THE EFFECT OF BUTYRATE-SUPPLEMENTED TOTAL PARENTERAL NUTRITION ON MUC2 MRNA AND GOBLET CELL CHEMOTYPES IN A SHORT BOWEL SYNDROME NEONATAL PIGLET MODEL

BY

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THESIS

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ABSTRACT

Background: Compromised barrier function and bacterial translocation are common complications of short bowel syndrome (SBS) and parenteral nutrition (PN). Although mucins help prevent intestinal bacteria translocation, total mucin production has been shown to decrease during PN infusion. Butyrate administration may be a mucin fortifying strategy as it has been shown to increase mucin production in vivo and ex vivo.

Objective: We tested the hypothesis that infusion of TPN supplemented with 9 mM butyrate will enhance protective goblet cell chemotypes in the small intestine, specifically sulfomucins, that enhancement of protective goblet cell chemotypes in the SCFA-supplemented group will be observed, since this group also contains 9 mM butyrate, that butyrate-supplemented TPN will have a greater effect on the small intestine than the large intestine, while butyrate supplementation at 60 mM will have a greater effect on the large intestine than the small intestine. We also hypothesize that muc2 mRNA abundance will increase in the small intestine with butyrate and SCFA-supplemented TPN infusion, whereas colonic muc2 mRNA abundance will increase in the colon with 60 mM butyrate administration.

Methods: Forty-eight hour old piglets underwent 80% proximal jejunouileal resection and were randomized to one of four groups: control total parenteral nutrition (TPN), TPN supplemented with 60 mM SCFA (36 mM acetate, 15 mM propionate, and 9 mM butyrate; SCFA), 9 mM butyrate (9Bu) or 60 mM butyrate (60Bu). Animals were further randomized to an acute (12 hour) or chronic (72 hour) time point. muc2 mRNA abundance, total goblet cell number, and total sulfomucin, sialomucin, acidomucin, and neutral mucin goblet cell numbers were ascertained.

Results: Sulfomucin chemotypes were increased in the jejunal villi of the 9Bu group compared to control (treatment main effect, p=0.047). Acidomucin chemotypes in ileal crypts were greater in the 60Bu group than the control group (treatment main effect, p=0.038). In the colonic crypts, SCFA
groups tended to have greater acidomucin chemotypes than control at 12h while 60Bu group tended to have greater acidomucin chemotypes per depth than control at 72h (p=0.060; Table 2.3). \textit{muc2} mRNA was increased in jejunal tissues in the 9Bu group compared to control with 270% and 30% increases in \textit{muc2} mRNA at 12 and 72 hours, respectively (p=0.010).

**Conclusion:** The 9 mM Bu treatment increased protective goblet cell chemotypes and \textit{muc2} mRNA in the jejunum while 60 mM Bu increased protective goblet cell chemotypes in the ileum and colon. The 9Bu treatment was the most effective at upregulating \textit{muc2} mRNA abundance and sulfomucin chemotypes. Butyrate-supplemented PN may be a therapeutic strategy for bolstering the epithelial barrier by increasing mucin production in neonates with SBS on PN.
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LIST OF ABBREVIATIONS

ANOVA     analysis of variance
CaCo2     human epithelial colorectal adenocarcinoma cell line
CCD-18Co  intestinal colonic myofibroblast cell line
cDNA      complementary deoxyribonucleic acid
Cl.16E    clonal derivative of HT29 cell line
ddH₂O     double distilled water
DEPC      diethylpyrocarbonate
DMEM      dulbecco’s modified eagle medium
DNA       deoxyribonucleic acid
dNTP      deoxyribonucleotide triphosphate
EGF       epidermal growth factor
EN        enteral nutrition
EST       expressed sequence tag, NCBI database
FAE       follicle associated epithelium
FITC      fluorescein isothiocyanate
FSR       fractional synthetic rate
GALT      gut associated lymphoid tissue
GH        growth hormone
GLP-2     glucagon-like peptide 2
Glut-2    glucose transporter 2
HIDAB     high iron diamine alcian blue
HT29      human colon adenocarcinoma cell line
IBD       inflammatory bowel disease
IF        intestinal failure
ITF       intestinal trefoil factor
IL-10/-   interleukin-10 knockout
IL-10     interleukin-10
JAM       junction adhesion molecule
LS174T    human colon carcinoma cell line
M cell    microfold cell
µg        microgram
µL        microliter
MLN       mesenteric lymph nodes
mM        millimolar
MODS      multiple organ dysfunction syndrome
mRNA      messenger ribonucleic acid
muc2      mucin 2
muc2/-    mucin 2 knockout
Na⁺K⁺ATPase sodium potassiam adenosine triphosphatase
Na S1     sodium sulfate transporter
NaS/-/-   sodium sulfate transporter knockout
NCBI      National Center for Biotechnology Information
PASAB     periodic acid schiff’s alcian blue
PGE1      prostaglandin E1
PGE2      prostaglandin E1
PN        parenteral nutrition
<table>
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<tr>
<td>PNALD</td>
<td>parenteral nutrition-associated liver disease</td>
</tr>
<tr>
<td>RER</td>
<td>rough endoplasmic reticulum</td>
</tr>
<tr>
<td>RIN</td>
<td>RNA integrity number</td>
</tr>
<tr>
<td>RLMβ</td>
<td>resistin-like molecule β</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNase</td>
<td>ribonuclease</td>
</tr>
<tr>
<td>rt-PCR</td>
<td>real time polymerase chain reaction</td>
</tr>
<tr>
<td>SBBO</td>
<td>small bowel bacterial overgrowth</td>
</tr>
<tr>
<td>SBS</td>
<td>short bowel syndrome</td>
</tr>
<tr>
<td>SCFA</td>
<td>short chain fatty acid</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SED</td>
<td>subepithelial dome</td>
</tr>
<tr>
<td>SGLT-1</td>
<td>sodium glucose transporter 1</td>
</tr>
<tr>
<td>siG A</td>
<td>secretory immunoglobulin A</td>
</tr>
<tr>
<td>T84</td>
<td>human colon carcinoma cell line</td>
</tr>
<tr>
<td>TPN</td>
<td>total parenteral nutrition</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>vWF</td>
<td>von Willebrand factor</td>
</tr>
<tr>
<td>wt</td>
<td>wild type</td>
</tr>
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<td>ZO-1</td>
<td>zona occludin-1</td>
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CHAPTER 1
LITERATURE REVIEW

INTRODUCTION

Short bowel syndrome (SBS) is the most common cause of intestinal failure in infants (Goulet and Ruemmele, 2006) and occurs in 4.4% of preterm infants with only a 73% to 89.7% survival rate (Quiros-Tejeira et al., 2004; Wales et al., 2004). Treatment often includes nutrition management in the form of parenteral nutrition (PN), which can extend the life of a patient with SBS for many years (Dudrick et al., 1968; Wilmore and Dudrick, 1968; Dudrick and Allen, 1971). However, long-term administration of PN can compromise quality of life and can result in potentially fatal primary complications such as parenteral nutrition-associated liver disease (PNALD), recurrent sepsis, and uncontrolled fluid losses, or secondary complications such as bacterial translocation, barrier dysfunction, muted intestinal adaptation, and multiple organ dysfunction syndrome (MODS) (Johnson et al., 1975; Morin et al., 1978; Ford et al., 1984; Deitch, 1992; Inoue et al., 1993; Conour et al., 2002; Yang et al., 2002; Ziegler et al., 2002; Dahly et al., 2003; Kansagra et al., 2003; Wiest and Rath, 2003; Kumpf, 2006; Grau et al., 2007; Jan, 2008).

Within the first line of defense against invading pathogens—in innate immunity—is intestinal mucous, made predominantly of the glycoprotein mucin. Decreases in mucin abundance or protective goblet cell chemotypes can leave the epithelial barrier exposed to bacteria and bacterial products which can lead to bacterial translocation (Gork et al., 1999). In addition, direct contact of harmful luminal contents to the epithelial lining promotes inflammation, which can further impair barrier function (Yang et al., 2002; Yang et al., 2008) and increase the risk of bacterial translocation. Further, it has been shown that parenteral nutrition-associated liver disease possibly results from bacterial translocation (Sondheimer et al., 1998). Because the liver is responsible for clearing toxins from the body, liver disease can lead to MODS. Bolstering one of the initial defenses, mucin, may
improve the outcome of SBS patients by preventing bacteria-induced mucosal inflammation and further complications.

Mucin abundance is decreased by the typical nutritional management of SBS, PN administration (Iiboshi et al., 1994; Bertolo et al., 1998; Law et al., 2007). However, PN increases protective goblet cell chemotypes (Conour et al., 2002). The short chain fatty acid (SCFA) butyrate enhances mucin production \textit{in vitro} and \textit{ex vivo} (Finnie et al., 1995; Willemsen et al., 2003; Hatayama et al., 2007; Burger-van Paassen et al., 2009), but its direct effect on mucin \textit{in vivo} and its effect via parenteral administration has not yet been elucidated. This study analyzes the effect of parenteral butyrate and SCFA administration on both mucin mRNA and goblet cell chemotypes in a SBS model piglet receiving total parenteral nutrition (TPN).

THE NEONATAL INTESTINE

\textit{Intestinal Cells}

Four main layers of tissue compose the intestinal wall: the mucosa, submucosa, muscularis externa, and serosa (Saladin, 2004). The mucosa layer is closest to the lumen and is comprised of a luminal layer of epithelial cells followed by the lamina propria and muscularis mucosa layers (Madara and Trier, 1994). The anatomy of the small intestine, from the nature of the folds to the surface of the cells, creates a vast area that allows for digestion and absorption of nutrients. The folds of Kerckring occur along the mucosa of the small intestine, and upon this surface area are projections called villi, fingerlike protrusions of the mucosal layer. Extending from the surface of each absorptive cell of the villi are microvilli which contain the brush border enzymes necessary for digestion. Crypts of Lieberkühn—similar to pores—descend from the epithelium to the muscularis mucosa (Saladin, 2004).
Stem cells located in the lower half of the crypts differentiate into one of five kinds of epithelial cells: enterocytes, goblet cells, enteroendocrine cells, paneth cells, and M cells, also known as microfold cells (Leedham et al., 2005). Epithelial cells migrate from the crypt to the tip of the villus where they are sloughed off by luminal contents and consequently digested. Enterocytes are responsible for continuous absorption of macromolecules and micronutrients from the lumen and transportation of the contents to the circulation and lymph, and they are present in the villi and the upper half of the crypt epithelium (Kimmich and Randles, 1975; Iuldashev et al., 1978; Saladin, 2004). Goblet cells are located in these same areas as enterocytes; however, they descend further into the crypts and have the primary role of mucin production and secretion (Moe, 1953; Forstner et al., 1973). Enteroendocrine cells are found along the epithelium and are responsible for releasing peptide hormones of both endocrine and paracrine function. Paneth cells are the only epithelial cell line present at the base of the crypts and extend about halfway up the crypt walls and secrete defensins, lysozyme, and phospholipase to protect against harmful luminal contents (Trier, 1963; Qu et al., 1996; Ayabe et al., 2000). Last, M cells are found in the follicle associated epithelium (FAE) of Peyer’s patches in the ileum and are responsible for antigen sampling and transportation (see below; Owen and Jones, 1974).

Epithelial cells are held together by tight junctions which are located between cells at the apical end of the cell, and desmosomes, located directly beneath tight junctions. Tight junctions restrict paracellular transport (Claude and Goodenough, 1973) and consist of the specific proteins zonula occludens-1 (ZO-1; Stevenson et al., 1986), cingulin (Citi et al., 1988), junctional adhesion molecules (JAM; Martin-Padura et al., 1998), claudin-1 and claudin-2 (Furuse et al., 1998), and occludin (Furuse et al., 1993). Desmosomes are more important for cell to cell adhesion than paracellular restriction and are comprised of the protein E-cadherin (Boller et al., 1985). Tight junction structure is very plastic in nature and can be modulated by physiological, physical, and
pathological occurrences. For example, intestinal inflammation has been shown to reduce barrier function by causing tight junction transmembrane proteins to internalize and by disrupting the protein’s membrane affiliations (Bruewer et al., 2003).

*Enteric Immune System*

The intestine has extensive immunological function, as it is in contact with both the external environment (e.g. food and ingested bacteria) and the internal resident microbiota. The innate immune system is a highly conserved type of immunity across organisms, and operates as both the first line of defense against pathogens and as an activator of the acquired immune system, which is present in more advanced organisms and involves recognition of foreign substances from previous encounters (Yuan and Walker, 2004). The intestine bridges to the acquired immune system via M cell and dendritic cell interaction.

The intestinal immune system is termed GALT, or gut associated lymphoid tissue, and consists of oval bundles of lymphoid tissue known as Peyer’s patches which are covered by follicular associated epithelium (FAE; Figure 1.1). Harmful luminal products or bacteria are endocytosed or phagocytosed, respectively, by M cells in the FAE and are trafficked to the base of the cell (Owen and Jones, 1974; Neutra et al., 1996). The bases of M cells are expanded to form a pocket like invaginations containing T cells, B cells, and macrophages (Farstad et al., 1994); however, it has not been clearly shown that these lymphocytes and macrophages are within the M cell plasma membrane. In addition, the M cell pocket is only characteristic of a mature M cell; newborn piglets have immature M cells that have not yet formed the pocket like invaginations (Kido et al., 2003). Below the FAE, in the lamina propria, is the subepithelial dome (SED) which contains strategically placed dendritic cells, presumably to begin processing M cell admitted antigens for presentation to T cells (Kelsall and Strober, 1996). Below the SED are lymphoid follicles (Owen, 1977) containing B
lymphocyte germinal centers (Butcher et al., 1982). Between the follicles are the interfollicular regions that are rich in T cells and macrophages (Kelsall and Strober, 1996). Typically, dendritic cells are responsible for the connection between the innate immune system and the acquired immune system by recognizing antigens engulfed by the M cells and presenting them to T cells (Langhoff and Steinman, 1989) located in either the M cell pocket (Pappo, 1989; Pappo et al., 1991), the lamina propria, or the nearest mesenteric lymph node (MLN; Holt et al., 1994). However, M cells also have processes that protrude from their basal membrane and extend as far as 10 µm and potentially make contact directly to lymphoid tissue (Giannasca et al., 1994). In addition, epithelial-associated dendritic cells have been shown to extend between intestinal epithelial cells, forming tight junctions with these cells, and directly sampling antigens from the lumen without the help of the M cell (Rescigno et al., 2001; Niess et al., 2005). These epithelium associated dendritic cells are also capable of traveling to the MLNs (Schulz et al., 2009).

Bacterial translocation has been established as a transcellular process, specifically through FAE M cells (Grutzkau et al., 1990; Jones et al., 1994; Jensen et al., 1998); however, there has been recent conjecture that bacterial translocation may also occur paracellularly (Nadler et al., 2000). In this *ex vivo* study by Nadler and colleagues, pinocytosis and phagocytosis were alternatively inhibited with no change noted in bacterial translocation meaning that paracellular transport is a likely mechanism for bacterial translocation. Although, both mechanisms of transcellular transport were not inhibited at the same time meaning that when one is restricted, the other mechanism may compensate. Further research in the area of paracellular bacterial translocation is needed.

