulting decrease in ileal villus height and crypt depth with GLP-2 therapy may have resulted from a greater extent of enterocyte apoptosis relative to proliferation. In this study, cellular activity does not provide a sound explanation for the observed changes in microscopic intestinal architecture.

In rodents, Scaglia et al. have evaluated pancreatic β cell proliferation and apoptosis during the neonatal period [178]. During the first month following birth, Scaglia et al. found that β cell replication of pre-existing β cells declines, while the frequency of apoptotic β cells increases from days 1-13, peaks at days 13-17, and then gradually declines until day 31. Interestingly, despite an increase in the frequency of β cell apoptosis and a decrease in β cell replication, pancreatic β cell mass, overall pancreatic mass, and animal body weight increase during the first month of the neonatal
period, while β cell size is mostly unchanged. Although this study evaluated the proliferative and apoptotic activity of pancreatic β cells during the early neonatal period of rodents, the concepts derived from this study may shed light on what is observed in neonatal piglets undergoing intestinal adaptation and growth.

In a similar manner to rodent pancreatic β cells, the proliferative and apoptotic activity of piglet intestinal epithelial cells may change over the course of the neonatal period. If enterocyte proliferation in neonatal piglets declines over time, while apoptosis increases, the time at which intestinal tissue is sampled will impact the results of enterocyte proliferative and apoptotic frequency. In the study conducted by Burrin et al., GLP-2 administration was associated with an increase in intestinal epithelial cell proliferation and a decrease in apoptosis [127]. Piglets in this study were terminated after 7 days for collection of intestinal specimens, while piglets in this thesis were maintained for 14 days. If intestinal samples were obtained at an earlier time point during the trial period, different results with regard to crypt cellular apoptosis and proliferation may have been observed. In addition to the time at which intestinal tissue is obtained, there are several other factors specific to a neonatal piglet model of SBS that may confound the effect of treatment on enterocyte proliferation and apoptosis, including normal piglet development and growth during the neonatal period, surgery, remnant intestinal anatomy, and nutrition. Determining the contribution of each of these factors on intestinal cellular activity would be a valuable pursuit for future studies.

Finally, it is worth mentioning that even though no statistically significant differences in villus apoptosis for all surgical and treatment groups were observed, when cleaved caspase-3 immunoreactivity for GLP-2 treated piglets relative to saline treated
piglets in the same surgical group were evaluated, JI and JC treated animals had lower fold of control cleaved caspase-3 immunoreactivity than sham animals. That is, relative crypt cellular apoptosis in GLP-2 treated animals compared to saline treated animals within the same surgical group was lower in JI and JC piglets as compared to sham piglets. Despite the presence of several factors that may impact intestinal epithelial cell activity, this finding provides some support to the second hypothesis of this thesis.

5.4.ii Exogenous GLP-2 and intestinal GLP-2 receptor mRNA expression

Currently, there are few studies that have examined the effects of exogenous GLP-2 administration on GLP-2R expression. Dube et al. demonstrated no differences in distal jejunal GLP-2R mRNA expression between mice administered exogenous human (Gly²)GLP-2 or saline [135], while de Heuvel et al. found an increase in GLP-2R mRNA expression in rodent colonic submucosal enteric neurons exposed to human GLP-2 [139].

In this thesis, in order to understand the relationship between endogenous and exogenous GLP-2 and the GLP-2R in different anatomical regions of the gastrointestinal tract, jejunal, ileal, and colonic GLP-2R mRNA expression was measured in piglets with and without intestinal surgery that were either administered saline or GLP-2 therapy. GLP-2R mRNA expression was found to be higher in jejunal, ileal, and colonic tissue for JI piglets treated with GLP-2 as compared to saline treated animals. Ileal GLP-2R mRNA expression was higher in sham GLP-2 treated animals relative to sham saline animals, and even though jejunal and colonic GLP-2R mRNA expression was not significantly different between sham GLP-2 and saline treated animals, there was a trend towards higher expression in the former group. Lastly, colonic GLP-2R mRNA expression was higher in JC GLP-2 treated animals relative to JC saline animals, and
even though jejunal GLP-2R mRNA expression was not significantly different between JC GLP-2 and saline treated animals, there was a trend towards higher expression in the former group. These observations suggest that exogenous GLP-2 administration increases GLP-2R mRNA transcripts in all intestinal anatomical regions of JI neonatal piglets (jejunum, ileum, and colon), and increases GLP-2R mRNA expression in some regions of sham piglets (ileum) and JC piglets (colon). In order to determine whether or not an increase in GLP-2 mRNA expression corresponds with an increase in GLP-2R protein, GLP-2R protein from the different anatomical regions of the small intestine would need to be isolated, and this would be a worthy pursuit for future studies.

