SPECIAL ARTICLE

Emerging Therapies for Intestinal Failure

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Given the immeasurable human distress and health care burden associated with intestinal failure, medical therapies aimed at intestinal rehabilitation are needed. Following massive small-bowel resection, the residual intestine is known to adapt structurally and functionally in an attempt to compensate for the resected portion. However, parenteral nutrition may be associated with many short- and long-term complications, including prevention of intestinal adaptation and promotion of mucosal atrophy due to lack of stimulus provided by oral or enteral nutrition. However, data herein demonstrate that the addition of butyrate, a short-chain fatty acid produced in the colon by dietary fiber fermentation, stimulates intestinal adaptation when added to parenteral nutrition, indicating that current solutions could be formulated to optimize intestinal adaptation and to reduce dependence of individuals with intestinal failure receiving long-term parenteral nutrition regimens. *Arch Surg. 2010;145(6):528-532*

It is truly a pleasure for me to participate in this symposium honoring Stanley J. Dudrick, MD, and his pivotal contributions to our practice of clinical nutrition. Like those who spoke before me, I am grateful for the strong influence that he has used to positively mentor me; however, I differ from those same individuals because I did not inherit his influence as a birthright associated with my academic pedigree. In fact, I first met Dr Dudrick as a young graduate student from Canada, who was at my first US meeting. I did not know a soul! On his own initiation, Dr Dudrick kindly stayed after my minisymposium presentation and discussed my research with me. He told me how important this work was and encouraged me to "keep it up" because the future of our field depended on young scientists such as myself. I have heard these words from him many times since then, and over time I began to believe in myself as that scientist that he saw. I tell this story to demonstrate how gracious Dr Dudrick is with his mentoring influence. One does not need to have gone to a certain school or trained with a particular individual. Dr Dudrick evaluates each individual for where they are at and gives them encouragement and his very best advice. As he said, we are responsible for the future of our discipline; however, armed with the confidence and wisdom that he has shared with us, I am confident that we will strive to honor his legacy with our strongest commitment.

With this commitment in mind, I would like to discuss the work of my research group that focuses on intestinal failure. The hallmarks of intestinal failure are diarrhea, dehydration, electrolyte disturbances, malabsorption, and progressive malnutrition. The development of parenteral nutrition (PN) by Dr Dudrick is considered the most important factor responsible for saving the lives of individuals with severe intestinal failure. Howard et al[1] observed that more than 75% of individuals receiving home PN survive longer than 3 years, and there are several anecdotal reports of individuals who have been receiving PN for more than 30 years. Complications exist, but the long-term viability of this therapy provides a window for us to amend the intestinal deficit, while the patient remains in a well-nourished and functioning state. Contemporary strategies for amending this deficit include intestinal transplantation and intestinal rehabilitation.

Stimulating the process of intestinal adaptation is a strategy to increase the sur-

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face area and functional capacity of the residual small intestine following massive small-bowel resection. Most of us are familiar with the marked trophic effect that enteral nutrients have on small intestinal mucosa. Examples of jejunoileal bypass from the days when this was used as a method of bariatric surgery provide us with a clear demonstration of the hypertrophied functioning intestine compared with the atrophied bypassed segment. Observations such as these led to an area of research that focuses on what hormones or factors could be added to PN to provoke the adaptive responses observed in the intestine whereby enteral nutrients are processed. Among the many stimuli that have been studied to date, discussed herein are the effects provided by short-chain fatty acids (SCFAs) and glucagonlike peptide 2 (GLP-2).

Nordgaard et al[2] sought to understand what attributes were important to maximize energy absorption in individuals with intestinal failure by analyzing the importance of the following 2 factors: (1) the presence of a functional colon and (2) the consumption of a high-carbohydrate diet. Among humans with short-bowel syndrome, they determined that energy absorption could be maximized by consuming a high-carbohydrate diet; however, the benefit was only achieved when a functional colon was present. There was no advantage to a high-fiber diet in patients with a jejunostomy. They theorized that the colon acted as a scavenging digestive organ in states of malabsorption owing to the presence of intestinal microbiota that served to ferment malabsorbed carbohydrate and soluble fiber. This fermentation process that allowed for energy capture from these substrates was termed *carbohydrate salvage* and is dependent on the colonic epithelium to presumably absorb and oxidize the SCFAs produced by fermentation.