Because M cells function to transport bacteria and bacterial products for immune function, they consequently have absent or minimal microvilli and greatly shortened glycocalyx (Bye et al., 1984; Neutra et al., 1999). Mucous, a viscoelastic gel-like covering over the intestinal epithelium is made mainly from goblet cell mucin secretion. Because goblet cell secretions could block M cell
engulfment, they are rare in the FAE of human intestinal cells and have been precisely calculated to account for only 0.5% of the cells in porcine FAE (Fujimura et al., 1992; Beier and Gebert, 1998). It may seem that increases in \textit{muc2} mRNA, and potentially more Muc2 protein and mucous production, or more protective goblet cell chemotypes would not likely affect bacterial translocation since M cells are primed for bacterial engulfment and have few neighboring protective goblet cells. However, mucous is still important in preventing bacteria-induced inflammation in the epithelium apart from the FAE. As previously mentioned, inflammation can result in barrier function damage. Furthermore, although definitive paracellular transport of bacteria and bacterial products has not been established, protective mucin types and mucous abundance are correlated with decreased bacterial translocation \textit{in vitro} and \textit{in vivo}, demonstrating the advantage of specific mucins in protection against bacterial translocation (Gork et al., 1999; Sakamoto et al., 2004; Dawson et al., 2009).

\textit{Mucins}

\textit{Mucins and Intestinal Function}

Mucous is a semi-permeable viscoelastic gel coating that protects the intestinal epithelium from enzymatic, chemical, and mechanical damage by providing barrier function making it essential in innate immunity. The main component of mucous is mucin, which is secreted from goblet cells; however, mucous also consists of other secretory components that confer protection to the intestine. Secretory immunoglobulin A (sIgA), a mucosal antibody secreted from intestinal plasma cells (Perkkio and Savilahti, 1980), is present in the intestinal mucous (Hamer et al., 2009). Paneth cells contribute to intestinal defense by secreting the microbiocidal peptides lysozyme (Fleming, 1922; Trier, 1963), defensins (Ayabe et al., 2000), and phospholipase (Qu et al., 1996). In addition to mucin, goblet cells secrete intestinal trefoil factor (ITF; Podolsky et al., 1993), a peptide thought to
safeguard the epithelial layer by encouraging healing upon insult (Thim, 1989; Wright et al., 1990; Alison et al., 1995). In ITF-deficient mice, the administration of dextran sulfate sodium—an agent that typically causes only mild intestinal injury—caused death from severe colitis (Mashimo et al., 1996). Goblet cells also secrete resistin-like molecule \( \beta \) (RLM\( \beta \)), a protein that promotes inflammation (Nair et al., 2008). While excessive inflammation can damage the epithelial layer, some inflammation is necessary and is a natural reaction against pathogens.

Mucins are generally categorized into two groups: secreted mucins (gel-forming and non-gel-forming) and membrane-anchored mucins. The majority of intestinal mucous is made of secretory gel-forming mucins, and contains predominantly Muc2, a mucous protein encoded by the \( \text{muc2} \) gene (Gum et al., 1989; Griffiths et al., 1990). Muc2 is generally considered to be a secretory rather than a membrane bound mucin, but some have found that Muc2 contributes to two different layers: one fraction completely detached from the epithelium that is easily washed away with saline and a fraction that is adherent to the epithelium (Johansson et al., 2008). The loose layer of mucous is thickest in the colon and thinnest in the jejunum. The adherent layer is continuous and firm in the colon but is thinner or non-existent in the small intestine (Atuma et al., 2001).

Mucins are heavily glycosylated proteins composed of approximately 75%-80% carbohydrate and 20-25% amino acids (Carlstedt et al., 1993). While Muc2 contains a significant amount of tandemly repeated, heavily O-glycosylated regions (Toribara et al., 1991) made mainly of serine, threonine, and proline (Fahim et al., 1987; Huan et al., 1992), the mucin ends are unique and have fewer serine and threonine residues and contain more cysteine residues (Gum et al., 1992). On average, there are 4 monosaccharides on the carbohydrate side chains (Carlstedt et al., 1993), which sometimes contain sulfate (Carlstedt et al., 1993) and sialic acid (Hansson et al., 1991) residues.

The structure of mucin is central to its function. The cysteine-rich ends of mucins serve to aid polymerization of mucin monomers into oligomers, which has been demonstrated by treating
mucins with disulfide-specific reducing agents and observing a subsequent loss of viscosity and gel-forming ability in the mucin (Tabachnik et al., 1981). In addition, the cysteine-rich ends are very similar to von Willebrand factor (vWF) (Gum et al., 1994), a glycoprotein found in endothelium, essential in blood coagulation. Cysteine-rich ends are crucial to oligomerization and packaging of vWF into granules, suggesting by analogy that cysteine-rich ends may be important to intestinal mucin polymerization and packaging as well. (Xu et al., 1992).

Their heavily glycosylated core gives mucins their expanded and viscoelastic structures and allows for solubility in the intestinal lumen. Removal of the carbohydrate moieties results in a denatured protein, demonstrating that the glycosylated side chains are essential to mucin structure (Shogren et al., 1989). In addition, it has been calculated that mucins with 2 or more carbohydrate residues per side chain results in a 2.5-3 fold increase in size in solution compared to a non-glycosylated mucin (Shogren et al., 1986). The expanded structure means that mucins overlap and become entangled, giving rise to the viscoelastic properties of mucin (Shogren et al., 1989). Viscoelasticity of intestinal mucous is necessary to withstand the mechanical stress of both the muscular force of the intestine, such as peristalsis, and the movement of particles across the mucous and epithelium. In other words, the viscoelasticity of mucous ultimately protects the epithelium from mechanical damage (Bansil et al., 1995). Because mucous serves as a physical barrier, it also protects the epithelium against chemical and enzymatic damage. Lastly, the sulfate and sialic acid residues create a high density negative surface charge which binds water, further enhancing viscoelastic properties.

In addition to the protection that mucin provides against the chemical, enzymatic, and mechanical stress of normal gastrointestinal function, they also provide protection from bacteria ingested or residing in the intestine. First, mucin protects the epithelium by trapping bacteria that subsequently get excreted with the loose layer of mucous in the feces. In a study by Johansson and
colleagues (2008), it was found that in the colon, the loose layer of mucin, which is excreted in the feces, is colonized by bacteria, while the inner layer is almost devoid of bacteria, possibly reflecting the effective barrier mucin provides. Further, in this study, muc2−/− mice had bacteria in direct contact with the colonic epithelium, demonstrating the necessity of mucin in preventing bacterial contact with the epithelium and possibly even preventing bacterial translocation (Johansson et al., 2008).

Secondly, mucin serves as a physical barrier that is difficult for the bacteria to break down. The type of carbohydrate side chains on mucin plays a role in the protectiveness of the mucin against bacterial penetration. Upon bacterial introduction in vitro to germ-free rat mucin, sulfomucins and neutral mucins were 40% and 55% degraded, respectively, at 4 hours while sialomucins were 90% degraded at 1 hour and almost completely degraded at 4 hours (Fontaine et al., 1998). Thus, sulfomucins and neutral mucins confer a more protective effect in the intestine than sialomucins because they are more slowly degraded by bacteria, therefore the established continuum of mucin protection is as follows: sulfomucins > neutral mucins >>> sialomucins.

An earlier study also suggested the benefit of acidomucins (both sialomucins and sulfomucins) over neutral mucins. The colonization of germ-free rats with rat-specific intestinal microbiota triggered an initial release of neutral mucins with a progressive shift towards acidic mucin secretion observed over the following 4 months (Enss et al., 1992). In this study, the researchers suggested that the replacement of neutral mucins with acidomucins over this time frame was in response to the development of gastrointestinal microbiota and was possibly a form of maturation of the gastrointestinal tract (Enss et al., 1992).

Several studies confirm the protective nature of sulfomucins (Sakata and von Engelhardt, 1981; Deplancke et al., 2000; Conour et al., 2002; Dawson et al., 2009). Mice deficient in sulfate transporters had higher levels of sialomucin than sulfomucin levels compared to control mice, and
these mice had greater susceptibility to toxin-induced colitis and exhibited decreased intestinal barrier function (Dawson et al., 2009). This is most likely attributed to the lack of sulfate residues provided intracellularly by sulfate transporters resulting in decreased sulfate addition during post-translational mucin modification (Dawson et al., 2009). Further, it has been demonstrated that sulfomucins increase in concentration from the duodenum to the colon, mirroring the same pattern of bacterial concentration from proximal to distal bowel (Sakata and von Engelhardt, 1981; Deplancke and Gaskins, 2001; Conour et al., 2002). The increase of sulfomucins in areas of higher microbial density suggests the importance of sulfomucins in barrier defense against resident and infectious microbial populations.

As previously mentioned, mucins serve to trap bacteria. The action of trapping bacteria also allows bacteria to congregate which has been shown in vitro to aid in the growth of biofilms on fixed Caco-2 human epithelial cells (Bollinger et al., 2003). While this may seem contrary to the purpose of trapping bacteria, and while mucous can aid in the biofilm formation of harmful species (Bollinger et al., 2003), biofilms have also been shown to be beneficial to the intestine and host health (Jones and Versalovic, 2009). In addition to binding each other, bacteria bind receptor sites on mucin which serve to compete with bacterial receptor sites on the underlying epithelium (Wadolkowski et al., 1988; Conway et al., 1990). Scientists have demonstrated that some pathogens have adapted to using these binding sites as temporary attachment while moving towards the epithelial layer (Drumm et al., 1988; McCormick et al., 1988; Conway et al., 1990). These same scientists also demonstrated that the pathogens lost their piliation, binding ability to epithelial cells, or the ability to tumble and swim after colonizing intestinal mucous. So it may be that intestinal mucous aids pathogens in reaching the epithelium, but may inhibit the bacteria’s ability to actually bind to the epithelium. If enough mucous is present, it is likely that excretion of the pathogens
while they are attached to the mucous can occur before the rate of replication of pathogens becomes threatening (Forstner, 1994).

**Synthesis and Excretion of Mucins**

Muc2 production appears to occur solely in goblet cells (Van Klinken et al., 1995). Within the goblet cells, mucins are synthesized similarly to most packaged secretory glycoproteins. They are first synthesized on ribosomes and then transported to the rough endoplasmic reticulum (RER) where they are folded and oligomerized. Carbohydrate side chain additions begin cotranslationally via sulfotransferases, sialytransferases, and glycosyltransferases at the ribosomal level, continue at the RER (Strous, 1979), and the main carbohydrate side chain additions occur within the Golgi complex (Dekker and Strous, 1990). Condensing granules containing the mucins bud off from the trans-Golgi and mucins are increasingly condensed with the help of increasing intragranular concentrations of cationic calcium (Paz et al., 2003). As goblet cells mature, granules are separated from the inner surface of the plasma membrane by a thin web of actin filaments. The mucins are released when the granule’s membrane fuses with the cell plasma membrane, creating a fusion pore, and then releasing the mucin via exocytosis through a dialating pore (Breckenridge and Almers, 1987). This process can be either constitutive or regulated.

In constitutive pathways, granules are transported directly to the plasma membrane and release mucin without any signal, only requiring contact with the membrane. Several cell types have been shown to release small amounts of glycoprotein continuously via the constitutive pathway while also retaining the ability to upregulate mucin secretion in response to an environmental signal (regulated pathway; Gumbiner and Kelly, 1982; Sporn et al., 1986; Arvan and Chang, 1987; Burgess and Kelly, 1987). The mechanism regarding the movement of mucins out of granules is not fully elucidated, but one hypothesis is that the smaller calcium ions escape first after the fusion pore
opens, leaving highly negative mucins to strongly repel each other, which pushes them out of the granule (Forstner, 1994). The hydrophilic mucins then become a viscous gel. Complete refilling of the goblet cell takes approximately 60-120 minutes (Specian et al., 1990). Once full, the goblet cell is then ready to release more mucin. The protection provided by mucous is most important in those with intestinal problems, such as intestinal failure, since many of these conditions predispose the intestine to further damaging events such as bacterial translocation into the systemic circulation or organs.

**INTESTINAL FAILURE**

*Etiology of Intestinal Failure*

Intestinal failure (IF) is defined as the critical reduction of functional bowel below the minimum amount needed for adequate absorption of the basic metabolic requirement without oral or intravenous supplementation (Jeejeebhoy, 2005). This decrease in function can result in severe malabsorption without supplementation and can be caused either by short bowel syndrome (SBS) which results from surgical resection of the bowel or intestinal failure which is non-functioning (but not necrotic) sections of the bowel that don’t necessarily need to be removed. Other causes of IF, besides SBS, include severe extended Hirschsprung’s disease (a motility disorder), chronic intestinal pseudo-obstruction, mesenteric venous thrombosis, necrotizing enterocolitis, gastroschisis, midgut volvulus, desmoids tumor, intestinal atresia, trauma, and Crohn’s disease (Hyman et al., 1994; Nishida et al., 2002). Conditions like these in which functional surface area, motility and/or obstruction are issues, intravenous supplementation is often necessary since enteral nutrition (EN) would not be viable. In the past, severe malabsorption plagued individuals with IF. However, patients with intestinal failure are now living longer due to the advent of PN in the mid-1970’s and vast improvements to PN over the last 3 decades (Langnas, 2008).
Intestinal Failure in Infants

IF is more common in preterm infants than term infants, as preterm infants typically suffer from decreased gastric motility, fat malabsorption, and digestive enzyme deficiencies such as lactase deficiency (Klaus and Fanaroff, 2001; American Academy of Pediatrics Committee on Nutrition, 2004; MacLean and Fink, 1980). This means the immature gastrointestinal system of a premature infant is likely predisposed to intestinal failure. Intestinal insults requiring resection can occur prenatally, neonatally, or postnatally. Prenatal etiologies of IF include atresia, malrotation, midgut volvulus, segmental volvulus, abdominal wall defects, gastroschisis, and extensive Hirschsprung’s disease. Midgut volvulus can occur during both neonatal and postnatal development, especially in a preterm infant whose gastrointestinal system is still developing. Other neonatal etiologies of IF include necrotizing enterocolitis, arterial thrombosis, and venous thrombosis. Postnatal origins of IF include complicated intussusceptions, arterial thrombosis, inflammatory bowel disease, post-traumatic resection, and extensive angioma (Goulet and Ruemmele, 2006).

Complications

Basic Complications

SBS has a relatively low incidence of about 3.1 per 1000 of term infants and 43.6 per 1000 of premature infants (Wales et al., 2004). There are many complications of SBS that are life-threatening, and the survival rates are alarmingly low, with only 73% to 89.7% of infants surviving (Quiros-Tejeira et al., 2004; Wales et al., 2004; Goulet et al., 2005). Better survival rates are significantly associated with remaining post-resection bowel lengths >38 cm, remaining ileocecal valve, remaining entire colon, and with primary anastomosis rather than an ostomy performed during resection (Quiros-Tejeira et al., 2004). Additionally, ostomy reversal is associated with increased survival (Quiros-Tejeira et al., 2004).
One of the functional complications of SBS is reduced digestive and absorptive capacity. SBS patients have decreased absorption of all nutrients, even easily digested carbohydrates (Olesen et al., 1999). Oftentimes, management of decreased absorption involves PN since most premature infants have neither the reflexes to suckle nor a mature gastrointestinal system to absorb nutrients from enteral nutrition (EN) delivered via tube feedings. Unfortunately, though PN has prolonged the lives of many infants suffering from SBS, it can cause further declines in intestinal function and can create many other complications by its administration. PN is associated with both morphological and functional decline of the intestine, termed intestinal atrophy (Johnson et al., 1975; Inoue et al., 1993). PN can have life threatening complications associated with long term use such as liver disease, septicemia or bacterial translocation, or loss of venous access (Pierro et al., 1996; Sondheimer et al., 1998; de Buys Roessingh et al., 2008). Fluid balance is also an issue in SBS patients depending on what part and how much of the intestine was resected. PNALD is most commonly related to cholestasis in the pediatric population and has been shown to occur in 67% of infants on long term PN after intestinal resection of which 17% progressed to liver failure (Sondheimer et al., 1998). In this study, bacterial or fungal infection of the blood, urine, peritoneal fluid, cellulitis aspirate, or cerebrospinal fluid preceded all instances of cholestasis, with most of these bacteria implicated in infection being common gastrointestinal bacteria, leading one to believe that bacterial translocation could have been the factor in some or many of these cases of cholestasis and liver failure (Sondheimer et al., 1998).