It is important to note that even though GLP-2R mRNA expression was higher in all anatomical regions of JI GLP-2 treated animals (relative to saline treated JI piglets), improvements in only morphological and histological outcomes of intestinal adaptation were observed in JI animals, while improvements in clinical, morphological, and histological outcomes of intestinal adaptation were observed in JC GLP-2 treated animals that only had a corresponding increase in colonic GLP-2R mRNA expression relative to JC saline treated animals. Differences in GLP-2R expression may not be responsible for the differences in outcomes of intestinal adaptation observed between JI and JC GLP-2 treated neonatal piglets. For instance, there may be a downstream mediator of GLP-2, such as IGF-1, that is more prominently expressed in JC GLP-2 treated neonatal piglets. To test this theory, it would be valuable to measure endogenous IGF-1 levels in sham, JI, and JC neonatal piglets with and without exogenous GLP-2 administration. Furthermore, a GLP-2R knockout piglet model of SBS that demonstrates improved outcomes of intestinal adaptation with exogenous administration of IGF-1, which are otherwise absent
with exogenous GLP-2 administration, would also support the role of IGF-1 as a
downstream mediator of GLP-2.

5.4.iii  Total endogenous PYY levels and in proximal-intestinal and distal-intestinal
resection neonatal piglet models of SBS

In addition to GLP-2, intestinal L cells also secrete PYY in response to nutrient
ingestion [76]. In order to understand the effect of remnant intestinal anatomy and
exogenous GLP-2 administration on intestinal L cell function, PYY levels were measured
in this study. Firstly, the effect of remnant intestinal anatomy on L cell function was
gleaned from total endogenous PYY levels at 50% enteral feeds and at termination in
saline treated animals. No differences in endogenous PYY levels among the different
saline treated and surgical groups were observed at either 50% enteral feeds or at
termination. Again, it is worth mentioning that at 50% enteral feeds, the percentage of
calories from enteral nutrition is the same for all animals, and hence the confounding
effect of enteral nutrients on PYY levels is eliminated. Hence, at 50% enteral feeds, the
absence of any differences in PYY levels among the different surgical groups suggests
that remnant intestinal anatomy does not affect L cell function. At termination, however,
since enteral nutrient load is variable, enteral nutrients confound the effect of remnant
intestinal anatomy on endogenous PYY levels, and it is challenging to understand the
effect of remnant anatomy alone on these levels.

Secondly, total endogenous PYY levels in GLP-2 treated animals were measured
to understand the effect of exogenous GLP-2 administration on intestinal L cell function
in the different surgical models of SBS. At 50% enteral feeds, no differences in total
endogenous PYY levels were observed among the different GLP-2 treated surgical
groups. This suggests that when enteral nutrient load was the same for all surgical
groups, exogenous GLP-2 did not have an effect on L cell function regardless of intestinal anatomy. However, at termination, JI and JC GLP-2 animals had significantly lower levels of total endogenous PYY compared to sham GLP-2 treated animals; therefore, at the end of the trial period, exogenous GLP-2 and remnant intestinal anatomy have an effect of endogenous PYY secretion, and possibly L cell function. Again, it must be noted that at termination enteral nutrient load varies between each surgical group, and varies within each surgical group (varies for each individual animal), and therefore, enteral nutrients confound the effect of remnant intestinal anatomy and exogenous GLP-2 administration on total endogenous PYY levels.