The SCFAs are a group of gastrointestinal-specific fuels that are a product of anaerobic bacterial metabolism of dietary fiber and resistant carbohydrate. Acetate, propionate, and butyrate are produced intraluminally in an almost constant molar ratio of 60:25:15 and account for approximately 85% of formed SCFAs.[3] Among their various properties, SCFAs stimulate sodium and water absorption in the colon, are quickly absorbed by intestinal mucosa, are high in caloric content, are readily metabolized by intestinal epithelium and liver, and are trophic to the intestinal mucosa.[4] In rats, the addition of SCFAs to PN has been shown to prevent PN-associated mucosal atrophy[5] and to enhance structural markers of adaptation to small-bowel resection.[6,7] Confirming work by Koruda et al,[6] a study by Tappenden et al[8] revealed that systemic SCFAs increase nutrient uptake and messenger RNA (mRNA) abundance of the basolateral hexose transporter glucose transporter 2 (GLUT2) following intestinal resection in rats. These findings are unique because they indicate that a specific nutrient (ie, SCFA) may modulate the gene expression and transport capacity of other nutrients (ie, glucose, galactose, and fructose). Furthermore, the observed increase in nutrient uptake indicates that systemic nutrients (ie, those provided to the basolateral side of the enterocyte, as in total PN) can increase the transport of luminal substrates (via brush border transporters) provided by oral or enteral nutrition. The concept that SCFAs can be provided intravenously to prepare the intestinal brush border for effective digestion and absorption of enteral nutrients is particularly attractive for patients with intestinal failure.

The most direct interpretation of the ability of the colon to harvest energy from fermented carbohydrates in the form of SCFAs implies a certain advantage to an individual unable to digest and absorb consumed nutrients in the small intestine. However, we postulated that these products of colonic fermentation may be involved in the regulatory process wherein the distal intestine initiates humoral signals that stimulate the adaptation to begin. In essence, a feedback mechanism whereby malabsorbed substrates reaching the distal intestine initiate the cascade that prompts the proximate intestine to expand its digestive and absorptive capacity is a powerful scenario. Furthermore, literature citing a complex carbohydrate diet's role in increasing energy absorption of those with short-bowel syndrome were adults only,[2] and the potential effect in pediatric intestine captured our attention given the many children with intestinal failure. Finally, only cocktails of SCFAs had been shown to be intestotrophic to that point,[5-8] and given the tight correlation of butyrate with intestinal proliferation, we postulated that it was the effective SCFA among those commonly studied.

In a project funded by the National Institutes of Health (Bethesda, Maryland), we sought to test the hypothesis that total PN supplemented with butyrate alone will rapidly increase the indexes of intestinal adaptation in the neonate and that these adaptive responses will be associated with increased plasma GLP-2 concentration. Neonatal piglets (n=126) were obtained from a local swine producer within 24 hours of birth and underwent superior vena cava cannulation, swivel placement, and 80% proximal jejunoileal resection. Littermate piglets with similar body weights were randomized to 1 of the following 4 treatment groups: (1) standard PN, (2) standard PN plus 60mM SCFAs (30mM acetate, 15mM propionate, and 9mM butyrate), (3) standard PN plus 9mM butyrate, or (4) standard PN plus 60mM butyrate. Within each diet group, animals were further randomized (6 per group) to receive infusions at various time points after surgery to allow for examination of short-term (4, 12, or 24 hours) and long-term (3 or 7 days) adaptations.