Secondary Complications

Bacterial translocation can be a life-threatening secondary complication of either SBS and/or PN administration and is the passage of viable or non-viable bacteria and bacterial products from the intestinal lumen through the epithelium and into the mesenteric lymph nodes, liver, spleen,
kidney, and bloodstream (Wells et al., 1988; Berg, 1999). Some researchers have found increased rates of bacterial translocation in rats receiving TPN compared to rats fed either rat chow ad libitum or TPN solution orally (Alverdy et al., 1988; Eizaguirre et al., 2001). However, other groups have found that TPN reduces barrier function but does not increase bacterial translocation in piglets (Nakasaki et al., 1998; Kansagra et al., 2003). As mentioned above, Sondheimer and colleagues (1998) speculated that bacterial translocation is a likely cause of cholestasis and PNALD. Bacterial translocation has been associated with MODS in other studies as well (Deitch, 1990; Sullivan et al., 1991; Zhi-Yong et al., 1992; Fukushima et al., 1995). The risk of bacterial translocation has been proposed to increase due to decreased epithelial barrier function (see below) caused by PN administration (Deitch et al., 1995).

It has been recently demonstrated that bacterial translocation is also correlated with small bowel bacterial overgrowth (SBBO; Cole et al., 2010). The mechanisms are not necessarily mutually exclusive, although the relationship between barrier function, SBBO, and bacterial translocation has not been clearly defined. SBBO is an increase in the total number of bacteria or of certain bacteria and can result in nutrient or micronutrient malabsorption (e.g. Vitamin B₁₂), gas generation with bloating, abdominal pain, diarrhea, altered motility, and kidney stones. SBBO is a complication of both SBS and PN and may decrease the effectiveness of weaning patients off of PN. Inflammation is often a result of SBBO, either due to certain bacterial species igniting an inflammatory response once entering the mucosa, or the host’s overreaction to absorbed bacterial antigens (Dibaise et al., 2006). TPN has specifically been shown to predispose neonatal piglets to overgrowth of *Clostridium perfringens*, a detrimental bacteria that has been implicated in bacterial translocation (Meddah et al., 2001; Deplancke et al., 2002).

Mucosal inflammation may contribute to the pathogenesis of bacterial translocation. Direct contact of harmful luminal contents to the epithelial lining promotes inflammation which can
further impair barrier function (Yang et al., 2002; Yang et al., 2008) and potentially increase the risk of bacterial translocation. Activation of pro-inflammatory cytokines from leukocytes, macrophages, and mast cells (Saladin, 2004) has been shown to impair mucosal integrity and barrier function by negatively affecting tight junction proteins (Bruewer et al., 2003).

Adequate mucin content is essential for proper barrier function and decreased mucin content can increase the risk of bacterial translocation. Dawson and colleagues (2009) found that sulfate transporter (NaS1) deficient (NaS1−/−) mice had a marked decrease in sulfomucin content. Without a correctly functioning NaS1 transporter, there are fewer sulfate residues available for mucin sulfation. NaS1−/− mice had a significant 24% increase in macromolecule permeability. Upon infection with Campylobacter jejuni, 60% of NaS1−/− mice experienced bacterial translocation presenting with Campylobacter jejuni in the liver three days after infection while none of the NaS1+/+ mice presented with bacterial translocation. Interestingly, the NaS1−/− and NaS1+/+ mice had similar mucosal integrity against resident microbiota, indicating that the decrease in barrier function was only in regards to pathogenic bacterial infection. Along with the decrease in sulfomucin content, an increase in sialomucin content of the NaS1−/− mice was noted. However, mRNA levels of mucins normally expressed in the mouse ileum were unchanged, demonstrating that any role that mucin levels played in increased bacterial translocation and intestinal macromolecular permeability in the NaS1−/− mice was likely related to a decrease in the sulfomucin to sialomucin ratio (Dawson et al., 2009). However, total mucin production was not measured; thus, increases in bacterial translocation in the NaS1−/− mice could be due to total decreased mucin abundance rather than solely decreased relative sulfomucin content.

The relationship between inflammation and sulfomucins has also been investigated. Muc2−/− mice downregulate several genes, including essential mucin synthesis enzymes such as sulfotransferases (Yang et al., 2008). Downregulation of sulfotransferases will result in decreases in
sulfomucin content. This study also demonstrated that Muc2−/− mice developed tumors through an inflammation linked pathway and that Muc2 deficiency resulted in subclinical chronic inflammation (Yang et al., 2008). Makkink and colleagues (2002) discovered that IL-10−/− mice had sulfate depleted mucins compared to control mice, and that IL-10−/− mice had correspondingly inflamed colons. IL-10 is an anti-inflammatory cytokine first described in 1989 (Fiorentino et al., 1989). The lack of IL-10 could easily cause inflammatory conditions such as inflammatory bowel disease (IBD) or colitis, and Makkink and colleagues’ findings suggest that decreased mucin sulfation could be part of the mechanism by which intestinal inflammatory conditions arise. Evidence also demonstrates that administration of IL-10 can decrease TPN-associated declines in tight junction proteins in mice (Sun et al., 2008), suggesting that a pro-inflammatory response is at least partly responsible for reduced barrier function, either by tight junction dysfunction or by decreases in mucin sulfation.

Mucins, Bowel Resection, and Nutrition Support

The fractional synthetic rate (FSR) is the rate of incorporation of a precursor into a product per unit of product mass and has been used in many studies to analyze protein synthesis and incorporation. The FSR of Muc2 in enterally fed human preterm infants after small bowel resection was 67.2%, which is one of the highest FSRs determined in humans to date. This was assessed by analyzing labeled threonine incorporation into mucins (Schaart et al., 2009).

The effect of nutritional support on the rate of mucin synthesis is debated. Conour and colleagues (2002) found an increase in the number of acidomucin expressing goblet cells in newborn piglets administered TPN. However, they did not quantify mucin abundance, so mucin decreases may be happening concomitantly with goblet cell number stasis or increases.

Recent reports demonstrated definitive decreases in total mucin content or mucin production with TPN administration. After administration of fluorescein isothiocyanate (FITC)-
dextran through a gastroduodenal tube, FITC-dextran descended between the villi in the TPN administered rats while increased mucous gel in the chow-fed group prevented the FITC-dextran dye from filling the inter-villi spaces and retained the dye to the lumen area (Iiboshi et al., 1994) demonstrating the increased mucous gel in the chow fed rats. This group also found that FITC-dextran plasma levels were elevated in TPN-administered rats compared to chow-fed rats, indicating increased permeability in the intestines of rats receiving TPN. Another study in which rats were administered TPN intravenously or fed with the elemental TPN solution orally, epithelial bound mucin and mucin within the goblet cells was significantly decreased in the jejunum, with a similar trend seen in the ileum (Spaeth et al., 1994). In addition to nutrition support, micronutrient supplementation affects mucin production as well. Comparing adequate threonine EN piglets, adequate threonine PN piglets, and deficient threonine EN piglets, PN resulted in lower mucin production in the duodenum, jejunum, and colon (Law et al., 2007).

Lastly, if nutrition support is not tolerated, mucin production can ultimately be hindered in an infant with SBS. If a preterm infant is in a state of protein-energy malnutrition, the infant can have decreased mucin production, resulting in increased susceptibility to bacterial translocation and infection (Sherman et al., 1985).

**MANAGEMENT OF INTESTINAL FAILURE**

The main goals in nutritional and medical management of intestinal failure are to maintain fluid, electrolyte and nutrient balances and to reduce side effects of treatment. Managing SBS in infants is critical as an infant is undergoing rapid development and has high nutritional requirements. Further, preterm infants have increased nutritional and medical needs because they have to catch up in growth to their term counterpart. Nutritional supplementation is based on the etiology of intestinal failure, the extent and location of resection, if resected, and the clinical lab values of the
patient to ascertain vitamin, mineral, and macronutrient deficiencies. Medical management often includes drug therapies such as anti-motility agents to allow the intestine adequate time for digestion and absorption. Intestinal transplantation or bowel lengthening procedures may be considered when long term TPN complications have occurred (O'Keefe et al., 2006; Jan, 2008).

EN in patients with compromised intestinal function is preferred, if tolerated, to avoid the complications associated with PN. If an infant is placed on PN, then management should include as much EN as possible to prevent PN-associated atrophy, PN dependence, and the aforementioned PN complications. Intestinal adaptation is the ability of the intestine to increase absorptive area and to recover functional abilities of this area to adapt for the loss of intestine post-resection (Althausen et al., 1950; Dowling and Booth, 1966; Bell et al., 1973; Stein and Wise, 1978; Curtis et al., 1984). Unfortunately, PN administration nullifies intestinal adaptation post-resection due to the lack of luminal stimulation provided by oral or enteral feeding (Morin et al., 1978; Ford et al., 1984; Ziegler et al., 2002). Dahly and colleagues (2003) demonstrated after 70% midjejunoileal resection that rats administered TPN had significantly greater mucosal hyperplasia by 8 hours, continuing through 7 days post-resection compared to transection controls. However, orally fed resected rats had an enhanced adaptive response compared to the rats administered TPN. This study is an example of intestinal adaptation occurring even with the administration of PN, but it should be noted that the orally fed rats still experienced greater adaptation demonstrating the benefit of luminal stimulation that EN or oral diets provide.

Threonine is an extremely important part of management in the SBS infant. The mucin core is made of mostly serine, threonine, and proline residues, thus these amino acids are essential for adequate mucin production. Studies with rats have been conducted to test mucin production with reduced threonine intake which have resulted in steady production of \textit{muc2} mRNA but decreases in mucin protein abundance, demonstrating that the effect of reduced threonine availability is at the
translational level of mucin production (Faure et al., 2005). Deficient threonine administration in piglets results in decreased mucin accumulation in the duodenum and colon compared to the adequate threonine fed counterparts (Law et al., 2007). In this study, adequate threonine resulted in stable numbers of goblet cells; however, these goblet cells were hypertrophied—or filled with more mucin—compared to deficient threonine fed piglets. In addition, adequate threonine goblet cells had more heterozygous chemotypes or contained a greater mix of mucins (Law et al., 2007). These studies suggest that adequate threonine supplementation plays a role in appropriate mucin production.

Altogether, PN has many basic complications; it reduces barrier function, promotes SBBO, decreases mucin abundance and ultimately increases the risk of bacterial translocation across the intestinal mucosa. The interconnected nature of inflammation, mucins, SBBO and barrier function in the process of bacterial translocation is evident, and as in all disease, prevention is the ideal management. Provision of EN in tolerable amounts is the best management for SBS as it will help to preserve mucosal integrity, barrier function, and mucin production, which together will reduce the risk of inflammation and bacterial translocation.

MODULATION OF MUCIN PRODUCTION

*Modulation via Stress, Nutritional Supplementation, and Growth Factors*

As just discussed, nutrition support and threonine can both affect the amount and type of mucin produced. Mucin can be affected by stress and other dietary factors as well. Mice exposed to repeated cold stress initially had an increase in mucin glycoprotein content after one stress exposure; however, goblet cell numbers were significantly decreased after just one stress exposure (Pfeiffer et al., 2001). This suggests that goblet cells can increase the rate in which they produce mucin in response to stress and become hypertrophied, or engorged, with mucin. Because cell development,
differentiation, and travel take a couple of days, hypertrophy is the most expected response to increase mucin in acute time frames in situations of normal proliferation. Even though stress elicits a beneficial response in mucin production, stress also causes a decrease in total goblet cell number. This study highlights the importance of reducing stress on an infant with intestinal failure so that goblet cell number is not reduced. In addition, more research needs to be conducted on how to increase mucin production without inducing goblet cell-reducing stress. One of these methods is by butyrate administration which will be discussed shortly.

Fiber can modulate mucin production. Rats that were administered TPN intravenously or fed with the TPN solution orally showed significantly decreased epithelial bound mucin and mucin within the goblet cells in the jejunum and the ileum. However, upon administration of 3.3 g cellulose per day, mucin concentration levels tend to increase, demonstrating that certain fiber may upregulate mucin production. Celluose, however, decreased unadhered luminal mucin abundance, probably because cellulose administration decreased transit time, removing unbound luminal mucin (Spaeth et al., 1994).

As discussed above, adequate provision of dietary threonine is crucial in intestinal mucin synthesis. Increasing oral threonine intake to 150% of requirements significantly increases mucin production in the jejunum (Faure et al., 2005). Another potential dietary modulator in mucin production is β-casomorphin-7, a milk degradation peptide resulting from the digestion of β-casein, which has been show to increase mucin production in vitro in rat and human goblet cells, demonstrating the potential of dairy products to improve mucosal protective function (Zoghbi et al., 2006).

Along with nutrition supplementation, growth factors—such as epidermal growth factor (EGF)—are important for intestinal growth. EGF is important for cell growth, proliferation, and differentiation (Cohen and Carpenter, 1975), and when provided luminally, decreases PN-associated
atrophy in rats administered TPN. In addition, EGF accelerates goblet cell maturation and mucin production and normalizes expression of tight junction proteins, all of which improve intestinal barrier function (Clark et al., 2006).

Modulation via the Short Chain Fatty Acid, Butyrate

Short chain fatty acids (SCFAs) are a subgroup of fatty acids including acetic, propionic, isobutyric, butyric, isovaleric, valeric, and caproic acids. The salts or ester of these acids are acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and caproate, respectively. The latter are produced \textit{in vivo} by fermentation of non-starch polysaccharides such as hemicelluloses, pectin, gums, and mucilages by the anaerobic microflora of the cecum and colon. The main SCFAs produced are acetate, propionate, and butyrate, which make up 83% of endogenous SCFAs. SCFAs in human colon are generally found to be at a 60:25:15 molar ratio of acetate, propionate, and butyrate, respectively (Cummings, 1984; Cummings and Englyst, 1987; Bourquin et al., 1992). Fermentative end products, however, can be influenced by the type of fermentable substrate available (Mortensen et al., 1988; Bourquin et al., 1992), the type of microbial species present in the intestine (Macfarlane and Gibson, 1995), and by intestinal transit time (Macfarlane et al., 1992). The opposite is also true, in that the type of fermentable substrate provided can influence the microbiota present (Wang and Gibson, 1993). Thus, the type of fiber, a common source of SFCA production, chosen to supplement EN or PN is important.

SCFAs can contribute about 10% of maintenance energy requirements in pigs (Imoto and Namioka, 1978). The production is hypothesized to be similar in humans due to the similarity between human and pig gastrointestinal tracts (Sangild, 2006). The energy contribution of SCFAs in humans has been estimated at about 10-15% (McNeil, 1984). Butyrate is preferentially absorbed
over propionate and acetate at a ratio of 90:30:50, and approximately 70-90% of butyrate produced in the intestines is used solely by the intestinal epithelium (Cummings, 1984).