5.4.iv Total endogenous PYY levels, small intestinal length, and enteral nutrients

Peptide YY release occurs in response to nutrient ingestion [76]. Consequently, it was expected that a positive correlation between percent calories consumed as enteral nutrients and total endogenous PYY levels would be observed in this study. However, enteral nutrient levels at termination did not correlate with total endogenous PYY levels in sham, JI, and JC saline treated groups. The absence of a positive correlation between enteral nutrient levels and endogenous PYY levels at termination may have resulted from a confounding effect of small intestinal length (which also varied for each individual animal) at this point during the trial.

Since gastrointestinal L cells are predominantly found in the ileum and colon [77], an observation of lower levels of endogenous PYY in JC piglets lacking ileal L cells (distal-intestinal resection animals) compared to JI piglets with remnant ileal L cells (proximal-intestinal resection animals) was expected. Furthermore, it was expected that a positive correlation between small intestinal length and total endogenous PYY levels
would be observed. However, small intestinal length did not correlate with total endogenous PYY levels in sham, JI, and JC saline treated groups. The absence of a positive correlation between small intestinal length and endogenous PYY levels at termination may have resulted from a confounding effect of enteral nutrient level (which varied for each individual animal) at this point during the trial.

5.4.v Glucagon-like peptide-2 and peptide YY secretion and metabolism

In order to understand the effect of remnant intestinal anatomy and exogenous GLP-2 administration on L cell secretion, endogenous bioactive GLP-2 levels and total endogenous PYY levels were also evaluated over the trial period (surgery, 50% enteral feeds, and termination) for the different surgical and treatment groups. Endogenous bioactive GLP-2 levels increased over the trial period for JI saline animals, and bioactive GLP-2 levels in JI animals were higher at 50% enteral feeds and at termination with initiation, and continuation, of GLP-2 therapy. PYY levels did not change over the course of the trial period for both JI saline and JI GLP-2 treated groups. In JC saline treated animals, endogenous bioactive GLP-2 levels were higher at termination than at the time of surgery, and there was a trend towards increasing endogenous levels over the trial period. In JC GLP-2 treated animals, however, bioactive GLP-2 levels were lower at termination than at 50% enteral feeds, despite continual administration of exogenous GLP-2. Furthermore, total endogenous PYY levels for JC GLP-2 treated animals were lower at termination than at the time of surgery. These findings may suggest that exogenous GLP-2 in JC animals is associated with an increase DPP-IV activity; however, if this was the case, in contrast to the observed decrease in both bioactive GLP-2 and total PYY levels over the trial period, only a reduction in bioactive GLP-2 levels should have
been observed, since the PYY assay used in this study measures both active and inactive PYY. Alternatively, these findings might suggest that the either renal clearance of circulating GLP-2 and PYY is improved in JC GLP-2 treated animals or that the endogenous contribution of GLP-2 and PYY by colonic L cells is reduced in JC GLP-2 treated piglets.
CHAPTER 6

CONCLUSIONS
6 Conclusions

Endogenous GLP-2 levels were found to be higher in proximal-intestinal resection neonatal piglet models of SBS that have remnant ileum (type 1 remnant anatomy) as compared to non-resected surgical controls, while endogenous GLP-2 levels in distal-intestinal resection neonatal piglet models of SBS that lack remnant ileum (type 2 remnant anatomy) were found to be similar to non-resected controls. Exogenous human GLP-2 improved morphological and histological outcomes of intestinal adaptation in proximal-intestinal resection and distal-intestinal resection neonatal piglet models of SBS; however, improved clinical outcomes were only observed with GLP-2 administration in the distal-intestinal resection neonatal piglet model. Since exogenous GLP-2 administration had the greatest impact on intestinal adaptation in the distal-intestinal resection neonatal piglet model of SBS, which represents the type 2 anatomical subtype of SBS, and the type 2 anatomical subtype represents the most common remnant anatomy in neonatal SBS, these preliminary results support a role for GLP-2 therapy in pediatric SBS, and particularly for patients who have undergone a distal-intestinal resection and lack remnant ileum.
CHAPTER 7