A critical feature of this experimental design was the neonatal piglet model of intestinal failure with nutritional support. The neonatal piglet is an excellent animal model for the human infant because of similar pathways of nutrient digestion, absorption, and metabolism.[9-12] In addition, the neonatal piglet is a commonly used model for the parenterally fed infant[13-15] and is known to be a suitable model for investigating therapeutic modalities for short-bowel syndrome in humans.[16-20] Heemskerk et al[18] validated the piglet intestinal failure model to ensure that it resembled the complications of short-bowel syndrome in human neonates and demonstrated that the model induces impaired body weight gain and adapts the remaining bowel at the tissue and cellular levels. In addition, the neonatal piglet experiences clinical complications comparable to those of the human neonate receiving PN such as intestinal atrophy, failure to thrive, and catheter sepsis.[21] Furthermore, the full-term neona-

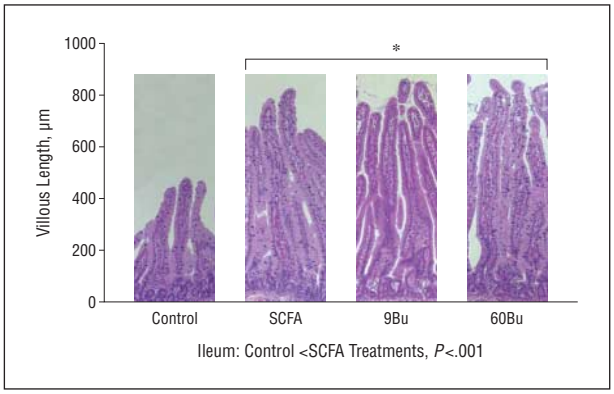

Figure 1. Ileal villous length was increased (*$P<.001$) following treatment with control parenteral nutrition or supplementation with a short-chain fatty acid (SCFA) cocktail, 9mM butyrate (9Bu), or 60mM butyrate (60Bu). Modified from Bartholome et al.[23]

tal piglet has a body composition similar to that of the premature human (23-31 weeks' gestation), permitting more invasive methods of investigation while maintaining clinical relevance.[22] Whereas a human infant doubles its birth weight by 4 to 6 months, the piglet doubles its birth weight in the first 7 to 10 days of life, providing a rapid model of growth and development for focused investigations.[15,22]

A fundamental observation clarified by this research is that SCFAs enhance structural and functional attributes of intestinal adaptation in the neonatal intestine after resection, as was previously shown to occur in the adult rat intestine.[6] Intestinal adaptation was observed using various structural aspects (intestinal weight or length, mucosal weight or composition, morphologic structure, and epithelial kinetics) and functional aspects (disaccharidase activity, ion and nutrient transport, and mRNA and protein abundance of enterocyte-associated nutrient transporters). These effects are detailed in the literature,[23] and representative data demonstrating these adaptations are shown in **Figure 1** and **Figure 2**.

Butyrate is the SCFA responsible for the increase in structural and functional adaptations following intestinal resection in neonatal piglets, as evidenced by almost identical effects of 3 SCFAs (36mM acetate, 15mM propionate, and 9mM butyrate) compared with 9mM butyrate alone. Structural and functional attributes of intestinal adaptation revealed that butyrate was the SCFA responsible for the adaptive results observed with the SCFA cocktail. This point is clearly shown in Figure 1, wherein ileal villous length was increased ($P<.001$) in all 3 SCFA-supplemented groups compared with the control PN group following massive small-bowel resection in neonatal piglets. This expansion of the villous epithelium was due to an increase in epithelial proliferation, as demonstrated by an increased ($P<.001$) number of proliferation cell nuclear antigen–stained cells per crypt (Figure 2), supported by similar increases ($P=.004$) in mucosal DNA concentration. No difference was observed between the SCFA cocktail-, 9mM butyrate-, or 60mM butyrate-treated groups in any of the dependent variables assessed.