Butyrate has been shown in vitro to modulate muc2 expression (Augenlicht et al., 2003; Willemsen et al., 2003; Gaudier et al., 2004; Hatayama et al., 2007; Burger-van Paassen et al., 2009). Paradoxically, low levels (1-2 mM) of butyrate stimulate muc2 expression (Willemsen et al., 2003; Hatayama et al., 2007; Burger-van Paassen et al., 2009), while higher levels of butyrate (5 mM-15 mM) either halt butyrate-stimulated increases of muc2 expression or even trigger mucous secretion (Barcelo et al., 2000; Burger-van Paassen et al., 2009). Bathing the human colon cancer cell line, LS174T, in 1-2 mM butyrate for 36 hours results in a 2-fold increase in muc2 mRNA and an 8-fold increase in Muc2 protein (Hatayama et al., 2007). This was corroborated by Burger-van Passaan and colleagues (2009) when 1 mM butyrate produced a 2-fold increase in muc2 mRNA levels when administered to LS174T cells. Also in this study, 5-15 mM butyrate showed a return of muc2 mRNA to basal levels. Additionally, 0.05 mM to 1 mM butyrate results in increased muc2 expression in monolayers of T84 cells, and cocultures of T84 and LS174T cells with CCD-18Co cells (Willemsen et al., 2003). In this study, butyrate was also shown to significantly increase prostaglandin E1 (PGE1) and decrease prostaglandin E2 (PGE2) concentrations in all dosage groups of cocultured cells. Prostaglandins are lipid compounds that come from the eicosanoid family of paracrine secretion (Saladin, 2004). They have been implicated in the regulation of mucous secretion (Plaisancie et al., 1998) and have been shown to be released from myofibroblasts, or a cell similar to both muscle and collagen, underneath the epithelial layer (Mahida et al., 1997). An increased PGE1/PGE2 ratio has been thought to be beneficial because PGE1 is more potent than PGE2 at stimulating muc2 expression in vitro (Willemsen et al., 2003).

Butyrate does not produce stimulatory effects in muc2 expression in all cell lines. muc2 expression is actually reduced upon butyrate bathing in Cl.16E cells (Augenlicht et al., 2003), a clonal
derivative of HT29 cells that are polarized and characterized by expression of the $muc2$ gene and mucin synthesis and secretion (Augeron and Laboisse, 1984; Capon et al., 1992; Velcich et al., 1995). Upon standard culturing of the Cl.16E cells, $muc2$ accumulated at day 7 and increased to a maximum level at day 15. At this point, these cells were treated with 5 mM butyrate which resulted in a 50% reduction in $muc2$ mRNA levels and a decrease in Muc2 apomucin after 24 hours (Augenlicht et al., 2003). These results seem to indicate that butyrate has a negative effect on goblet cells; however, the results can be explained by the butyrate concentration used and by referring to studies by others. Augenlicht and colleagues consistently used butyrate at the concentration of 5 mM, which as previously mentioned, has been shown to be inhibitory on $muc2$ expression (Burger-van Paassen et al., 2009). Also, Gaudier and colleagues (2004) found that in a glucose rich medium, Cl.16E cells show no increase in $muc2$ expression upon 2 mM butyrate treatment; however, in the absence of glucose, $muc2$ expression increased 23-fold above basal level. Thus, the metabolism of butyrate as a carbon source may play a role in the induction of Muc2 production. Augenlicht and colleagues (2003) bathed their Cl.16E cells in Dulbecco’s Modified Eagle Medium (DMEM) which depending on the solution, may contain glucose. Because the presence of glucose in butyrate administration also effects $muc2$ expression (Gaudier et al., 2004), it is possible that the lack of effect of butyrate on Muc2 levels found by Augenlicht and colleagues was due to the potential presence of glucose in the cell medium.

Thus, while 1 and 2 mM butyrate levels increase $muc2$ expression, 5 mM butyrate administration has been shown to reduce $muc2$ mRNA expression to baseline levels (Augenlicht et al., 2003; Burger-van Paassen et al., 2009). In addition to $muc2$ expression and mucin production, butyrate affects mucin secretion. Barcelo and colleagues (2000) demonstrated in vivo that 5 mM luminal butyrate administration more than doubled mucin secretion in isolated vascularly perfused rat colons. Mucins are secreted as protective measure in the intestine, and 5 mM butyrate appears to
trigger that protective response because that level is possibly perceived by the intestine as damaging or butyrate may serve as a signal for regulated mucin secretion.

One *ex vivo* study analyzed the effects of butyrate administration on mucin production. Butyrate administration of 0.1 mM resulted in a 476% mucin synthesis increase in comparison to the control tissue in fresh human colonic biopsies. The increase was nullified in the presence of sodium bromo-octanoate (an inhibitor of beta-oxidation), which reduces the ability for cells to metabolize fatty acids such as butyrate, furthering the hypothesis that metabolism of butyrate by colonocytes is an important mechanism in mucin synthesis (Finnie et al., 1995).

There have not been any *in vivo* studies conducted analyzing the effects of butyrate administration on mucin production, nor have there been any studies analyzing parenteral butyrate administration on mucin production, but butyrate has had strong positive effects on mucin production when administered luminally *in vitro* and *ex vivo*. The one *in vivo* study by Barcelo and colleagues did not ascertain the affects of luminal butyrate on mucin production or *muc2* expression; but studies in which fiber was administered *in vivo* (as previously discussed, fiber fermented by enteric bacteria produce short chain fatty acids) mucin production was increased. Satchithanandam and colleagues (1996) found that rats fed 10% psyllium had increased colonic luminal mucin, or loosely bound mucin. They also suggest that total mucin levels—which include membrane adherent mucins—were increased, but their determination methods of total mucin included protein assays of the scrapings of the entire mucosal layer (Satchithanandam et al., 1996). Increases in protein content in this case could be due to increases in villus height or other mucosal factors as butyrate positively affects factors other than mucin when administered luminally *in vivo* (Scheppach et al., 1992; Scheppach et al., 1997; Andoh et al., 1999; Vernia et al., 2000). Gaudier and colleagues have shown that cells may need to metabolize butyrate to increase mucin production and *muc2* expression; however, since butyrate positively affects so many factors when administered luminally *in vivo* (in
which glucose availability cannot be eliminated), butyrate may still be able to produce positive effects in the presence of glucose.

Administration of butyrate parenterally has been shown to enhance several indices of the intestinal structure and function. Parenterally provided butyrate enhances glucose transporter-2 (glut-2) mRNA, sodium glucose transporter-1 (slc5a1) mRNA, Na+,K+-adenosine triphosphatase (Na+,K+-ATPase) mRNA (a critical basolateral enterocyte transporter), proglucagon mRNA (a precursor to GLP-2 and incretins), plasma GLP-2 (an intestinotrophic peptide), glucose absorption, villus height, proliferation and has been shown to decrease apoptosis (Tappenden et al., 1997; Tappenden and McBurney, 1998; Bartholome et al., 2004). Because of the positive effects of butyrate seen in both luminal and parenteral administration, parenteral butyrate and SCFA administration holds promise as an intestinal mucin modulator as well, potentially providing protection from intestinal damage and bacterial translocation. This is the first study to describe the effects of parenterally supplied butyrate and SCFAs on muc2 mRNA expression and goblet cell chemotypes in a SBS model.
RESEARCH OBJECTIVE

Protective mucins are a crucial part of intestinal barrier defense. Mucins are even more important in neonates with SBS on PN since both SBS and PN increase the risk for bacterial translocation.

Several nutritional modulators of mucin production have been studied, with butyrate being effective in upregulating mucin production \textit{in vitro} and \textit{ex vivo}; however the effect of butyrate \textit{in vivo} is unknown. This research aims to analyze the effect of parenteral butyrate administration on mucin-producing goblet cell chemotypes in the small and large intestine using a PN-supported neonatal piglet SBS model. The data from this research explores the following:

1) the effects of \textbf{SCFA-supplemented TPN} on goblet cell chemotypes and \textit{muc2} mRNA in a massive small bowel resection neonatal piglet model;

2) the effects of \textbf{butyrate-supplemented TPN}, at doses of 9 mM and 60 mM, on goblet cell chemotypes and \textit{muc2} mRNA in a massive small bowel resection neonatal piglet model;

Because butyrate has been documented to improve so many factors of gastrointestinal structure and function, we hypothesize that infusion of TPN supplemented with 9 mM butyrate will enhance protective goblet cell chemotypes in the small intestine, specifically sulfomucins. Similarly, we expect enhancement of protective goblet cell chemotypes in the SCFA-supplemented group too, since this group also contains 9 mM butyrate. Further, we hypothesize that butyrate-supplemented TPN will have a greater effect on the small intestine than the large intestine, while butyrate supplementation at 60 mM will have a greater effect on the large intestine than the small intestine because the large intestine is more accustomed to SCFA and may need a larger dose to confer a benefit. As such, we expect \textit{muc2} mRNA abundance to increase in the small intestine with butyrate
and SCFA-supplemented TPN infusion, whereas we expect colonic \textit{muc2} mRNA abundance to increase in the colon with 60 mM butyrate administration.
Figure 1.1: Peyer’s patch in the neonatal piglet. The M cell does not contain the basolateral pocket that is characteristic of mature M cells. The antigen is present at the luminal surface of the FAE, and upon M cell engulfment, a dendritic cell recognizes the antigen as a pathogen and transports the antigen to the lamina propria in or beyond the SED or to the nearest MLN via the afferent lymphatic system.
CHAPTER 2
THE EFFECT OF BUTYRATE-SUPPLEMENTED TOTAL PARENTERAL NUTRITION ON muc2 mRNA AND GOBLET CELL CHEMOTYPES IN A SHORT BOWEL SYNDROME NEONATAL PIGLET MODEL

ABSTRACT

Background: Compromised barrier function and bacterial translocation are common complications of short bowel syndrome (SBS) and parenteral nutrition (PN). Although mucins help prevent intestinal bacteria translocation, total mucin production has been shown to decrease during PN infusion. Butyrate administration may be a mucin fortifying strategy as it has been shown to increase mucin production in vivo and ex vivo.

Objective: We tested the hypothesis that infusion of TPN supplemented with 9 mM butyrate will enhance protective goblet cell chemotypes in the small intestine, specifically sulfomucins, that enhancement of protective goblet cell chemotypes in the SCFA-supplemented group will be observed, since this group also contains 9 mM butyrate, that butyrate-supplemented TPN will have a greater effect on the small intestine than the large intestine, while butyrate supplementation at 60 mM will have a greater effect on the large intestine than the small intestine. We also hypothesize that muc2 mRNA abundance will increase in the small intestine with butyrate and SCFA-supplemented TPN infusion, whereas colonic muc2 mRNA abundance will increase in the colon with 60 mM butyrate administration.

Methods: Forty-eight hour old piglets underwent 80% proximal jejunuoileal resection and were randomized to one of four groups: control total parenteral nutrition (TPN), TPN supplemented with 60 mM SCFA (36 mM acetate, 15 mM propionate, and 9 mM butyrate; SCFA), 9 mM butyrate (9Bu) or 60 mM butyrate (60Bu). Animals were further randomized to an acute (12 hour) or chronic (72 hour) time point. muc2 mRNA abundance, total goblet cell number, and total sulfomucin, sialomucin, acidomucin, and neutral mucin goblet cell numbers were ascertained.
Results: Sulfomucin chemotypes were increased in the jejunal villi of the 9Bu group compared to control (treatment main effect, p=0.047). Acidomucin chemotypes in ileal crypts were greater in the 60Bu group than the control group (treatment main effect, p=0.038). In the colonic crypts, SCFA groups tended to have greater acidomucin chemotypes than control at 12h while 60Bu group tended to have greater acidomucin chemotypes per depth than control at 72h (p=0.060; Table 2.3). *muc2* mRNA was increased in jejunal tissues in the 9Bu group compared to control with 270% and 30% increases in *muc2* mRNA at 12 and 72 hours, respectively (p=0.010).

Conclusion: The 9 mM Bu treatment increased protective goblet cell chemotypes and *muc2* mRNA in the jejunum while 60 mM Bu increased protective goblet cell chemotypes in the ileum and colon. The 9Bu treatment was the most effective at upregulating *muc2* mRNA abundance and sulfomucin chemotypes. Butyrate-supplemented PN may be a therapeutic strategy for bolstering the epithelial barrier by increasing mucin production in neonates with SBS on PN.

INTRODUCTION

Within the first line of defense against invading pathogens—innate immunity—is intestinal mucous, the majority of which is secretory gel-forming mucins, predominantly Muc2, a mucous protein encoded by the *muc2* gene (Gum et al., 1989; Griffiths et al., 1990). Mucins are classified as acidomucins or neutral mucins. Acidomucins have either sulfate or sialic acid residues on their carbohydrate moieties and are called sulfomucins and sialomucins, respectively, whereas neutral mucins have no such residues (Hansson et al., 1991; Carlstedt et al., 1993). Sulfomucins and neutral mucins confer a more protective effect in the intestine than sialomucins because they are more slowly degraded by bacteria, therefore the established continuum of mucin protection is as follows: sulfomucins > neutral mucins >>> sialomucins (Fontaine et al., 1996).
Several studies confirm the protective nature of sulfomucins (Sakata and von Engelhardt, 1981; Deplancke et al., 2000; Conour et al., 2002; Dawson et al., 2009). It has been demonstrated that sulfomucins increase in concentration from the duodenum to the colon, mirroring the pattern of bacterial concentration from proximal to distal bowel (Sakata and von Engelhardt, 1981; Deplancke and Gaskins, 2001; Conour et al., 2002). Sulfated mucins have been shown to be essential in protecting against bacterial translocation (Dawson et al., 2009).

Muc2 is crucial in preventing bacterial contact with the epithelial lining (Johansson et al., 2008). Decreases in mucin abundance or in protective goblet cell chemotypes leave the epithelial barrier exposed to bacteria and bacterial products, which can lead to bacterial translocation (Gork et al., 1999). Direct contact of harmful luminal contents to the epithelial lining promotes inflammation, which can further impair barrier function (Yang et al., 2002; Yang et al., 2008) and increase the risk of bacterial translocation. Bolstering mucin quality and abundance may improve the outcome of SBS patients by preventing bacteria-induced mucosal inflammation and further complications such as bacterial translocation.

Mucin abundance is decreased by PN administration, which is the primary nutritional therapy for individuals with intestinal failure (Iiboshi et al., 1994; Bertolo et al., 1998; Law et al., 2007). The SCFA butyrate enhances mucin production in vitro and ex vivo (Finnie et al., 1995; Willemsen et al., 2003; Hatayama et al., 2007; Burger-van Paassen et al., 2009), but its direct effect on mucin chemotype and abundance in vivo and systemically administered as a component of PN has not been elucidated. This study analyzes the effect of parenteral butyrate and SCFA administration on both muc2 mRNA abundance and goblet cell chemotypes in a TPN-supported neonatal piglet with SBS. We hypothesized that infusion of TPN supplemented with 9 mM butyrate would enhance protective goblet cell chemotypes in the small intestine, specifically sulfomucins. Similarly, we expected enhancement of protective goblet cell chemotypes in the SCFA-supplemented group.
too, since this group also contained 9 mM butyrate. Further, we hypothesized that butyrate-supplemented TPN would have a greater effect on the small intestine than the large intestine, while butyrate supplementation at 60 mM would have a greater effect on the large intestine than the small intestine because the large intestine is more accustomed to SCFA and may need a larger dose to confer a benefit. As such, we expected muc2 mRNA abundance to increase in the small intestine with butyrate and SCFA-supplemented TPN infusion, whereas we expected colonic muc2 mRNA abundance to increase in the colon with 60 mM butyrate administration.