FUTURE DIRECTIONS
7 Future directions

Although the results of this research have promising implications for future clinical work, there are several issues that remain to be sorted out in the laboratory. Firstly, there were many trends observed in this thesis that did not reach statistical significance. The calculation used to determine sample size for this study was based on an expected difference in small intestinal length. In future work, it would be worthwhile to determine sample size based on more than one primary outcome, and a larger number of animals in each group may produce results with statistically significant differences.

Secondly, in this particular model of SBS (where calories delivered by the enteral route vary between, and within, surgical and treatment groups), the confounding effect of enteral nutrients on intestinal adaptation must be determined. In future work, by maintaining the same amount of enteral nutrition in all arms of the study (pair-feeding), the effect of proximal or distal-intestinal surgery on intestinal adaptation can be more clearly understood. Previously, Burrin et al. have shown that 60% of nutritional energy delivered by the enteral route is adequate to observe a rise in endogenous GLP-2 and PYY levels (as compared to GLP-2 levels for animals receiving 0% enteral nutrition) [179]. More specifically then, in future work, by gradually advancing and maintaining all animals at 60% enteral nutrition, the effect of intestinal surgery and remnant intestinal anatomy on intestinal adaptation and endogenous GLP-2 and PYY secretion can be understood without the confounding effect of enteral nutrient load.

Thirdly, an attempt to understand the effects of remnant intestinal anatomy and exogenous GLP-2 on L cell secretion was made without directly measuring L cell populations. Immunohistochemical staining of L cells in the normal gastrointestinal tract
of humans, pigs, and rodents have demonstrated that intestinal L cells are predominantly found in the distal intestine and colon of these animals [29, 30]. Knowing this, one would expect to observe lower levels of endogenous GLP-2 and PYY, and fewer L cells in a distal-intestinal resection neonatal piglet model of SBS in which remnant ileum is not present. For future studies, it would be worthwhile perform immunohistochemistry techniques to obtain L cell profiles in JI and JC neonatal piglets to determine if intestinal surgery and remnant intestinal anatomy have an effect on L cell populations in various anatomical regions of the gastrointestinal tract.

Fourthly, even though an attempt was made to examine certain mechanistic aspects of the actions of GLP-2, including the effect of GLP-2 on intestinal cellular proliferation and apoptosis, as well as on GLP-2R mRNA expression, understanding the mechanisms of action of GLP-2 in general, and in neonatal piglet models of SBS in particular, should be the main focus of future studies. There are multiple mediators involved in the actions of GLP-2, and the trophic effects of GLP-2 on the intestinal epithelium are likely exerted indirectly via downstream growth factors [112]. Possible downstream mediators for the intestinotrophic actions of GLP-2 include IGF-1, ErB ligands, and KGF [136]. It would be worthwhile to measure at least some of these downstream mediators in future studies.

Finally, in neonatal piglet models of SBS, exogenous GLP-2 may have an effect on other outcomes of functional adaptation that were not investigated in this study, including intestinal permeability and blood flow. Therefore, in future work, it would be worthwhile to measure these outcomes by performing in vivo intestinal permeability
studies with lactulose or mannitol, and by examining intestinal mucosal eNOS expression as a measure of intestinal blood flow.

Although a Phase 1 clinical trial investigating the safety and dosing of GLP-2 in infants and children with intestinal failure is currently underway (NCT01573286), the heterogeneity of the disease process, difficulties in obtaining invasive tissue samples, other methodological constraints, and ethical considerations make it difficult to perform mechanistic research on human neonates. It is important to obtain an understanding of the mechanisms of action of GLP-2 in order to facilitate knowledge of optimal dosing, timing and duration of therapy, and drug toxicity. Hence, there continues to be a role for studying potential therapies for pediatric SBS using a large animal model that adequately mimics the clinical setting of neonatal SBS.
REFERENCES


-