Intestinal adaptation was affected at short- and long-term time points by butyrate; however, structural and functional adaptations did not occur in parallel. Examination of the results obtained in this study[23] revealed that many structural attributes of intestinal adaptation were altered by butyrate, with increases ($P<.001$) observed as early as 4 hours after treatment (Figure 2). However, this observation was not widespread, revealing a dichotomy between the structural and functional adaptations assessed. For example, ileal peptide transporter 1 (PepT1) transport was approximately half that of the control group after 3 days of butyrate treatment; by day 7 of butyrate treatment, PepT1 activity was increased almost 8-fold compared with that of the control group (**Figure 3**). Data such as these demonstrate that the process of intestinal adaptation can be augmented within hours of stimulation; however, they underscore the concept that structural and functional adaptations are regulated by distinct signals and likely represent a lag associated with differentiation of newly proliferated cells in the crypt to those expressing functional proteins, such as PepT1 within the upper villous region. The use of a time course to uncouple the short- and long-term timing of the structural and functional adaptations after resection remains an important component of future work.

Butyrate affects intestinal function in short- and long-term manners. Despite the data in Figure 3 showing that some functional attributes (such as PepT1 activity) may require epithelial differential and time for the effects of butyrate to be fulfilled, other evidence revealed short-term effects of butyrate on nutrient transport.[23] However, a novel observation in our laboratory demonstrated that physiologic concentrations of luminal butyrate (10mM) increase ($P<.05$) sodium-glucose cotransporter 1 activity more than 2-fold within 5 minutes of exposure (**Figure 4**).[24] We are testing the hypothesis that the mechanism whereby butyrate mediates this response is via facilitation of recruitment of intracellular pools of SGLT-1 to the brush border membrane.

Butyrate administration increases GLP-2 plasma concentration, thereby revealing a potential regulatory mechanism. At all short- and long-term time points studied after intestinal resection in neonatal piglets, plasma GLP-2 concentration was increased ($P=.007$) in the groups treated with supplemental total PN (SCFA, 9mM butyrate, and 60mM butyrate) compared with the control group (**Figure 5**).[23] However, despite our initial hypothesis that this increase in GLP-2 was the mechanism whereby butyrate increased intestinal adaptation, the data we generated led us to postulate that, although some of the effects observed with butyrate may have been mediated by GLP-2, others are likely due to the direct effects of butyrate. For example, our work studying the GLUT2 promoter found that butyrate activated this promoter, whereas GLP-2 did not. Similarly, the acute effects of butyrate on SGLT-1 activity (Figure 4) were not recapitulated with GLP-2 treatment. Finally, using a rat intestinal resection model, we determined that inhibiting GLP-2 activity via use of the truncated GLP-2 metabolite (3-33), shown to be a receptor antagonist,[25] only partially inhibited specific structural and functional attributes of intestinal adaptation.[26]

Butyrate alone is also responsible for activation of the GLUT2 promoter. The conclusion that butyrate is the effective SCFA regarding intestinal adaptation was sup-