MATERIALS AND METHODS

Experimental Design

Piglets were obtained at 48 hours of age from the Imported Swine Research Laboratory at the University of Illinois at Urbana-Champaign. Piglets underwent superior vena cava cannulation, swivel placement and 80% proximal jejunuoileal resection and were then randomized by initial body weight (1.77 ± 0.16 kg, n = 120) to one of four groups: control TPN (TPN), TPN supplemented with 60 mM SCFA (36 mM acetate, 15 mM propionate, and 9 mM butyrate; SCFA), 9 mM butyrate (9Bu) or 60 mM butyrate (60Bu). Animals were further randomized to an acute 12 hour (12h) or a chronic 72 hour (72h) infusion time point, at which point samples were harvested and dependent variables assessed.

Surgery

The surgical protocol was approved by the Laboratory Animal Care Advisory Committee at the University of Illinois at Urbana-Champaign. Aseptic technique and sterile equipment were used at all times. Piglets were anesthetized with isoflurane (98% oxygen, 2% Isoflurane; Baxter Pharmaceutical Products, Inc., Deerfield, Illinois). Betadine (10% povidone-iodine; Purdue Frederick Co., Norwalk, Connecticut) and lidocaine (2% lidocaine HCl, 20mg/ml; Abbott
Laboratories, North Chicago, Illinois) were applied to the surgical sites prior to jugular
catheterization, laporotomy, and catheter externalization. A 3.5 French polyvinyl chloride catheter
(Bergen-Brunswig, Lack Zurick, Illinois) was inserted into the external jugular vein through a 3-cm
incision in the right clavicle region. The catheter was inserted 6-cm into the external jugular into the
superior vena cava for TPN infusion. After placement, the catheter was flushed with 5 mL of
heparinized saline (20 U/ml; Sigma-Aldrich Co., St. Louis, Missouri) and subcutaneously tunneled
and externalized in the subscapular region.

All piglets also received an 80% proximal jejunoileal resection, as follows. A laparotomy was
performed to the right of the umbilicus and 15-cm of jejunum distal to the ligament of Treitz and
75-cm of ileum proximal to the ileoceccal junction were measured. Following vessel stricture, the
measured small intestine was excised, weighed and measured, and an anastomosis performed to
reestablish bowel continuity. The peritoneum, muscularis, and subcutaneous layers were sutured
separately. Piglets’ activity levels were monitored during surgical recovery. Piglets received Naxcel®
antibiotic (3 mg/kg BW; SmithKline Beeham Corp., Philadelphia, Pennsylvania) prior to surgery and
during the entire study period. Piglets also received buprenorphine analgesic (.01 mg/kg BW; Henry
Schein Inc., Melville, New York) intramuscularly every 12 hours for the first 24 hours post-surgery
to minimize discomfort.

Animal Care and Housing

Piglets were fitted with jackets and swivel tethers (Alice King Chatham Medical Arts,
Hawthorne, California) to protect infusion lines while allowing the piglets to move freely in their
cages. Piglets were individually housed in metabolic cages in the Edward R. Madigan laboratory
animal care facility at the University of Illinois at Urbana-Champaign. Radiant heaters were placed
at the top of the cages to maintain temperature of about 34°C.
Parenteral Nutrition Solutions

PN solutions were prepared and sterilized daily in a laminar flow hood. The solutions contained dextrose, amino acids, lipids, and all essential minerals and vitamins (Table 2.1). SCFA and butyrate were added to the PN solutions at the concentrations previously mentioned. Volumetric infusion pumps (IMED 980; St. Louis, Missouri) were used to infuse PN. A stress factor or 20% was included in calculating energy and protein needs resulting in an infusion of 307 Kcal/Kg/d and 15.5 g amino acids/kg/d. The piglets received nutrition support continuously throughout the duration of the study. Nutrition needs were calculated daily and PN infusions rates adjusted accordingly to ensure adequate supply of energy and nutrients for growth and recovery from surgery.

Sample Collection

At euthanasia, the intestines were promptly removed. Proximal to the ligament of Treitz was considered the duodenum. Distal from this ligament to the anastomosis was considered the jejunum, and distal to the anastomosis and proximal to the ileocecal valve was considered the ileum. The colon was removed as well. All segments were flushed with ice-cold saline and samples from each segment were fixed in 10% buffered formalin. Formalin-fixed tissue was dehydrated in ethanol and infiltrated and embedded in paraffin wax. Blocks of tissue were sectioned to approximately 5 μm thickness at the Veterinary Medicine Center for Microscopic Imaging and sections were placed on polylysine slides for optimal adhesion during staining procedures.

Measurement of muc2 RNA

Total cellular RNA was isolated from jejunal, ileal, and colonic samples using the guanidium isothiocyanate phenolchloroform method of Chomczynski (1993) and as previously described in our
lab (Bartholome et al., 2004). RNA concentration and purity after isolation were determined using a Thermo Scientific NanoDrop 2000 (Thermo Scientific, Wilmington, DE). RNA quality was determined by running all samples through ethidium bromide RNA gel electrophoresis and examining under UV illumination using a Fotodyne Incorporated 3-3100 UV light box (Fotodyne Incorporated, Hartland, WI). In addition, 12 samples with varying 18S and 28S band intensities in gel electrophorosis were chosen for analysis in an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA) at the W.M. Keck Center for Comparative and Functional Genomics (University of Illinois at Urbana-Champaign, Urbana, IL) to further ascertain RNA integrity. The sample with the weakest 18S and 28S band under UV illumination after RNA gel electrophoresis produced a RNA integrity number (RIN) of 7.30 in the Agilent 2100 bioanalyzer. An RIN number is assigned by Agilent Technologies’ bioanalyzer software and is a representation of the entire electrophoretic trace of an RNA sample including degradation products. An RIN of greater than 7 is recommended for optimal rt-PCR (real time-polymerase chain reaction) results. Thus, all samples were of high quality and ideal for rtPCR.

Reverse transcription was carried out in a Gene Mate® Genius thermocycler (ISC Bioexpress, Kaysville, Utah) as a 20 µL total volume containing 5µg RNA reconstituted in 10 µL DEPC water, 1 µL random primer, 1 µL dNTP, 4 µL 5x First-Strand Buffer, 2 µL 0.1 M DTT, 1 µL RNaseOut, and 1 µL SuperScript II Reverse Transcriptase. All of these reagents were obtained from Invitrogen Corporation. rt-PCR consisted of a total reaction volume of 10 µL containing 2 µL DEPC water, 2 µL cDNA template, 5 µL of TaqMan® Universal PCR Master Mix, 0.5 µL eukaryotic 18S rRNA endogenous control primer and probe 20x (Applied Biosystems, Carlsbad, CA), .5 µL Muc2 Custom Made Taqman® Gene Expression Assays PCR primer and probe (Applied Biosystems, Foster City, CA). rt-PCR was completed using a Taqman ABI 7900 real time PCR machine (Applied Biosystems, Carlsbad, CA).
The oligonucleotide primers specific for Muc2 cDNA detection were made by Applied Biosystems Custom Made Taqman® Gene Expression Assays. The custom probe was designed using the National Center for Biotechnology Information (NCBI) nucleotide and protein databases and Primer Express 2.0 (Applied Biosystems, Carlsbad, CA). Because pig DNA for muc2 has not yet been elucidated, a search was completed for mouse muc2 mRNA through the nucleotide database in NCBI. Gi accession number 149258274 was chosen because it did not contain long segments of repeated codons. Because mucins commonly have similar protein backbones, especially in the center of the molecule, choosing a gene sequence with long segments of repeated codons would decrease specificity of the designed primer. A nucleotide blast was then completed comparing this chosen gene against sus scrofa expressed sequence tags (ESTs) with a low complexity filter to mask repeat regions. An 81% identity was found with EST: BX671371 which was then blasted against refseq RNA to establish if this EST matches muc2 mRNA already elucidated for other species. A significant alignment of 88% identity was found with NM 002457 or Homo sapiens muc2 mRNA. Then a blastx—protein comparison—of EST BX671371 against non-redundant protein sequences was performed resulting in 25 matches of significant alignment with elucidated muc2 proteins. The first 8 matches were 85%-87% similar to EST BX671371, with the 15% difference likely due to species differences in mucin proteins. Accession numbers CAD54416 (Muc2 protein Mus muscularis-922 amino acids) and AAA59861.1 (Muc2 protein Homo sapiens-131 amino acids) were blasted against each other with only an 82% alignment, demonstrating the interspecies variation of mucin proteins. BX671371 was entered into Primer Express 2.0 software, with temperature, cytosine and guanine content, and primer length at default settings, and the primer set with lowest penalty was chosen for custom primer and probe design. The resulting primer set requested from Applied Biosystems is as follows:

Forward: 5’ – TCCAAGCCTCTCCCTGCAT – 3’
Reverse: 5’ – ACAGCCACCAGGTCTCATTCTC – 3’
Probe: 5’ – CAGACTTCGATCCTCCCA – 3’

The rt-PCR product of pooled cDNA of all samples were run on a 1.8% agarose gel with a 50 base pair DNA ladder (New England Biolabs, Ipswich, MA) and visualized under UV illumination on a Molecular Imager® ChemiDoc™ XRS with Quantity One 4.6.3 software (Bio-Rad Laboratories, Hercules, CA) to determine the length of the amplicon to verify amplification of the target. One band was present for 18S mRNA while no band was seen for muc2 (Figure 2.1).

Measurement of Mucin Protein: Acidic versus Neutral Mucins

Samples used for goblet cell chemotype counting were formalin-fixed, sliced to 5 µm thickness and placed on polylysine slides. Samples were stained with periodic acid alcian blue (PASAB) carbohydrate stain technique (Mowry, 1956; Carson, 1990). Sections were deparaffinized, stained for 5 minutes with alcian blue, rinsed for 5 minutes in running tap water and 5 minutes in ddH₂O. They were then incubated for 10 minutes in 1.0% periodic acid, and rinsed again for 5 minutes in running tap water and ddH₂O, sequentially. A 10-minute incubation in Schiff’s reagent was conducted, followed by another sequential rinse of tap water, then ddH₂O. Lastly, slides were counterstained with Harris’s hematoxylin with acetic acid for 15 seconds, rinsed in running tap water for 10 minutes, dehydrated, and cover-slipped. PASAB stain colors neutral mucins pink and acidic mucins blue (Figure 2.2). Another scientist blinded the histologist to the sample origin. Up to 10 well-oriented and intact villi and crypt units were identified in each sample, length and depth measured, and goblet cells counted. Goblet cells were counted, then categorized to one of the following 5 groups: 1.) 100% acidomucin; 2.) 75% acidomucin and 25% neutral mucin; 3.) 50% acidomucin and 50% neutral mucin; 4.) 25% acidomucin and 75% neutral mucin, and; 5.) 100% neutral mucin. The mixed goblet cells (Figure 2.3) were partitioned into the group that most
closely matched its composition. In addition, to obtain solely acidomucin and neutral mucin values of all goblet cells, calculations of the mixed goblet cells—those containing both acidomucin and neutral mucin components—were completed to distribute acidomucin and neutral mucin components into either the 100% acidomucin chemotype or 100% neutral mucin chemotype groups. Images were obtained using a Zeiss Axioskop 40 microscope and a Zeiss AxioCam MRc 5 camera (Carl Zeiss IMT Corporation, Maple Grove, MN). Length and depth measurements of crypts and villi were obtained using AxiovisionLE Software (Release 4.7.2, Carl Zeiss). Images for publication were obtained using an Olympus BX51 microscope, an Olympus DP70 digital camera and DP Manager software (Olympus America Inc., Center Valley, PA).

**Measurement of Sulfomucin versus Sialomucin Goblet Cell Chemotypes**

Samples used for goblet cell chemotype counting were formalin-fixed, sliced to 5 µm thickness and placed on polylysine slides. Samples were stained with high iron diamine alcian blue (HIDAB) carbohydrate stain technique, as previously described (Spicer, 1965; Spicer et al., 1965; Carson, 1990), except incubation in high iron diamine solution was limited to 16 hours rather than 18 hours to reduce non-specific staining. Following high iron diamine stain, slides were washed in running tap water for 5 minutes and stained with alcian blue (pH 2.5) for 5 minutes, washed in running tap water for 2-3 minutes, counterstained with Nuclear Fast Red (Sigma Aldrich, St. Louis, MO) for 1-2 minutes, washed in running tap water for 5 minutes, dehydrated, and cover-slipped. HIDAB stain colors sulfated mucins brown and sialated mucins blue (**Figure 2.4**). Another scientist blinded the histologist to the sample origin. Up to 10 well-oriented and intact villi and crypt units were identified in each sample, length and depth measured, and goblet cells counted. Goblet cells were counted in one of the 5 following groups: 1.) 100% sulfomucin; 2.) 75% sulfomucin and 25% sialomucin; 3.) 50% sulfomucin and 50% sialomucin; 4.) 25% sulfomucin and
75% sialomucin; and 5.) 100% sialomucin. These mixed goblet cells (Figure 2.5) were partitioned into the group that most closely matched its composition. Calculations were carried out and imaged captured as described in the previous section.

Statistical Analysis

Data were analyzed using the mixed model in SAS statistical software (version 9.1; SAS Institute, Cary, NC). Analysis was completed using analysis of variance (ANOVA) for a split-plot design. Sources of variation include litter (block), time (main plot), and treatment (subplot). Residuals were output from the mixed model and the Shapiro-Wilk test was used to test for normality. Spearman correlation tests were performed on the residuals to test for homogeneity. Log or reciprocal transformations were performed for any data that was not normal or had unequal distribution of residuals. After p-values were obtained, data was retransformed to acquire the correct mean estimate and the standard error of retransformed data is presented as an average of standard error (SE) values calculated from both the upper and lower retransformed confidence intervals of group means. If the main effects for time or treatment, or the interaction of time*treatment were significant, the following post hoc comparisons were conducted: control versus 9Bu at 12h, control versus 60Bu at 12h, control versus SCFA at 12h, control versus 9Bu at 72h, control versus 60Bu at 72h, and control versus SCFA at 72h. These comparisons were completed for rt-PCR results, total goblet cell number, sulfomucin versus sialomucin chemotype abundance, and acidomucins versus neutral mucins chemotype abundance. Significant data are identified as p≤0.050 and trends identified by p≤0.100. Data were analyzed as one-tailed t-tests given the directional hypothesis being tested.
RESULTS

Body and Organ Weights

As previously reported by our research group, piglet body weight did not differ between control piglets and those receiving SCFA and butyrate supplementation at any point during the study (Bartholome et al., 2004). In addition, organ weights did not differ between groups at the conclusion of the study (Bartholome et al., 2004).

Total Goblet Cell Number

In the jejunal villi, 12h total goblet cell number per height tended to be greater than 72h total goblet cell number (time main effect, p=0.074; Table 2.2). In the ileal crypts, 12h goblet cell number per depth was significantly greater than that at 72h (time main effect, p=0.0004; Table 2.2). No significance was noted in the colon (Table 2.2).

Acidomucin and Neutral Mucin Goblet Cell Chemotypes

Within the jejunal and ileal crypts, 12h neutral chemotypes per crypt depth (in µm) were significantly greater than at 72h (p=0.005, p=0.012, respectively; Table 2.3). Within the ileal crypt, 12h acidomucin chemotypes per depth were also greater at 12h than at 72h (p=0.0003). Also within the ileal crypt, acidomucin chemotypes per depth were greater in the 60Bu group than the control group (treatment main effect, p=0.038; Figure 2.6). In the colonic crypts, SCFA groups tended to have greater acidomucin chemotypes per depth than control at 12h while 60Bu group tended to have greater acidomucin chemotypes per depth than control at 72h (p=0.060; Table 2.3).

Within the ileal villi, SCFA and 60Bu acidomucin chemotypes per villus height (in µm) were greater than control at 12h; however, these differences were not sustained at 72h (p=0.031; Table
No significant differences or trends were noted in the jejunum for either neutral mucin chemotypes or acidomucin chemotypes per height (Table 2.4).

**Sulfomucin and Sialomucin Goblet Cell Chemotypes**

Within the ileal crypts, sialomucin chemotypes per depth tended to be greater at 12h than at 72h (time main effect, p=0.054; Table 2.5). Within the colonic crypts, control sialomucin chemotypes per depth were greater than 9Bu, 60Bu, and SCFA at 72h (p=0.039; Table 2.5; Figure 2.8). No differences were noted in the jejunal crypts.

Within the jejunal villi, 9Bu had greater sulfomucin chemotypes per height than control (treatment main effect, p=0.047; Table 2.6; Figure 2.9). Also within the jejunal villi, sialomucin chemotypes per height tended to be greater at 72h than at 12h (p=0.058; Table 2.6). Within the ileal villi, control groups had greater sialomucin chemotypes than SCFA groups at 12h; however, this difference was not seen at 72h (p=0.0004; Table 2.6).

**Muc2 mRNA Abundance**

Within the jejunum, muc2 mRNA was greater in the 9Bu group than the control group (treatment main effect, p=0.010; Table 2.7; Figure 2.10). Within the ileum, muc2 mRNA was greater at 72h than at 12h (p=0.017; Table 2.7). No significance was noted in the colon.

**DISCUSSION**

The goal of this study was to investigate the effect of butyrate and SCFA administration via parenteral nutrition on mucin production in the jejunum, ileum, and colon. Our study revealed significant increases at the transcriptional level of mucin production and significant increases in protective goblet cell chemotypes upon butyrate supplementation. This study used a parenterally-
supported SBS neonatal piglet model that is recognized as an ideal model for gastrointestinal disease in the human infant for two reasons. First, the timing of gastrointestinal maturation is quicker than humans, but clusters in a similar ratio, meaning the pig is a close reflection of human maturation sequences but matures in much less time, naturally lending itself to the research setting (Sangild, 2006). Further, piglets have shown similar intestinal adaptation to humans post-resection (Heemskerk et al., 1999). Thus, due to the interspecies similarities in the gastrointestinal tract, the piglet’s mucin responses to resection, PN, and butyrate administration will likely be similar to humans’ response.

In this study, the number of acidomucin goblet cell chemotypes per crypt were nearly double that of the neutral mucin chemotypes in the colon of the control piglets which mirrors the natural state of mucin ratios seen in neonatal life in humans (Filipe et al., 1989; Deplancke and Gaskins, 2001), further demonstrating the validity of the piglet model for study of goblet cell development and chemotypes.

Gel electrophoresis of pooled cDNA samples produced a faint 18S band; however no muc2 band was present. The 18S band was estimated to be 18 ng while the lowest amount present in the DNA ladder was approximately 27 ng. Even though 40 uL of rt-PCR amplicon was injected into the sample well, the 18S band was still faint. rt-PCR demonstrated by CT counts that 18S was exponentially greater in quantity than muc2. Thus, we believe that muc2 is at a concentration that is high enough to be detected by rt-PCR, but not high enough to be visible on a gel. However, rt-PCR verified amplification of muc2. Additionally, Taqman primer and probe sets are highly sensitive and specific, and NCBI blasts confirmed that the primer and probe sets have significant alignment with Sus scrofa ESTs and muc2 mRNA of other species demonstrating that the primer and probe sets would amplify Sus scrofa muc2.
Sulfomucin goblet cell chemotypes increased within the jejunal villi of the 9Bu group. As previously mentioned, sulfomucins are the most protective against bacterial degradation, with 40% degraded at 4 hours after bacterial inoculation, followed by neutral mucins with 55% degraded at 4 hours post-inoculation, and followed lastly by sialomucins which are 90% degraded at 1 hour and almost completely degraded at 4 hours (Fontaine et al., 1998). With a 37.3% increase of villus sulfomucin chemotypes at 12h and a 10.7% increase at 72h, the 9Bu group likely has an improved protective barrier against bacteria than the control group. The increase in sulfomucin chemotype in the 9Bu group was mirrored by significant jejunal $muc2$ mRNA increases in the 9Bu group. A 270% increase in $muc2$ mRNA was noted at 12h and a 30% increase was observed at 72h. Enhancement by 9Bu at the transcriptional level and enhancement of protective goblet cell chemotypes were greater at the acute time point (12h) than the chronic time point (72h), suggesting that butyrate administration may be more beneficial for the jejunum immediately after small bowel resection. Because 9Bu had positive effects but SCFA did not (SCFA is 60mM total concentration SCFA with 9mM butyrate), 60mM total concentration may be inhibitory in the jejunum. This will be discussed in more depth shortly.

The distal small bowel did not show any significant improvements versus control with 9Bu treatment. In the ileum, 60Bu significantly enhanced crypt acidomucin chemotypes compared to control (treatment main effect). There was a 16.4% increase at 12h and a 32.6% increase at 72h. In addition, 60Bu and SCFA enhanced villus acidomucin chemotypes at 12h by 75.5% and 44.7%, respectively. These data indicate that butyrate supplementation of 60Bu is most beneficial for the ileum and that benefits are seen at both acute and chronic time points. They also indicate that since 60Bu and SCFA—both 60mM treatments, but differing butyrate concentrations—had positive effects, that concentration of short chain fatty acids, rather than type of short chain fatty acid may be the mechanism by which the mucin is enhanced in the ileum. Conversely, since 60Bu produced a
greater increase in acidomucin chemotypes than the SCFA group, one could also argue that butyrate was the source of mucin enhancement, and that the greater concentration of butyrate may have increased the benefit.

Colonie goblet cell abundance and chemotype was not altered by the 9Bu treatment. However, SCFA and 60Bu tended to increase acidomucin chemotypes at 12h and at 72h, respectively. This again suggests that the distal bowel may need greater butyrate concentrations than the jejunum to confer benefits.

There are a couple possibilities for why the jejunum may have responded positively to 9Bu while the ileum and colon responded positively to 60Bu. The 9mM dosage of butyrate administered to the piglets averaged 2.21mM/hour for the average piglet weight, while the 60mM butyrate dosage averaged 14.9mM/hour. As discussed previously, 1-2 mM of butyrate has been shown to be beneficial upon luminal administration, while 5-15 mM has been shown to be detrimental (Barcelo et al., 2000; Willemsen et al., 2003; Hatayama et al., 2007; Burger-van Paassen et al., 2009). No studies have been conducted on an upper limit of butyrate administered parenterally; however, it seems there may be an upper limit of butyrate tolerance for the jejunum, at least. Because bacterial concentrations increase from proximal to distal bowel, the ileum is accustomed to greater SCFA concentration than the jejunum. Therefore, it is conceivable that a higher concentration of butyrate is needed—even when administered parenterally—to stimulate beneficial effects in the ileum versus the jejunum. Furthermore, because the jejunum is exposed to less fermentative products such as butyrate, 60mM butyrate may be beyond the tolerable level of short chain fatty acids for the jejunum. As previously shown, plasma concentrations of SCFA are not elevated in this piglet model with administration of 60 mM SCFA, 9 mM butyrate, and 60 mM butyrate (Bartholome et al., 2004), indicating that either the upper limit was not reached with these dosages, or an upper limit cannot be defined by remaining plasma concentrations. Rather, upper limits may need to be defined by
measured structural and functional indices of the intestine such as protective goblet cell chemotype abundance.

Sialomucins have been shown to be less protective than sulfomucins and neutral mucins (Fontaine et al., 1998). In this study, control groups had greater sialomucin chemotypes than the SCFA group at 12h in the ileum and control groups in the colon had greater sialomucin chemotypes than all treatment groups at 72h. Sialomucin chemotypes only comprise 2% of the total colonic goblet cells in these tissues, so although the decrease in sialomucin chemotypes was seen, it was a negligible decrease as sialomucins are such a minor component of large intestinal mucin. Further, humans have an even greater ratio of sulfomucins/sialomucins than piglets (Deplancke and Gaskins, 2001). Due to sialomucins not being as protective as other mucins, sialomucin chemotypes composing a small percentage of total colonic goblet cells, and the greater sulfomucin/sialomucin ratio in humans, decreases in sialomucins by SCFA and butyrate administration are statistically but not clinically significant. Although neither a decrease nor an increase in sialomucins is clinically significant, additional mucins no matter how easily degraded could only be beneficial as long as there is no concomitant decrease in sulfomucins or neutral mucins. A significant increase in sialomucin chemotypes was noted in the jejunal villus with 9Bu, which could only be a beneficial adaptation.

Significant time differences were noted in that jejunal crypt neutral mucin chemotypes and ileal crypt neutral mucin, acidomucin and sialomucin chemotypes were greater at 12h than at 72h (time main effect). Because there were no treatment differences, the apparent decrease in goblet cell chemotypes from 12-72h were not due to SCFA or butyrate administration. Rather, there was a trend for a decrease in jejunal crypt total goblet cells and a significant decrease in ileal crypt total goblet cells, showing that this is likely a natural physiological change seen in piglets recovering from massive bowel resection. The decrease of total goblet cell number at 72h can be explained by the effect parenteral nutrition has on mucin production and other indices of intestinal functioning.
previously mentioned. It may be that the upregulation in mucins provided by butyrate treatment or adaptation cannot outweigh the increasingly damaging effects that PN administration has on mucosal function and morphology over time. As we have previously reported, this piglet model experiences an increased rate of crypt cell proliferation with SCFA and butyrate administration compared to control piglets; however, all piglets have a decreased rate of crypt cell proliferation at 72h compared to 12h demonstrating that administration of SCFA and butyrate may not be able to overcome the negative effects of PN administration over time (Bartholome et al., 2004).

The regulation of mucin expression has not been established in this study or in other literature. In this study, ileal \textit{muc2} was greater at 72h than at 12h, but ileal crypt goblet cells were greater at 12h than at 72h with no significant differences noted in the ileal villi. This suggests that goblet cell chemotype development is regulated at the translational level. However, jejunal regulation of mucin expression appears to be transcriptionally regulated. \textit{muc2} mRNA abundance in the jejunum was increased by 9Bu treatment with a concomitant increase in jejunal sulfomucin and sialomucin chemotypes, indicating that an increase in mucin gene transcription may lead to an increase in mucin protein translation. Further study needs to be conducted to clarify the mechanism(s) of mucin and goblet cell chemotype regulation and how these methods may vary along the length of the intestinal tract.

In summary, with adequate nutritional provision, upregulations of protective goblet cell chemotypes and \textit{muc2} expression occurred with butyrate and SCFA administration. The 9Bu treatment produced the best improvement of the jejunal mucin profile, while SCFA and 60Bu treatments improved the ileal mucin profile. Because of the discrepancy in benefit between treatments, the ideal treatment may depend on the etiology of intestinal failure and resulting bowel resection. A larger remaining jejunum than ileum post-resection may have greater benefit from the
9Bu treatment while a greater remaining ileum versus jejunum post-resection may receive greater benefit from the SCFA or 60Bu treatments.

In conclusion, SCFA-supplemented TPN enhances protective goblet cell chemotypes in the neonatal intestine of a SBS model piglet, and butyrate-supplemented TPN enhances both $muc2$ mRNA and protective goblet cell chemotypes in the neonatal intestine of a SBS model piglet. Therefore, supplementation of TPN with SCFA or butyrate may benefit infants with SBS by increasing protective goblet cell chemotypes and $muc2$ expression, potentially decreasing PN-associated complications such as bacterial translocation.
Table 2.1: Composition of parenteral nutrient solutions

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>TPN¹</th>
<th>TPN + 9Bu²</th>
<th>TPN + 60Bu²</th>
<th>TPN + SCFA²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose¹⁴ (ml/L)</td>
<td>235.3</td>
<td>231.9</td>
<td>216.2</td>
<td>224.2</td>
</tr>
<tr>
<td>Amino acids¹⁵ (ml/L)</td>
<td>594.0</td>
<td>594.0</td>
<td>594.0</td>
<td>594.0</td>
</tr>
<tr>
<td>Lipids⁴,⁶ (ml/L)</td>
<td>133.3</td>
<td>133.3</td>
<td>133.3</td>
<td>133.3</td>
</tr>
<tr>
<td>Acetic acid⁷ (mmol/L)</td>
<td>__</td>
<td>__</td>
<td>__</td>
<td>36.0</td>
</tr>
<tr>
<td>Propionic acid⁷ (mmol/L)</td>
<td>__</td>
<td>__</td>
<td>__</td>
<td>15.0</td>
</tr>
<tr>
<td>n-Butyric acid⁷ (mmol/L)</td>
<td>__</td>
<td>9.0</td>
<td>60.0</td>
<td>9.0</td>
</tr>
<tr>
<td>MTE-6⁸,⁹ (µl/L)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Iron dextran¹⁰ (µl/L)</td>
<td>111.0</td>
<td>111.0</td>
<td>111.0</td>
<td>111.0</td>
</tr>
<tr>
<td>Calcium gluconate⁹,¹¹ (µl/L)</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Vitamin A + E + D¹² (µl/L)</td>
<td>23.5</td>
<td>23.5</td>
<td>23.5</td>
<td>23.5</td>
</tr>
<tr>
<td>Vitamin K¹³ (µl/L)</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Vitamin C¹⁴,¹⁵ (µl/L)</td>
<td>320.0</td>
<td>320.0</td>
<td>320.0</td>
<td>320.0</td>
</tr>
<tr>
<td>Vitamin B complex¹⁴,¹⁶</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Folic acid⁷ (µl/L)</td>
<td>28.0</td>
<td>28.0</td>
<td>28.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Biotin⁷,¹⁸ (µl/L)</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Sterile water (ml/L)</td>
<td>34.4</td>
<td>37.0</td>
<td>48.2</td>
<td>41.4</td>
</tr>
</tbody>
</table>

¹TPN represents a standard total parenteral nutrient solution providing; energy, 1 Kcal/ml; carbohydrate, 100mg/ml; amino acids 50.5 mg/ml; lipid 40 mg/ml.
²TPN + 9Bu, TPN + 60Bu, and TPN + SCFA represent standard total parenteral nutrient solution plus the short-chain fatty acids listed.
³50% dextrose (50 g dextrose/100 ml).
⁵8.5% Trasol®; (leucine 529 mg, phenylalanine 526 mg, lysine 492 mg, methionine 492 mg, isoleucine 406 mg, valine 390 mg, histidine 372 mg, threonine 356 mg, tryptophan 152 mg, alanine 176 mg, glycine 176 mg, arginine 880 mg, proline 356 mg, tyrosine 34 mg, Na⁺ 70 mM, K⁺ 60 mM, Mg²⁺ 5 mM, Ca²⁺ 70 mM, PO₄⁻ 30 mM, acetate 130 mM); Baxter Healthcare Corporation.
⁶30% Intralipid® (soybean oil 30g phospholipids 1.2g glycerine 1.7g)/100 ml; Baxter Healthcare Corporation.
⁷Sigma-Aldrich Co., St. Louis, Missouri.
⁸Contains: chromium .3 µmol/L; copper 25.2 µmol/L; iodine 1.9 µmol/L; manganese 21.6 µmol/L; selenium 1.0 µmol/L; zinc 492 µmol/L.
⁹Fujisawa USA, Inc. Deerfield, Illinois.
¹⁰Contains; elemental iron 100mg/ml; Rhone Merieux, Inc., Athens, Georgia.
¹¹Contains: calcium 2.14g/100 ml.
Table 2.1, continued