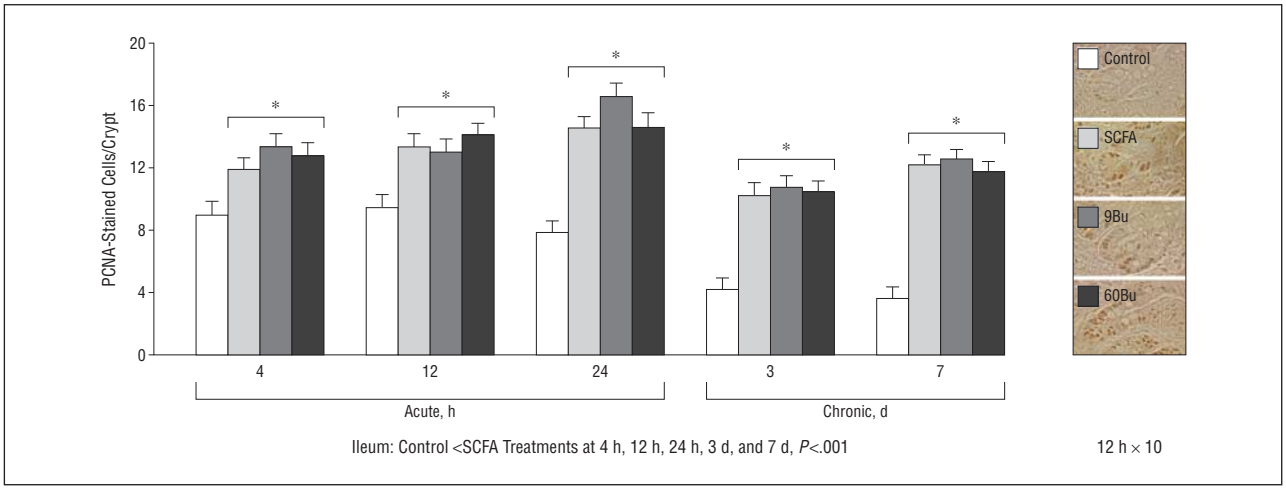

Figure 2. Ileal epithelial proliferation was increased (*$P<.001$) following treatment with control parenteral nutrition or supplementation with a short-chain fatty acid (SCFA) cocktail, 9mM butyrate (9Bu), or 60mM butyrate (60Bu). PCNA indicates proliferation cell nuclear antigen. Sections shown are from the 12th-hour time point and visualized at ×10 magnification strength. Modified from Bartholome et al.[23]

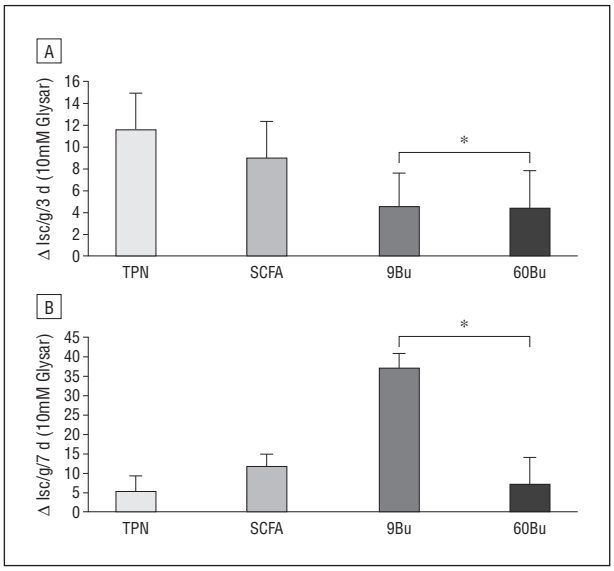

Figure 3. Activity of the ileal peptide transporter 1 following treatment with control total parenteral nutrition (TPN) or supplementation with 9mM butyrate (9Bu) or 60mM butyrate (60Bu) at 3 days (top) and 7 days (bottom) following intestinal resection in piglets. *$P<.05$, butyrate vs TPN or short-chain fatty acid (SCFA) treatments. Glysar indicates glycylsarcosine; Isc, short-circuit current.

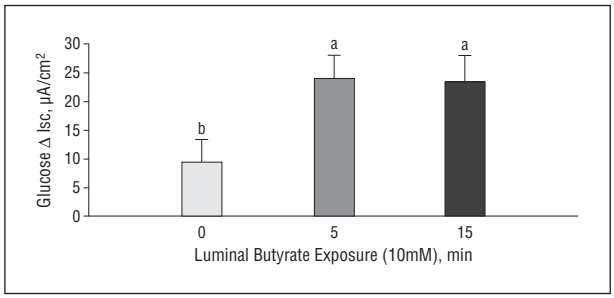

Figure 4. Ileal sodium-glucose cotransporter 1 activity is upregulated ($P<.05$) within 5 minutes of luminal exposure to physiologic concentrations of butyrate (10mM), as measured in modified Ussing chambers. Bars with different letters are significantly different from each other. Isc indicates short-circuit current.[24]