12Vital E®-A+D Durvet; Contains: vitamin A 2331 IU; vitamin E 233 IU; vitamin D 7 IU; Boehringer Ingelheim Animal Health, Inc., St. Joseph, Missouri.
13Contains; phytonadione 10 mg/ml; K-Ject Vetus Animal Health; Burns Veterinary Supply Inc., Rockville Centre, New York.
15Contains: sodium ascorbate 250 mg/ml.
16Contains: thiamin hydrochloride (B₁) 12.5 mg/ml; niacinamide (B₃) 12.5 mg/ml; pyridoxine hydrochloride (B₆); d-panthenol 5 mg/ml; riboflavin (B₂; as riboflavin 5’ phosphate sodium) 2 mg/ml; cyanocobalamin (B₁₂) 5 µg/ml.
17Contains: folic acid 5 mg/ml; American Pharmaceutical Partners, Inc., Los Angeles, California.
18Contains: d-biotin 20 mg/ml.
Table 2.2: Effect of short-chain fatty acid-supplemented total parenteral nutrition on total villus and crypt goblet cells following massive small bowel resection in neonatal piglets\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Control</th>
<th>9 Bu</th>
<th>60 Bu</th>
<th>SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VILLUS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.038 ± 0.006</td>
<td>0.042 ± 0.007</td>
<td>0.030 ± 0.004</td>
<td>0.036 ± 0.006</td>
</tr>
<tr>
<td>72h</td>
<td>0.036 ± 0.005</td>
<td>0.034 ± 0.005</td>
<td>0.039 ± 0.006</td>
<td>0.033 ± 0.005</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.025 ± 0.004</td>
<td>0.021 ± 0.006</td>
<td>0.021 ± 0.007</td>
<td>0.041 ± 0.007</td>
</tr>
<tr>
<td>72h</td>
<td>0.029 ± 0.006</td>
<td>0.036 ± 0.006</td>
<td>0.025 ± 0.005</td>
<td>0.027 ± 0.005</td>
</tr>
<tr>
<td><strong>CRYPT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum\textsuperscript{3}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.093 ± 0.007</td>
<td>0.095 ± 0.007</td>
<td>0.085 ± 0.007</td>
<td>0.101 ± 0.010</td>
</tr>
<tr>
<td>72h</td>
<td>0.076 ± 0.008</td>
<td>0.082 ± 0.007</td>
<td>0.088 ± 0.008</td>
<td>0.091 ± 0.007</td>
</tr>
<tr>
<td>Ileum\textsuperscript{4}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.096 ± 0.009</td>
<td>0.103 ± 0.00802</td>
<td>0.109 ± 0.008</td>
<td>0.106 ± 0.008</td>
</tr>
<tr>
<td>72h</td>
<td>0.073 ± 0.009</td>
<td>0.080 ± 0.008</td>
<td>0.094 ± 0.008</td>
<td>0.081 ± 0.008</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.126 ± 0.011</td>
<td>0.113 ± 0.011</td>
<td>0.120 ± 0.012</td>
<td>0.137 ± 0.013</td>
</tr>
<tr>
<td>72h</td>
<td>0.116 ± 0.013</td>
<td>0.121 ± 0.012</td>
<td>0.133 ± 0.015</td>
<td>0.123 ± 0.013</td>
</tr>
</tbody>
</table>

\textsuperscript{1} All values are mean ± SEM expressed as relative goblet cell number per depth and is adjusted to include neutral and acidic fractions of mixed cells. Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1.

\textsuperscript{2} Abbreviations: 9 mM butyrate (9Bu), 60 mM butyrate (60Bu), 60 mM short chain fatty acid (SCFA).

\textsuperscript{3} Within this dependent variable, 12h tended to be greater than 72h (time main effect, p=0.074).
Table 2.2, continued

4 Within this dependent variable, 12h is greater than 72h (time main effect, p=0.0004).
Table 2.3: Effect of short-chain fatty acid-supplemented total parenteral nutrition on neutral and acidic goblet cell chemotypes following massive small bowel resection in neonatal piglets \(^1,2\)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Control</th>
<th>9 Bu</th>
<th>60 Bu</th>
<th>SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NEUTRAL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum (^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.0383 ± 0.0043</td>
<td>0.0377 ± 0.0043</td>
<td>0.0284 ± 0.0043</td>
<td>0.0405 ± 0.0062</td>
</tr>
<tr>
<td>72h</td>
<td>0.0243 ± 0.0054</td>
<td>0.0235 ± 0.0043</td>
<td>0.0264 ± 0.0048</td>
<td>0.0247 ± 0.0045</td>
</tr>
<tr>
<td>Ileum (^4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.0285 ± 0.0039</td>
<td>0.0274 ± 0.0034</td>
<td>0.0309 ± 0.0039</td>
<td>0.0271 ± 0.0037</td>
</tr>
<tr>
<td>72h</td>
<td>0.0230 ± 0.0028</td>
<td>0.0233 ± 0.0029</td>
<td>0.0258 ± 0.0033</td>
<td>0.0231 ± 0.0029</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.0479 ± 0.0055</td>
<td>0.0380 ± 0.0055</td>
<td>0.0421 ± 0.0061</td>
<td>0.0410 ± 0.0070</td>
</tr>
<tr>
<td>72h</td>
<td>0.0356 ± 0.0068</td>
<td>0.0358 ± 0.0060</td>
<td>0.0296 ± 0.0080</td>
<td>0.0453 ± 0.0069</td>
</tr>
<tr>
<td><strong>ACIDIC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.0012 ± 0.0003</td>
<td>0.0013 ± 0.0003</td>
<td>0.0013 ± 0.0003</td>
<td>0.0015 ± 0.0006</td>
</tr>
<tr>
<td>72h</td>
<td>0.0010 ± 0.0003</td>
<td>0.0013 ± 0.0003</td>
<td>0.0011 ± 0.0003</td>
<td>0.0017 ± 0.0005</td>
</tr>
<tr>
<td>Ileum (^5,6)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>12h</td>
<td>0.0669 ± 0.0056</td>
<td>0.0746 ± 0.0051</td>
<td>0.0779 ± 0.0051</td>
<td>0.0691 ± 0.0056</td>
</tr>
<tr>
<td>72h</td>
<td>0.0516 ± 0.0056</td>
<td>0.0543 ± 0.0051</td>
<td>0.0684 ± 0.0051</td>
<td>0.0571 ± 0.0051</td>
</tr>
<tr>
<td>Colon (^7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.0779 ± 0.0070</td>
<td>0.0754 ± 0.0070</td>
<td>0.0779 ± 0.0078</td>
<td>0.0961 ± 0.0088</td>
</tr>
<tr>
<td>72h</td>
<td>0.0802 ± 0.0087</td>
<td>0.0856 ± 0.0078</td>
<td>0.1040 ± 0.0102</td>
<td>0.0785 ± 0.0090</td>
</tr>
</tbody>
</table>
Table 2.3, continued

1 All values are mean ± SEM expressed as relative goblet cell number per depth and is adjusted to include neutral and acidic fractions of mixed cells. Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1.

2 Abbreviations: 9 mM butyrate (9Bu), 60 mM butyrate (60Bu), 60 mM short chain fatty acid (SCFA).

3 Within this dependent variable, 12h was greater than 72h (time main effect, p=0.005).

4 Within this dependent variable, 12h was greater than 72h (time main effect, p=0.012).

5 Within this dependent variable, 12h was greater than 72h (time main effect, p=0.0003).

6 Within this dependent variable, 60Bu was greater than control (treatment main effect, p=0.038).

7 Within this dependent variable, SCFA tended to be greater than control at 12h and 60Bu tended to be greater than control at 72h (0.060).
<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Control</th>
<th>9 Bu</th>
<th>60 Bu</th>
<th>SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>NEUTRAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.01861 ± 0.00451</td>
<td>0.02178 ± 0.00594</td>
<td>0.01210 ± 0.00266</td>
<td>0.01490 ± 0.00407</td>
</tr>
<tr>
<td>72h</td>
<td>0.01370 ± 0.00301</td>
<td>0.01406 ± 0.00309</td>
<td>0.01425 ± 0.00313</td>
<td>0.01294 ± 0.00284</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.00006 ± 0.00003</td>
<td>0.00009 ± 0.00005</td>
<td>0.00006 ± 0.00004</td>
<td>0.00011 ± 0.00006</td>
</tr>
<tr>
<td>72h</td>
<td>0.00003 ± 0.00002</td>
<td>0.00006 ± 0.00003</td>
<td>0.00006 ± 0.00004</td>
<td>0.00004 ± 0.00003</td>
</tr>
<tr>
<td>ACIDIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.01862 ± 0.00318</td>
<td>0.01821 ± 0.00349</td>
<td>0.01730 ± 0.00269</td>
<td>0.02037 ± 0.00390</td>
</tr>
<tr>
<td>72h</td>
<td>0.01994 ± 0.00310</td>
<td>0.01885 ± 0.00293</td>
<td>0.01929 ± 0.00299</td>
<td>0.01885 ± 0.00293</td>
</tr>
<tr>
<td>Ileum³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.00930 ± 0.00199</td>
<td>0.01346 ± 0.00320</td>
<td>0.01632 ± 0.00441</td>
<td>0.02161 ± 0.00462</td>
</tr>
<tr>
<td>72h</td>
<td>0.01622 ± 0.00387</td>
<td>0.02027 ± 0.00437</td>
<td>0.01032 ± 0.00280</td>
<td>0.01533 ± 0.00376</td>
</tr>
</tbody>
</table>

¹ All values are mean ± SEM expressed as relative goblet cell number per depth and is adjusted to include neutral and acidic fractions of mixed cells. Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1
² Abbreviations: 9 mM butyrate (9Bu), 60 mM butyrate (60Bu), 60 mM short chain fatty acid (SCFA).
³ Within this dependent variable, SCFA and 60Bu were greater than control at 12h (p=0.031); however these differences were not sustained at 72h.
Table 2.5: Effect of short-chain fatty acid-supplemented total parenteral nutrition on crypt sulfomucin and sialomucin chemotypes per crypt depth following massive small bowel resection in neonatal piglets\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Control</th>
<th>9 Bu</th>
<th>60 Bu</th>
<th>SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SULFATED</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.06551 ± 0.01052</td>
<td>0.06391 ± 0.01052</td>
<td>0.05886 ± 0.00969</td>
<td>0.07864 ± 0.00969</td>
</tr>
<tr>
<td>72h</td>
<td>0.06376 ± 0.00963</td>
<td>0.06069 ± 0.00963</td>
<td>0.06384 ± 0.00958</td>
<td>0.05311 ± 0.01003</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.04090 ± 0.00991</td>
<td>0.03207 ± 0.00738</td>
<td>0.04613 ± 0.01605</td>
<td>0.04947 ± 0.01137</td>
</tr>
<tr>
<td>72h</td>
<td>0.04394 ± 0.01014</td>
<td>0.04626 ± 0.00995</td>
<td>0.04510 ± 0.00960</td>
<td>0.05370 ± 0.01213</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.13490 ± 0.00945</td>
<td>0.13690 ± 0.01059</td>
<td>0.13130 ± 0.01015</td>
<td>0.13350 ± 0.01015</td>
</tr>
<tr>
<td>72h</td>
<td>0.13470 ± 0.01189</td>
<td>0.13700 ± 0.01048</td>
<td>0.12280 ± 0.01409</td>
<td>0.13180 ± 0.01074</td>
</tr>
<tr>
<td>SIALYLATED</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.00008 ± 0.00017</td>
<td>0.00027 ± 0.00035</td>
<td>0.00045 ± 0.00048</td>
<td>0.00010 ± 0.00018</td>
</tr>
<tr>
<td>72h</td>
<td>0.00004 ± 0.00011</td>
<td>0.00013 ± 0.00019</td>
<td>0.00001 ± 0.00009</td>
<td>0.00019 ± 0.00026</td>
</tr>
<tr>
<td>Ileum\textsuperscript{3}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.05824 ± 0.00844</td>
<td>0.06547 ± 0.00844</td>
<td>0.05856 ± 0.00895</td>
<td>0.04849 ± 0.00844</td>
</tr>
<tr>
<td>72h</td>
<td>0.04580 ± 0.00799</td>
<td>0.04242 ± 0.00807</td>
<td>0.03523 ± 0.00867</td>
<td>0.04545 ± 0.00845</td>
</tr>
<tr>
<td>Colon\textsuperscript{4}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.00101 ± 0.00057</td>
<td>0.00051 ± 0.00062</td>
<td>0.00114 ± 0.00062</td>
<td>0.00084 ± 0.00062</td>
</tr>
<tr>
<td>72h</td>
<td>0.00304 ± 0.00070</td>
<td>0.00082 ± 0.00062</td>
<td>0.00004 ± 0.00081</td>
<td>0.00004 ± 0.00065</td>
</tr>
</tbody>
</table>
Table 2.5, continued

1 All values are mean ± SEM expressed as relative goblet cell number per depth and is adjusted to include sulfated and sialylated fractions of mixed cells. Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1
2 Abbreviations: 9 mM butyrate (9Bu), 60 mM butyrate (60Bu), 60 mM short chain fatty acid (SCFA).
3 Within this dependent variable, 12h tended to be greater than 72h (time main effect, p=0.054).
4 Within this dependent variable, control was greater than 9Bu, 60Bu, and SCFA at 72h (p=0.039).
Table 2.6: Effect of short-chain fatty acid-supplemented total parenteral nutrition on villus sulfomucin and sialomucin chemotypes per villus height following massive small bowel resection in neonatal piglets$^{1,2}$

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Control</th>
<th>9 Bu</th>
<th>60 Bu</th>
<th>SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SULFATED</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum$^3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.0343 ± 0.0038</td>
<td>0.0471 ± 0.0038</td>
<td>0.0395 ± 0.0034</td>
<td>0.0371 ± 0.0034</td>
</tr>
<tr>
<td>72h</td>
<td>0.0364 ± 0.0034</td>
<td>0.0403 ± 0.0034</td>
<td>0.0340 ± 0.0038</td>
<td>0.0351 ± 0.0035</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.0321 ± 0.0043</td>
<td>0.0317 ± 0.0038</td>
<td>0.0383 ± 0.0057</td>
<td>0.0343 ± 0.0045</td>
</tr>
<tr>
<td>72h</td>
<td>0.0280 ± 0.0031</td>
<td>0.0315 ± 0.0037</td>
<td>0.0290 ± 0.0031</td>
<td>0.0334 ± 0.0044</td>
</tr>
<tr>
<td><strong>SIALYLATED</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum$^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.0004 ± 0.0003</td>
<td>0.0008 ± 0.0005</td>
<td>0.0005 ± 0.0004</td>
<td>0.0004 ± 0.0003</td>
</tr>
<tr>
<td>72h</td>
<td>0.0010 ± 0.0006</td>
<td>0.0017 ± 0.0009</td>
<td>0.0006 ± 0.0004</td>
<td>0.0021 ± 0.0012</td>
</tr>
<tr>
<td>Ileum$^5$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.0027 ± 0.0006</td>
<td>0.0035 ± 0.0006</td>
<td>0.0019 ± 0.0006</td>
<td>0.0008 ± 0.0006</td>
</tr>
<tr>
<td>72h</td>
<td>0.0015 ± 0.0006</td>
<td>0.0005 ± 0.0005</td>
<td>0.0024 ± 0.0005</td>
<td>0.0021 ± 0.0006</td>
</tr>
</tbody>
</table>

$^1$ All values are mean ± SEM expressed as relative goblet cell number per depth and is adjusted to include sulfated and sialylated fractions of mixed cells. Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1.

$^2$ Abbreviations: 9 mM butyrate (9Bu), 60 mM butyrate (60Bu), 60 mM short chain fatty acid (SCFA).

$^3$ Within this dependent variable, 9Bu was greater than control, (treatment main effect, p=0.047).

$^4$ Within this dependent variable, 72h tended to be greater than 12h (time main effect, p=0.058).

$^5$ Within this dependent variable, control was greater than SCFA at 12h; however this difference was not seen at 72h (p=0.0004).
Table 2.7: Effect of short-chain fatty acid supplemented total parenteral nutrition on muc2 mRNA following massive small bowel resection in neonatal piglets\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Control</th>
<th>9 Bu</th>
<th>60 Bu</th>
<th>SCFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum\textsuperscript{3}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.571 ± 0.193</td>
<td>2.112 ± 0.788</td>
<td>0.775 ± 0.225</td>
<td>0.930 ± 0.269</td>
</tr>
<tr>
<td>72h</td>
<td>0.878 ± 0.254</td>
<td>1.138 ± 0.336</td>
<td>0.800 ± 0.232</td>
<td>0.578 ± 0.195</td>
</tr>
<tr>
<td>Ileum\textsuperscript{4}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.885 ± 0.264</td>
<td>0.723 ± 0.264</td>
<td>1.004 ± 0.264</td>
<td>0.562 ± 0.264</td>
</tr>
<tr>
<td>72h</td>
<td>0.883 ± 0.264</td>
<td>1.494 ± 0.264</td>
<td>1.052 ± 0.262</td>
<td>1.138 ± 0.262</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h</td>
<td>0.508 ± 0.187</td>
<td>0.461 ± 0.226</td>
<td>0.671 ± 0.083</td>
<td>0.593 ± 0.156</td>
</tr>
<tr>
<td>72h</td>
<td>0.636 ± 0.167</td>
<td>0.712 ± 0.187</td>
<td>0.446 ± 0.118</td>
<td>0.556 ± 0.146</td>
</tr>
</tbody>
</table>

\textsuperscript{1} All values are mean ± SEM expressed as \textit{muc2} mRNA/18S mRNA. Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1.

\textsuperscript{2} Abbreviations: 9 mM butyrate (9Bu), 60 mM butyrate (60Bu), 60 mM short chain fatty acid (SCFA).

\textsuperscript{3} Within this dependent variable, 9Bu was greater than control (treatment main effect, \(p=0.010\)).

\textsuperscript{4} Within this dependent variable, 72h was greater than 12h (\(p=0.017\)).
Figure 2.1: Gel electrophoresis. DNA ladder is present in the left lane with the bottom four bands being 50 base pairs, 100 base pairs, 150 base pairs, and 200 base pairs. The product of rt-PCR of the cDNA of all samples pooled is in the right lane and contains both 18S and muc2 amplicon. The 18S DNA amplicon is 187 base pairs which is present as a faint band. The muc2 amplicon is 64 base pairs and is not visible in the right lane.
**Figure 2.2:** Periodic acid Schiff’s alcian blue (PASAB) stain of ileal tissue from a 72h piglet receiving 9Bu treatment. Image is at 10x magnification. PASAB stain colors neutral mucins pink and acidomucins blue.
Figure 2.3: PASAB stain of ileal villi from a 72h piglet receiving 9Bu treatment. Image is at 40x magnification. PASAB colors neutral mucins pink and acidomucins blue. Goblet cells were counted in one of the 5 following groups: 1.) 100% acidic mucin; 2.) 75% acidic mucin and 25% neutral mucin; 3.) 50% acidic mucin and 50% neutral mucin; 4.) 25% acidic and 75% neutral mucin; and 5.) 100% neutral mucin. These mixed goblet cells were assigned to the group that most closely matched their composition.
Figure 2.4: High iron diamine alcian blue (HIDAB) stain of jejunal tissue from a 12h control piglet. Image is at 10x magnification. HIDAB stain colors sulfomucins brown and sialomucins blue.
75% sulfomucin; 25% sialomucin

50% sulfomucin; 50% sialomucin

**Figure 2.5:** High iron diamine alcian blue (HIDAB) stain of jejunal crypts of a 12h control piglet. Image is at 40x magnification. HIDAB stain colors sulfomucins brown and sialomucins blue. Goblet cells were counted in one of the 5 following groups: 1.) 100% sulfomucin; 2.) 75% sulfomucin and 25% sialomucin; 3.) 50% sulfomucin and 50% sialomucin; 4.) 25% sulfomucin and 75% sialomucin; and 5.) 100% sialomucin. These mixed goblet cells were assigned to the group that most closely matched their composition.
Figure 2.6: Effect of SCFA and Bu supplementation on ileal crypt acidomucin chemotypes. Data is expressed as relative goblet cell number per depth and is adjusted to include neutral and acidic fractions of mixed cells. *Adjusted acidic goblet cell number was greater in the 60Bu treatment group than control (treatment main effect, p=0.038). Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1.
Figure 2.7: Effect of SCFA and Bu supplementation ileal villus acidomucin chemotypes. Data is expressed as relative goblet cell number per height and is adjusted to include neutral and acidic fractions of mixed cells. *Significantly different than respective control group (p=0.031). Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1.
Figure 2.8: Effect of SCFA and Bu supplementation on colonic crypt sialomucin chemotypes. Data is expressed as relative goblet cell number per depth and is adjusted to include sulfated and sialated fractions of mixed cells. *Significantly different than respective control group (p=0.039). Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1.
Figure 2.9: Effect of SCFA and Bu supplementation on jejunal villus sulfomucin chemotypes. Data is expressed as relative goblet cell number per height and is adjusted to include sulfated and sialated fractions of mixed cells. *Sulfomucin chemotypes are greater in 9 Bu than control (treatment main effect, p=0.047). Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1.
Figure 2.10: Effect of SCFA and Bu supplementation on jejunal muc2 mRNA. Data is expressed as muc2 mRNA/18s mRNA. *The 9Bu group had greater muc2 mRNA than control, (treatment main effect, p=0.010). Data were analyzed statistically as one-tailed t-tests in split-plot design using the mixed model in SAS 9.1.
SBS and PN administration both contribute to intestinal barrier dysfunction and bacterial translocation, and these data demonstrate butyrate’s effectiveness in improving innate barrier defense and presents a promising treatment for decreasing rates of bacterial translocation. Within this study, exploring bacterial infection in the surrounding lymph nodes and the liver and kidneys would be an interesting avenue of study to elucidate whether the increases in protective goblet cell chemotypes observed in piglets receiving SCFA and butyrate supplementation decreased bacterial translocation. Additionally, to be sure that any decreases in bacterial infection are attributed to increases in protective mucins, tight junction protein abundance and mRNA expression could also be assessed.

Since PN is much more common than TPN, the effect of butyrate and SCFA administration on goblet cell chemotypes and muc2 in the presence of EN is an important relationship to establish. This avenue would also be important to explore since practitioners try to avoid TPN with most SBS infants and instead try to administer some EN to maintain as much bowel function as possible. Because butyrate has been shown to have profound effects on mucin production when administered luminally (Finnie et al., 1995; Willemsen et al., 2003; Gaudier et al., 2004; Hatayama et al., 2007), and PN is associated with decreased mucin production (Iiboshi et al., 1994; Bertolo et al., 1998; Law et al., 2007), the positive effects of PEN in conjunction with parenteral butyrate or SCFA administration on protective goblet cell chemotypes and muc2 mRNA could be additive.

To study the effects of butyrate administration in addition to EN, butyrate could clinically only be supplied parenterally, as butyrate is not tolerable as an oral solution. However, oral administration of tributyrin is a possibility. Tributyrin is a prodrug form of butyrate which means that it is inactive until metabolized, at which time it becomes an active metabolite. Tributyrin has
been shown to be tolerated in an adult clinical trial and 3 daily oral dosings can attain in vitro levels of butyrate seen in many studies (Edelman et al., 2003). Tributyrin confers positive effects on intestinal morphology by increasing villus height and crypt depth when administered with a fermentable substrate in pigs (Piva et al., 2002; Piva et al., 2008). Further, administration of a 1:1 mixture of triacetin and tributyrin in rats with 60% bowel resection and cecectomy has been demonstrated to significantly increase jejunal mucosa, all intestinal segmental weights, and all intestinal segmental protein levels (Kripke et al., 1991). While in this study it cannot be determined if the positive effects on intestinal adaptation are due to tributyrin rather than triacetin, this is expected to be the case due to all the literature stating specifically butyrate’s positive effects on intestinal adaptation (Tappenden et al., 1997; Bartholome et al., 2004; Hamer et al., 2008) and recent findings supporting butyrate’s relationship with proglucagon and intestinotrophic GLP-2 (Woodard and Tappenden, 2008).

Another method to increase butyrate availability to the intestinal epithelium is to increase its endogenous production. This can be done by feeding fermentable fiber along with PEN that is then digested by the resident microbiota to create SCFAs, including butyrate (Bourquin et al., 1993; Kapadia et al., 1995). Additionally, some fibers increase butyrate production more than others (Kapadia et al., 1995). This method may be less direct than administration of a prodrug; however, it has other benefits. Feeding certain fibers not only increases SCFA production, but can also change the composition of the intestinal microbiota by increasing the density of the resident microbes, which can decrease the growth of harmful microbes such as Clostridium difficile (May et al., 1994). Increasing butyrate production and potentially protective mucins could also decrease growth of harmful bacteria, which would be obviously beneficial in a short bowel syndrome infant at risk for bacterial translocation. Both animal studies and clinical studies exploring the effects of tributyrin and fiber administration are needed to validate the effectiveness of these methods on mucin production and on possible subsequent prevention of bacterial translocation.
In addition, the mechanism by which butyrate increases mucin production needs to be explored. It may be that butyrate increases or decreases an intermediate factor that affects mucin production such as affecting mRNA or protein expression of sulfotransferases or sialotransferases. If there is an intermediate factor, its discovery would allow expedited clinical targeting of increasing mucin production.

In summary, the area of mucin modulation via SCFAs and butyrate is novel and much is yet to be discovered. Butyrate is a promising supplement to potentially improve the clinical outcomes of the SBS pediatric population via improved barrier defense.
REFERENCES


Bartholome, A.L., Albin, D.M., Baker, D.H., Holst, J.J. and Tappenden, K.A. Supplementation of total parenteral nutrition with butyrate acutely increases structural aspects of intestinal


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FORMAL EDUCATION

6/2007 – 8/2010  Masters student in Division of Nutritional Sciences
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Expected Graduation: August 2010
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Advisor: Kelly A. Tappenden, PhD, RD
Thesis research: The Effect of Butyrate-Supplemented Total Parenteral Nutrition on muc2 mRNA and Goblet Cell Chemotypes in a Short Bowel Syndrome Neonatal Piglet Model.

University of Illinois at Urbana Champaign
Graduated May 2010

2001 - 2005  Bachelor of Science in Family and Consumer Sciences
Cum Laude with Honors
University of Georgia, Athens, GA
Major: Dietetics
GPA: 3.67/4.00

OTHER EDUCATION

The ACES Academy of Teaching Excellence
College of ACES Teaching College Course 2009
Graduated November 2009

The University of Illinois at Urbana Champaign, College of Business
Certificate in Business Administration
Graduated Spring 2009
SCHOLARSHIPS, AWARDS, AND HONORS

University of Illinois at Urbana-Champaign
2010  William Rose Endowed Award
2007-2009  Jonathan Baldwin Turner Fellowship
2009  Abbott Nutrition Scholarship for Certificate in Business Administration

University of Georgia, Athens, GA
2001-2005  HOPE Scholarship (full tuition)
           National Collegiate Scholar
           Presidential Scholar (4 semesters)
2004  Top 5% of class

RESEARCH EXPERIENCE

University of Illinois at Urbana Champaign
Laboratory of Kelly A. Tappenden, PhD, RD

June 2007 – June 2009  Research Fellow
July 2009 – Present  Research Assistant

Studies:
2009: Effect of Butyrate Supplementation on Mucin Production and Barrier Function in Short Bowel Syndrome Piglets Receiving TPN
Funding: National Institutes of Health
PI: Kelly Tappenden, PhD, RD
Selected for Oral Presentation at Experimental Biology Conference 2010 for the American Society for Nutrition symposia “Animal Models in Gastrointestinal Health and Disease”

2008: Evaluation of GATTEX Effects on Intestinal Development in Neonatal Piglets
Funding: NPS Pharmaceuticals
PI: Kelly Tappenden, PhD, RD

2008: Impact of Route of Delivery on the Response to Dietary Prebiotics in Neonatal Piglets
Funding: Bristol-Myers Squibb
PI: Sharon Donovan, PhD, RD
Role: Intestinal electrophysiology, ion transport and ion secretion

2007: Effect of Mode of Delivery and Early Nutrition on Intestinal Development in the Neonatal Piglet
Funding: Bristol-Myers Squibb
PI: Sharon Donovan, PhD, RD
Role: Intestinal electrophysiology, ion transport and ion secretion
**TEACHING EXPERIENCE**

2008  
University of Illinois at Urbana Champaign  
Research Apprentice Program Mentor

2005 – 2006  
Rockdale County Public Schools  
Substitute Teacher

2004 – 2005  
University of Georgia  
Teaching Assistant: Anatomy and Physiology Labs I and II

2002 – 2004  
Athens Clarke County Mentoring Program

**PROFESSIONAL EXPERIENCE**

**4-H Illini Food Science and Human Nutrition Summer Academy**  
**University of Illinois, Department of Food Science and Human Nutrition**

*Co-Coordinator*  
*January 2009 - July 2009*

*FSHN Summer Academy dates*  
*June 29th – July 1st 2009*

- Developed and implemented creative FHSN programming, devised budget, recruited professors
- Delivered lessons on anatomy, physiology, and molecular nutrition
- Generated interest for University of Illinois and for FSHN through program
- Worked with 4-H Extension Specialist, Deb Stocker, to create IRB to evaluate efficacy of program
- Students who participated in IRB evaluation marked “agreed” or “strongly agreed” on areas of high enjoyment of academy, retention of FSHN concepts from academy, and recommendation of academy to peers.

**Women, Infants, Children Program**  
**East Metro Health District, Newton County Health Department; Covington, GA**

*Nutritionist*  
*July 2006 – May 2007*

- Assess nutritional risk and supply nutrition education to clients
- Provide nutrition education to community through Nutrition Education Outreach Program
- Involved in statewide committee devoted to changing WIC counseling process

**PROFESSIONAL ORGANIZATIONS**

- American Dietetic Association
- American Society of Nutrition
- American Society of Parenteral and Enteral Nutrition
- University of Georgia Alumni Association
- Nutritional Sciences Graduate Student Association, *Secretary, 2008*