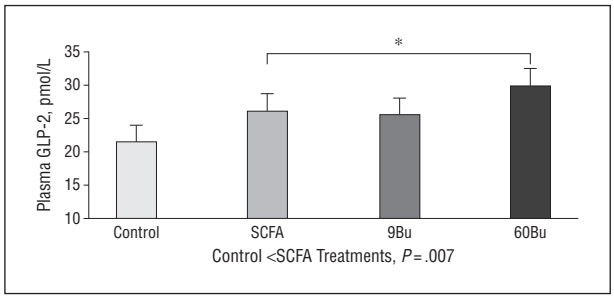

Figure 5. Plasma glucagonlike peptide 2 (GLP-2) concentration is increased (*$P=.007$) by all short-chain fatty acid (SCFA) supplements, including butyrate alone, following intestinal resection in neonatal piglets. 9Bu indicates 9mM butyrate; 60Bu, 60mM butyrate. Modified from Bartholome et al.[23]

ported by in vitro data examining upregulation of the human GLUT2 promoter by butyrate using Caco2-BBe monolayers. In an in vitro system devoid of GLP-2, butyrate increased GLUT2 mRNA abundance and induced promoter activity in a dose-dependent fashion ($P<.001$ for both), whereas acetate and propionate did not (**Figure 6**).[27] As such, focused experiments are needed to identify the critical components of intestinal adaptation and to yield conclusions regarding the relative contributions of butyrate and GLP-2 during the various phases of this process. It is possible that butyrate initiates adaptation directly; however, it is more likely involved in a cascade of adaptive mediators, of which GLP-2 is a strong candidate. Indeed, experimental evidence indicates that there are likely separate signals for short- vs long-term adaptation,[23] as there are for structural vs functional adaptation[23]; therefore, understanding signaling molecules during each of these phases requires further study.

In summary, the data using a relevant preclinical model of pediatric short bowel syndrome indicate that butyrate is the SCFA responsible for increased structural and functional indexes of adaptation and that these structural and functional adaptations of the residual intestine are altered at the short- (4 hours) and long-term (7 days) time points studied. Armed with this knowledge, we seek to establish clinically feasible methods for administering butyrate to human infants with intestinal failure to stimulate structural and functional adaptations of their small

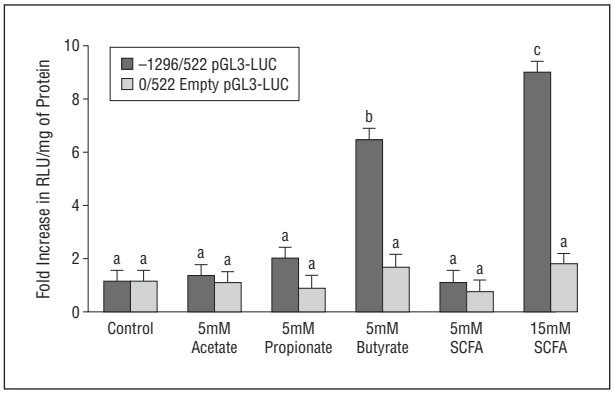

Figure 6. Response of the glucose transporter 2 promoter in Caco2-BBe cells to short-chain fatty acid (SCFA) treatment. Caco2-BBe monolayers were treated with SCFA for 48 hours before harvest. Butyrate increased ($P < .05$) promoter activation compared with all other treatments. Bars with different letters are significantly different from each other. RLU indicates relative light units. From Mangian and Tappenden.[27]

intestine. We postulate that the strategic provision of partial enteral nutrition, specifically formulated to augment butyrate production by the inclusion of prebiotics and probiotics, will promote structural and functional adaptations in neonatal piglets with short bowel syndrome by enhancing mucosal surface area and nutrient processing capacity, thereby preparing the residual small intestine for proper digestion and absorption of enteral nutrients. Indeed, the administration of butyrate directly to the intestinal mucosa, compared with systemically as in our previous investigations, may be even more efficacious. If the mechanisms by which butyrate stimulates intestinal adaptation are revealed using a clinically feasible administration strategy, then the timing, route, and composition of nutritional support could be optimized to promote intestinal adaptation in children with intestinal failure and to reduce their long-term dependence on PN. By enhancing the lives of children with intestinal failure, we honor the legacy of Stanley J. Dudrick, MD, wherein sustaining their lives became possible.

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